

METHODS OF TREATING DYSTROPHINOPATHIES

Technical field

- 5 The present invention relates, in general terms, to methods of treating dystrophinopathies and more specifically to methods of treating dystrophinopathies such as Duchenne and Becker's muscular dystrophy using LINC complex inhibitors.

Background

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Dystrophin is a structural protein that stabilises the membranes of skeletal and cardiac muscle. Duchenne muscular dystrophy (DMD) is the most frequent form of muscular dystrophy and is caused by mutations that result in loss of the dystrophin protein. The onset of DMD is in early childhood, resulting in progressive muscle wasting and immobility in the
15 early teens. Death occurs in the mid- to late 20s, primarily due to cardiomyopathy. Becker muscular dystrophy (BMD), which also arises from mutations in the dystrophin gene, results in the production of a partially functional dystrophin protein and typically presents with a milder phenotype. Various therapeutic options have been investigated to treat DMD, BMD and other dystrophinopathies arising from insufficient or dysfunctional dystrophin; these
20 range from gene replacement using a mini dystrophin gene to various small molecules to suppress the symptoms. None have yet been shown to be effective in treating DMD cardiomyopathy.

It would be desirable to overcome or ameliorate at least one of the above-described problems,
25 or at least to provide a useful alternative.

Summary

Disclosed herein is a method of treating or preventing a dystrophinopathy in a subject, the
30 method comprising administering an effective amount of a LINC complex inhibitor to the subject.

Disclosed herein is a LINC complex inhibitor, for use in treating or preventing a dystrophinopathy in a subject.

Disclosed herein is the use of a LINC complex inhibitor in the manufacture of a medicament for treating or preventing a dystrophinopathy in a subject.

- 5 Disclosed herein is a method of treating or preventing a myopathy and/or muscular dystrophy associated with a dystrophinopathy in a subject, the method comprising administering an effective amount of a LINC complex inhibitor to the subject.

Disclosed herein is a method of treating or preventing a dystrophinopathy in a cell, the
10 method comprising contacting the cell with an effective amount of a LINC complex inhibitor.

Brief description of the drawings

Embodiments of the present invention will now be described, by way of non-limiting
15 example, with reference to the drawings in which:

Figure 1 Characterisation of iPSC derived cardiomyocytes. A) RT-PCR using primers spanning exons 46-49 and 12-18 of human dystrophin gene showed reduced dystrophin expression in mutant cells. B) Representative immunofluorescence images of control 5171,
20 DMD-3429 and DMD-5169 Cardiomyocytes (CMs) showed positive staining of cardiac specific marker sarcomeric-actinin. Dystrophin protein expression was detected in control 5171-CM, and not in the DMD3429 and DMD5169 CMs. The antibody against ALFA detected DNSUN1-ALFA fusion protein expression in cell lines with DNSUN1-ALFA knock-in. Scale bar: 50µm.

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Figure 2 shows an analysis of video recordings of the contraction of iPSC-derived cardiomyocytes at day 11 after differentiation. A) Representative images from videos showing the time to the peak of contraction. The contractions of DMD-CM (3429 and 5169) are slower comparing with parental control (5171). B) Representative pictures from videos
30 of DMD-3429 and DMD-5169 subclones (set A) showed slow contractions were rescued following DNSUN1 knock-in. C) Fluorescent video recording of iPSC-CM DMD-3429 and DMD-5169 subclones with GCaMP6s knock-in (set B) showed slow contractions were rescued by DNSUN1 knock-in (Fluo4-AM staining). D) Summary analysis of the time to the peak of each contractions from the lines. The slow and irregular contractions in the

DMD-CM lines (DMD3429,3429-GCaMP6s,DMD5169, 5169-GCaMP6s) were rescued by DNSUN1 knock-in. (unpaired t-test, n=20, **p<0.01***p<0.001, ****p<0.0001),

Figure 3 shows the restoration of reduced contractility in DMD-CM by DNSUN1 knock-in.

- 5 The purified cardiomyocytes were stained with 5uM Fluo4-AM and the fluorescent intensity representing their Ca^{2+} cycling was quantified over the duration of the recordings. (A) Data from 20-30 single cell recordings were summarized and analysed. Compared to controls (5171), the DMD cardiomyocytes (3429) showed large variation in Ca^{2+} intensity and exhibited significantly increased beat to beat duration (t-test, p<0.001, n=20-30), the results
- 10 indicate DMD cardiomyocytes demonstrated arrhythmia and bradycardia, however, after DNSUN1 knock-in DMD lines (3429-DNSUN1), the reduced beating frequency was significantly improved (t-test, p<0.001, n=20-30). (B) The contractility of the cardiomyocytes were indicated by the amplitude of Ca^{2+} transient. A representative recording of 10sec live imaging showed reduced contractility of DMD-CM was rescued in
- 15 DNSUN1 knock-in DMD-CM to the level comparable to the control line.

Figure 4 shows the restoration of beating frequency of DMD heart organoids (HOs) by DNSUN1 knock-in. 10^5 cardiomyocytes were aggregated to form 1 heart organoid, with 12 organoids being generated from each line. Beating frequencies were recorded and monitored

- 20 over 28 days. Results showed that the DMD-HO (3429) demonstrated slow and arrhythmogenic beating frequency (A), that quickly decline and stop beating by day 21, while with the DNSUN1 knock-in, the contractions of HOs were significantly rescued starting from day7 till day 28(B). t-test, ***p<0.001, n=10-12.

- 25 **Figure 5** shows that muscle necrosis in the diaphragms of 21-day old mdx mice is reduced with the loss of SUN1. There was no difference in muscle necrosis between the quadriceps of mdx/mdx WT and mdx/mdx/SUN1^{-/-} mice, but significantly less myofibre necrosis in the mdx/mdxSUN1^{-/-} mice. (P=0.031, Student t-test).

- 30 **Figure 6** shows the establishment of Dox-inducible MYOD1 iPSCs. (A) Schematic representation of piggybac-inducible MYOD1 plasmid map and SKM differentiation and maturation protocols. (B) DMD-iPSCs were differentiated to myoblasts at Day 5 and myotubes at Day 7 upon doxycycline induction. (C) Immunostaining of iPSC-SKMs for skeletal muscle-specific markers (MyoG, MHC and a-actinin).

Figure 7 shows that AAV-DJ-DNSUN1 transduction rescued over-activation of TGF β profibrotic signaling in DMD-SKM. (A) Immunostaining of DMD and control myotubes for pSmad2. The intensity of pSmad2 were quantitated by ImageJ software. Results from three biological experiments are shown. Values represents mean $\pm$ SD (n=10 images and >500 CMs per line were analysed, ****p<0.0001, ***p<0.001). (B) Immunostaining of collagen I in DMD and control myotubes at Day 11 (after dox induction) showed that AAV-DJ delivery of DNSUN1 (on Day 5 after dox induction) significantly reduced intracellular collagen levels. Fluorescence intensity was quantitated by ImageJ software. Results from three biological experiments were pooled. Value represents mean $\pm$ SD (n=10 images and >500 CMs per line were analysed, ****p<0.0001, ***p<0.001). (C) Quantitative RT-PCR confirmation of collagen I expression in iPSC-SKM at Days 7 and 9 after Dox inductions. Relative expression of genes against GAPDH are analysed as mean $\pm$ SD (n = 3 biological replicates, *p<0.05, **p<0.01, ***p<0.001, unpaired two-tailed T-test).

Detailed description

Dystrophin is part of the cellular skeletal complex that extends from the extracellular matrix through the cell membrane to the nuclear membrane. The inventors have discovered that disruption of the LINC complex, which is a protein complex localised to the nuclear membranes, is surprisingly effective at treating myopathies and muscular dystrophies associated with a dystrophinopathy, e.g., Duchenne muscular dystrophy (DMD). DMD cell models were generated from induced pluripotent stem cells (iPSCs) derived from fibroblasts of DMD patients. The iPSCs were differentiated into skeletal muscle cells (myotubes) and cardiomyocytes, from which heart organoids were further derived. The cell models recapitulated defects seen in DMD and other dystrophinopathies; for example, the DMD cardiomyocytes exhibited significant arrhythmia and bradycardia, and the DMD myotubes exhibited constitutive over-activation of TGF β -dependent pro-fibrotic signalling. The inventors then delivered a gene encoding a dominant-negative SUN1 mini-protein (DNSUN1) to the model cells. SUN1 is a LINC complex protein and DNSUN1 was previously shown to be capable of disrupting LINC complex assembly. LINC complex disruption through expression of DNSUN1 significantly improved the contractility and ion channel gating of DMD cardiomyocytes and heart organoids to levels seen in normal cardiomyocytes. Similarly, expression of DNSUN1 in DMD skeletal muscle cells

significantly reduced pro-fibrotic signalling to levels seen in normal skeletal muscle cells. In addition, the inventors also showed that genetic ablation of SUN1 in a mouse DMD model (mdx) significantly reduced skeletal muscle necrosis in the diaphragm.

- 5 Accordingly, this disclosure provides methods of treating or preventing dystrophinopathies or dystrophinopathy-associated muscle disorders or conditions using a LINC complex inhibitor.

Disclosed herein is a method of treating or preventing a dystrophinopathy, or a myopathy
10 and/or skeletal muscular dystrophy associated with a dystrophinopathy, in a subject, the method comprising administering an effective amount of a LINC complex inhibitor to the subject.

Disclosed herein is a LINC complex inhibitor, for use in treating or preventing a
15 dystrophinopathy, or a myopathy and/or skeletal muscular dystrophy associated with a dystrophinopathy, in a subject.

Disclosed herein is the use of a LINC complex inhibitor in the manufacture of a medicament for treating or preventing a dystrophinopathy, or a myopathy and/or skeletal muscular
20 dystrophy associated with a dystrophinopathy, in a subject.

Disclosed herein is a method of treating a dystrophinopathy in a cell, the method comprising contacting the cell with an effective amount of a LINC complex inhibitor.

25 General definitions

The term “inhibitor” as used herein refers to an agent or composition which decreases or abolishes at least one function or biological activity of a target molecule or molecular complex. The inhibitor may decrease or abolish the level of an RNA or protein, for example,
30 by decreasing/abolishing expression of the RNA or protein, or decreasing the half-life of the RNA or protein. Alternatively, the inhibitor may decrease or abolish a biological activity of the RNA or protein, for example, by decreasing/abolishing interaction of the RNA or protein with a binding partner or a substrate. The inhibitor of a molecular complex may act on one or more components of the molecular complex, e.g., by decreasing/abolishing

expression of a component, or decreasing/abolishing assembly of the complex by disrupting intermolecular interactions. Alternatively, the inhibitor of a molecular complex may act on the assembled complex, e.g., by decreasing/abolishing interaction of the complex with a molecular target, or decreasing/abolishing signal transduction from the complex.

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The term “expression” refers to the biosynthesis of a gene product. For example, in the case of a coding sequence, expression involves transcription of the coding sequence into mRNA and translation of mRNA into one or more polypeptides. Conversely, expression of a non-coding sequence involves transcription of the non-coding sequence into a transcript only.

10 The term “expression” is also used herein to refer to the presence of a protein or molecule in a particular location and, thus, may be used interchangeably with “localisation”.

As used herein, a “site-specific nuclease (SSN) system” comprises one or more nucleases engineered or naturally capable of introducing a single- or double-strand break at a specific site in a nucleic acid sequence. The nuclease may act alone or in conjunction with guiding molecules such as RNAs or proteins to achieve precise targeting. The term “SSN system” encompasses both the nuclease and any cooperative molecules required to achieve sequence specificity and cleavage. Examples of SSN systems include, but are not limited to, zinc finger nuclease (ZFN) systems, transcription activator-like effector nuclease (TALEN) systems, meganuclease systems, and CRISPR-associated nucleases (Cas) systems.

The terms “disruption” and “disrupted” are used interchangeably herein to refer to any genetic modification that decreases or eliminates expression and/or the functional activity of the nucleic acid or an expression product thereof. For example, disruption of a gene includes within its scope any genetic modification that decreases or eliminates expression of the gene and/or the functional activity of a corresponding gene product (e.g., mRNA and/or protein). Genetic modifications include complete or partial inactivation, suppression, deletion, interruption, blockage, or down-regulation of a nucleic acid (e.g., a gene). Illustrative genetic modifications include, but are not limited to, gene knockout, inactivation, mutation (e.g., insertion, deletion, point, or frameshift mutations that disrupt the expression or activity of the gene product), or use of inhibitory nucleic acids (e.g., inhibitory RNAs such as sense or antisense RNAs, molecules that mediate RNA interference such as siRNA, shRNA, miRNA; etc.), inhibitory polypeptides (e.g., antibodies, polypeptide-binding partners, dominant

negative polypeptides, enzymes etc.) or any other molecule that inhibits the activity of the gene or level or functional activity of an expression product of the gene.

The terms “polynucleotide” and “nucleic acid” are used interchangeably herein to refer to a polymer of nucleotides, which can be RNA, mRNA, cRNA, cDNA or DNA. The term typically refers to a polymeric form of nucleotides of at least ten bases in length. The constituent nucleotides may be ribonucleotides, deoxynucleotides, a combination of both, or a modified form of either type of nucleotide. The term includes single- and double-stranded forms of DNA.

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The term “nucleotide” refers to a ribonucleotide or a deoxyribonucleotide or modified form thereof, as well as an analogue thereof. Nucleotides include species that comprise purines, e.g., adenine, hypoxanthine, guanine, and their derivatives and analogues, as well as pyrimidines, e.g., cytosine, uracil, thymine, and their derivatives and analogues. The term “nucleotide” is also meant to include what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, and nebularine. The term “nucleotide” is also meant to include the N3' to P5' phosphoramidate resulting from the substitution of a ribosyl 3' oxygen with an amine group. Further, the term nucleotide also includes those species that have a detectable label, such as for example a radioactive or fluorescent moiety, or mass label attached to the nucleotide.

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Nucleotide analogues include nucleotides having modifications in the chemical structure of the base, sugar and/or phosphate, including, but not limited to, 5' pyrimidine modifications, 8' purine modifications, modifications at cytosine exocyclic amines, and substitution of 5-bromo-uracil; and 2' sugar modifications, including but not limited to, sugar-modified ribonucleotides in which the 2'-OH is replaced by a group such as an H, OR, R, halo, SH, SR, NH₂, NHR, NR₂, or CN, wherein R is an alkyl moiety. Nucleotide analogues are also meant to include nucleotides with bases such as inosine, queuosine, xanthine, sugars such as 2'-methyl ribose, non-natural phosphodiester linkages such as methylphosphonates, phosphorothioates and peptides.

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Modified bases refer to nucleotide bases such as, for example, adenine, guanine, cytosine, thymine, uracil, xanthine, inosine, and queuosine which have been modified by the replacement or addition of one or more atoms or groups. Some examples of types of

modifications that can comprise nucleotides that are modified with respect to the base moieties include but are not limited to, alkylated, halogenated, thiolated, aminated, amidated, or acetylated bases, individually or in combination. More specific examples include, for example, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N,N,-
5 dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5-(2-amino)propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine, 7-methylguanosine, 2,2-dimethylguanosine, 5-methylaminoethyluridine, 5-methyloxyuridine, deazanucleotides such as 7-deaza-adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O- and N-alkylated purines and pyrimidines such as N6-methyladenosine, 5-methylcarbonylmethyluridine, 15 uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4,6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides
20 that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties may be, or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles.

25 As used herein, the terms "polypeptide", "peptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally-occurring amino acid, such as a chemical analogue of a corresponding naturally-occurring amino acid, as well as to naturally-occurring amino acid
30 polymers. These terms do not exclude polymers containing amino acid modifications, for example, glycosylations, acetylations, phosphorylations and the like. Soluble forms of the subject proteinaceous molecules are particularly useful. Included within the definition are also polypeptides containing one or more analogues of an amino acid including, for example, unnatural amino acids, and polypeptides with substituted linkages.

- The terms “treating” and “preventing” include: delaying or preventing the onset of symptoms of the disease, disorder or condition; reducing the severity of (i.e., alleviating) the symptoms of the disease, disorder or condition; reversing the symptoms of (i.e.,
- 5 ameliorating) the disease, disorder or condition; reducing morbidity of subjects having the disease, disorder or condition; reducing mortality of subjects having the disease, disorder or condition; delaying or preventing progression of the disease, disorder or condition (e.g., to a later stage); and/or otherwise inhibiting the symptoms or effects of the disease, disorder or condition for at least a period of time. It is to be understood that the terms “treating” and
- 10 “preventing” do not imply that the disease, disorder or condition, or a symptom or effect thereof, is permanently delayed, reduced, alleviated, ameliorated or otherwise inhibited and therefore also encompasses the temporary delay, reduction, alleviation, amelioration or otherwise inhibition of the disease, disorder or condition, or a symptom or effect thereof.
- 15 The term “administering” refers to contacting, applying, injecting, transfusing or providing an inhibitor as referred to herein to a subject.

- The term “pharmaceutically-acceptable” as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, etc., which are, within the scope of sound medical
- 20 judgment, suitable for use in contact with the tissues of the subject in question (e.g., a human subject) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, adjuvant, filler, buffer, preservative, antioxidant, lubricant, binder, stabiliser, solubiliser, surfactant, masking agent, colouring agent or flavouring agent of a composition
- 25 according to the present disclosure must also be “acceptable” in the sense of being compatible with the other ingredients of the formulation. Suitable carriers, diluents, excipients, adjuvants, fillers, buffers, preservatives, antioxidants, lubricants, binders, stabilisers, solubilisers, surfactants, masking agents, colouring agents and flavouring agents can be found in standard pharmaceutical texts, for example, Remington’s *The Science and*
- 30 *Practice of Pharmacy* (Ed. A. Adejare), 23rd Edition (2020), Academic Press.

The terms “subject”, “patient”, “host” or “individual” used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall

within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates (e.g., humans, monkeys and apes, and includes species of monkeys such as from the genus *Macaca* (e.g., cynomolgus monkeys such as *Macaca fascicularis*, and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), as well as marmosets (species from the genus *Callithrix*), squirrel monkeys (species from the genus *Saimiri*) and tamarins (species from the genus *Saguinus*), as well as species of apes such as chimpanzees (*Pan troglodytes*)), rodents (e.g., mice rats, guinea pigs), lagomorphs (e.g., rabbits, hares), bovinos (e.g., cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., pigs), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), avians (e.g., chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars etc.), marine mammals (e.g., dolphins, whales), reptiles (snakes, frogs, lizards etc.), and fish. In one embodiment, the subject is a human subject.

By “effective amount”, in the context of treating or preventing a condition is meant the administration of an amount of an agent or composition to an individual in need of such treatment or prophylaxis, either in a single dose or as part of a series, that is effective for the prevention of incurring a symptom, holding in check such symptoms, and/or treating existing symptoms, of that condition. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. For example, the term “an agent” includes a plurality of agents, including mixtures thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will

be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase “consisting essentially of”, and variations such as “consists essentially of” will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

Dystrophinopathies

As used herein, a dystrophinopathy refers to a disease caused by or resulting from a mutation in the dystrophin gene. Dystrophin is part of a large glycoprotein complex (the dystrophin-associated protein complex, or DPC) located on the cytoplasmic side of the plasma membrane of muscle cells and cardiomyocytes. In this complex, dystrophin functions in mechanically reinforcing the sarcolemma to withstand contraction-induced injury, while also stabilising the DPC, preventing its degradation. In the absence of dystrophin, DPC degradation occurs, resulting in several downstream detrimental effects that manifest at the cellular and tissue level. These effects include weakening of the sarcolemmal membrane, loss of membrane proteins, disrupted calcium homeostasis, up-regulation of inflammatory factors, and mitochondrial dysfunction. Cells (e.g., cardiac and skeletal muscle cells) and muscle tissue with dystrophinopathy may exhibit reduced contractility, dysfunctional contraction, and increased collagen deposition. In cardiomyocytes and heart tissue, dystrophinopathy can lead to bradycardia and arrhythmias. Untreated dystrophinopathy may ultimately lead to degeneration of skeletal and cardiac muscle fibres, muscle necrosis, and cardiac fibrosis.

Dystrophinopathy encompasses a range of conditions (ranging from mild to severe) including Duchenne muscular dystrophy, Becker muscular dystrophy, intermediate muscular dystrophy, and DMD-associated dilated cardiomyopathy (DCM). In some embodiments, at one end of the range, a dystrophinopathy is phenotypically associated with asymptomatic increases in serum concentrations of creatine phosphokinase (CK) and/or

muscle spasms with myoglobinuria. In some embodiments, at the other end of the range, dystrophinopathy is associated with muscle loss or degeneration (e.g., of skeletal and cardiac muscles), decreased muscle function, pseudohypertrophy of the tongue and calf muscles, a higher risk of neurological abnormalities, and a shortened lifespan. Mutations in the dystrophin gene (*DMD*) that give rise to dystrophinopathies may be accessed in clinical or genomic databases, such as the ClinVar or GenBank databases maintained by the National Center for Biotechnology Information, USA.

A given condition, disorder or pathology herein is “associated with” a dystrophinopathy if that condition, disorder or pathology is caused or exacerbated by the dystrophinopathy. Non-limiting examples of dystrophinopathy-associated myopathy and muscular dystrophy include cardiomyopathy, cardiac muscular dystrophy and skeletal muscle dystrophy.

LINC complex, SUN and KASH domain proteins

As used herein, a “LINC complex” refers to a polypeptide complex comprising one or more SUN domain-containing proteins and one or more KASH domain-containing proteins. LINC complexes connect the inner nuclear membrane (INM) and the outer nuclear membrane (ONM) of the nuclear envelope. SUN domain-containing proteins span the INM, and are associated with nuclear lamins and chromatin-binding proteins on the nucleoplasmic side of the INM, and with KASH domain-containing proteins on the perinuclear side of the INM. KASH domain-containing proteins span the ONM, and are associated with cytoskeletal structural components such as actin filaments, microtubule motors and intermediate filaments on the cytoplasmic side of the ONM, and with SUN domain-containing proteins on the perinuclear side of the ONM. SUN domain proteins function as transmembrane tethers for KASH domain proteins in the ONM. LINC complexes are formed by protein-protein interactions between SUN domain-containing proteins and KASH domain-containing proteins. The LINC complex may comprise non-covalent and/or covalent interactions between SUN domains and KASH domains.

A “SUN domain-containing protein” or “SUN domain protein” herein refers to any polypeptide containing a SUN (i.e., Sad1p, UNC-84) domain. SUN domains comprise ~175 residues and are highly conserved across eukaryotes. The domains are generally found at the C-terminus of SUN domain proteins and extend into the perinuclear space. SUN domain

proteins also contain nucleoplasmic domains which interact with structural components of the nucleoskeleton, such as lamins.

SUN domain proteins include but are not limited to SUN1, SUN2, SUN3 and SUN4. Human
5 SUN1 is the polypeptide identified by UniProtKB Q94901. Human SUN2 is the polypeptide identified by UniProtKB Q9UH99. Human SUN3 is the polypeptide identified by UniProtKB Q8TAQ9. Human SUN4 is the polypeptide identified by UniProtKB A9Z1W8. Reference to SUN1, SUN2, SUN3 and SUN4 in this specification includes reference to isoforms, variants and homologues thereof.

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A “KASH domain-containing protein” or “KASH domain protein” herein refers to any polypeptide containing a KASH (i.e., Klarsicht, ANC-1, Sync Homology) domain. The 50–60 amino acid KASH domain is generally found at the C-terminus of KASH domain proteins, and contains a single helix spanning the ONM and a ~30 amino acid region which
15 extends into the perinuclear space. KASH domain proteins also contain cytoplasmic domains which interact with cytoskeletal components, such as actin.

KASH domain proteins include but are not limited to Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 and KASH5. Human Nesprin-1 is the polypeptide identified by UniProtKB
20 Q8NF91 and encoded by the *SYNE1* gene. Human Nesprin-2 is the polypeptide identified by UniProtKB Q8WXH0 and encoded by the *SYNE2* gene. Human Nesprin-3 is the polypeptide identified by UniProtKB Q6ZMZ3 and encoded by the *SYNE3* gene. Human Nesprin-4 is the polypeptide identified by UniProtKB Q8N205 and encoded by the *SYNE4* gene. Human KASH5 is the polypeptide identified by UniProtKB Q8N6L0 and encoded by
25 the *KASH5* gene.

LINC complex inhibitors

The present disclosure provides inhibitors capable of inhibiting a LINC complex. The term
30 “LINC complex inhibitor” as used herein refers to an agent capable of decreasing or inhibiting at least one function or biological activity of a LINC complex. For example, a LINC complex inhibitor may: inhibit the formation of a LINC complex (i.e., inhibit LINC complex assembly); promote disruption and/or degradation of a LINC complex or a LINC

complex constituent (e.g., a LINC complex protein); or inhibit LINC complex activity and/or function.

5 The LINC complex inhibitor may be a nucleic acid (such as DNA, RNA or a combination of DNA and RNA), polypeptide, site-specific nuclease (SSN) system, small molecule, or a combination thereof. Where the inhibitor is a polypeptide or SSN system, methods herein may comprise administering a vector encoding the polypeptide or one or more components of the SSN system.

10 In some embodiments, the LINC complex inhibitor inhibits formation of a LINC complex. Inhibiting formation of a LINC complex may include one or more of: inhibiting expression of a gene encoding a LINC complex protein; genetically modifying a LINC complex protein to reduce or prevent its interaction with other LINC complex proteins; reducing the level of RNA encoding a LINC complex protein; increasing degradation of RNA encoding a LINC
15 complex protein; disrupting normal post-transcriptional processing (e.g., splicing, translation) of RNA encoding a LINC complex protein; reducing the level of a LINC complex protein; increasing degradation of a LINC complex protein; altering the subcellular localisation of a LINC complex protein; preventing interaction between two or more LINC complex proteins.

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In some embodiments, the LINC complex inhibitor inhibits gene and/or protein expression of a constituent protein of a LINC complex. Constituent proteins of LINC complexes include SUN domain-containing proteins and KASH domain-containing proteins. A constituent protein of a LINC complex may be referred to as “a LINC complex protein”. Non-limiting
25 examples of constituent proteins include SUN1, SUN2, Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 and KASH5.

In one embodiment, the LINC complex inhibitor is an inhibitor of SUN1. In one embodiment, the LINC complex inhibitor is an inhibitor of SUN2. In one embodiment, the LINC complex
30 inhibitor is an inhibitor of both SUN1 and SUN2. In one embodiment, the LINC complex inhibitor is a combination of an inhibitor of SUN1 and an inhibitor of SUN2. In one embodiment, the LINC complex inhibitor is an inhibitor of Nesprin-1. In one embodiment, the LINC complex inhibitor is an inhibitor of Nesprin-2. In one embodiment, the LINC complex inhibitor is an inhibitor of Nesprin-3. In one embodiment, the LINC complex

inhibitor is an inhibitor of Nesprin-4. In one embodiment, the LINC complex inhibitor is an inhibitor of KASH5.

In one embodiment, the LINC complex inhibitor is capable of binding to a LINC complex,
5 a LINC complex protein or an interaction partner for a LINC complex protein.

In one embodiment, the LINC complex inhibitor is capable of inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein. In one embodiment, the LINC complex inhibitor reduces or abolishes interaction between a SUN
10 domain-containing protein and a KASH domain-containing protein. In one embodiment, the LINC complex inhibitor reduces or abolishes interaction between a SUN domain-containing protein and a nucleoskeletal or nucleoplasmic component (such as a nuclear lamin or chromatin). In one embodiment, the LINC complex inhibitor reduces or abolishes interaction
15 between a KASH domain-containing protein and a cytoskeletal or cytoplasmic component (such as an intermediate filament or microtubule).

In some embodiments, the LINC complex inhibitor inhibits protein-protein interaction between: SUN1 and Nesprin-1, SUN1 and Nesprin-2, SUN1 and Nesprin-3, SUN1 and Nesprin-4, SUN1 and KASH5, SUN2 and Nesprin-1, SUN2 and Nesprin-2, SUN2 and
20 Nesprin-3, SUN2 and Nesprin-4, or SUN2 and KASH5.

Suitable inhibiting agents may be identified from a screen of compound, nucleic acid, peptide or polypeptide libraries. A given agent or composition may be evaluated for inhibitory properties using suitable assays. The assays may be, e.g., *in vitro* assays, such as
25 cell-based or cell-free assays, or *in vivo* assays, such as assays performed in non-human animals. Where assays are cell-based assays, they may comprise the use of cells engineered (e.g., via transfection/transduction) to express the constituent proteins of LINC complexes. The cells may be further engineered to express a candidate LINC complex inhibitor.

30 Polypeptide inhibitors

In some embodiments, the LINC complex inhibitor is a polypeptide. The inhibitory polypeptide may be a derivative of a LINC complex protein or an interaction partner of a LINC complex protein, i.e., the inhibitor may comprise or consist of a polypeptide fragment

of a LINC complex protein or an interaction partner of a LINC complex protein. Such a polypeptide inhibitor preferably displays competitive inhibition of interaction between a LINC complex protein and one or more other LINC complex constituents, and may thus be referred to as a “decoy”, “dominant-negative” or “mimetic” of the protein from which it is derived. In this way, such a polypeptide inhibitor can inhibit the formation of LINC complexes and/or disrupt existing LINC complexes via displacement of endogenous LINC complex constituents, forming complexes with reduced functionality or which are non-functional.

Accordingly, in some embodiments of the present methods, the LINC complex inhibitor comprises a polypeptide derived from a LINC complex protein. In one embodiment, the LINC complex inhibitor comprises a polypeptide derived from a SUN domain protein or a KASH domain protein. In one embodiment, the polypeptide is a dominant negative SUN domain-containing protein capable of inhibiting interaction between a SUN domain protein and a KASH domain protein. In one embodiment, the polypeptide is a dominant negative KASH domain-containing protein capable of inhibiting interaction between a KASH domain protein and a SUN domain protein.

In one embodiment, the LINC complex inhibitor comprises a polypeptide derived from SUN1 and/or SUN2. The polypeptide may disrupt SUN-KASH interactions by competing with endogenous SUN1 and/or SUN2 proteins for binding to their cognate KASH domain proteins.

In one embodiment, the polypeptide comprises or consists essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 1 or 3.

In one embodiment, the LINC complex inhibitor comprises a dominant negative SUN1 (DNSUN1) polypeptide. The dominant negative SUN1 polypeptide may comprise a SUN domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%,

or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 1.

In one embodiment, the polypeptide comprises or consists essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 2.

In one embodiment, the LINC complex inhibitor comprises a dominant negative SUN2 (DNSUN2) polypeptide. The dominant negative SUN2 polypeptide may comprise a SUN domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 3.

In one embodiment, the polypeptide comprises or consists essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 4.

In one embodiment, the LINC complex inhibitor comprises a polypeptide derived from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 and/or KASH5. The polypeptide may disrupt SUN-KASH interactions by competing with endogenous Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 and/or KASH5 proteins for binding to their cognate SUN domain proteins.

In one embodiment, the polypeptide comprises or consists essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 5 to 9.

30

In one embodiment, the LINC complex inhibitor comprises a dominant negative Nesprin-1 polypeptide. The dominant negative Nesprin-1 polypeptide may comprise a KASH domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 10 to 14.

92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 5.

5 In one embodiment, the LINC complex inhibitor comprises a dominant negative Nesprin-2 polypeptide. The dominant negative Nesprin-2 polypeptide may comprise a KASH domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 6.

10

In one embodiment, the LINC complex inhibitor comprises a dominant negative Nesprin-3 polypeptide. The dominant negative Nesprin-3 polypeptide may comprise a KASH domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 7.

20 In one embodiment, the LINC complex inhibitor comprises a dominant negative Nesprin-4 polypeptide. The dominant negative Nesprin-4 polypeptide may comprise a KASH domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 8.

25 In one embodiment, the LINC complex inhibitor comprises a dominant negative KASH5 polypeptide. The dominant negative KASH5 polypeptide may comprise a KASH domain comprising or consisting essentially of an amino acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to an amino acid sequence as set forth in SEQ ID NO: 9.

30

Other exemplary polypeptide inhibitors derived from SUN- and KASH-domain proteins are described in WO2021010898 and WO2023101607 (which are hereby incorporated by reference in its entirety).

Methods herein may comprise administering a nucleic acid vector encoding the polypeptide inhibitor. In one embodiment, the nucleic acid vector is a viral vector. The nucleic acid vector may be delivered in any suitable pharmaceutically acceptable delivery vehicle, including but not limited to a viral vector, liposome, micelle, polymeric nanoparticle, lipid nanoparticle and extracellular vesicle.

SUN domain sequence from SUN1

PQDVFKPTTSRLKQPLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLRELT
 10 LLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMSHLEDIL
 GKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTG
 CETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLR
 DLQLQILRNVTHHVSVTQQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQ
 DKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIIYP
 15 GNCWAFKGSQGYLVVRLSMMIHAAFTLEHIPKTLSPGTGNISSAPKDFAVYGLENE
 YQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYR
 FRVHGEPVK (SEQ ID NO: 1)

Exemplary dominant negative SUN1 (DNSUN1)

20 MKWVTFISLLFLFSSAYSSRGVFRRDGPSGGSRLEEELRRLTETGGDDPQDVFKPT
 TSRLKQPLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLRELTLLQKLQAR
 VDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMSHLEDILGKLREKSE
 AIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAV
 QERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILR
 25 NVTHHVSVTQQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVD
 FALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIIYPGNCWAFK
 GSQGYLVVRLSMMIHAAFTLEHIPKTLSPGTGNISSAPKDFAVYGLENEYQEEGQL
 LGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEP
 VKKDEL (SEQ ID NO: 2)

30 [Signal peptide underlined; ALFA-tag double-lined; Golgi retention signal boxed]

SUN2 luminal region including SUN domain

DEGWEARDSSPHFQAEQVRVMSRVHSLERRLEALAAEFSSNWQKEAMRLERLELR
 QGAPGQGGGGGLSHEDTLALLEGLVSRREAALKEDFRRETAARIQEELSALRAEH

QQDSEDLFKKIVRASQSESEARIQQLKSEWQSMTQESFQESSVKELRRLEDQLAGLQ
 QELAALALKQSSVAEEVGLLPQQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQ
 REEMQAQLRELESKILTHVAEMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIV
 KQALQRYSEDRIGLADYALESGGASVISTRSETYETKTALLSLFGIPLWYHSQSPR
 5 VILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKD
 FAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMATYQVVLRILTNWGH
 EYTCIYRFRVHGEPAH (SEQ ID NO: 3)

Exemplary dominant negative SUN2 (DNSUN2)

10 MKWVTFISLLFLFSSAYSSRGVFRRDGPALDEGWEARDSSPHFQAEQVRMSRVHSL
 ERRLEALAAEFSSNWQKEAMRLERLELRQGAPGGGGGLSHEDTLALLEGLVS
 RREAALKEDFRRETAARIQEELSALRAEHQQDSEDLFKKIVRASQSESEARIQQLKS
 EWQSMTQESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGLLPQQIQAV
 VRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTHVAEMQGK
 15 SAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASV
 ISTRSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRL
 LSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIKGFDEDLQQEGTLLGKFTYDQDG
 EPIQTFHFQAPTMATYQVVLRILTNWGHPEYTCIYRFRVHGEPAH KDEL (SEQ ID
 NO: 4)

20 [Signal peptide underlined; Golgi retention signal boxed]

KASH domain sequence from Nesprin-1

RGFLFRVLRAALPLQLLLLLLLIGLACLVPMSEEDYSCALSNNFARSFHPMLRYTNG
 PPPL (SEQ ID NO: 5)

25

KASH domain sequence from Nesprin-2

RSFLSRVVRAALPLQLLLLLLLLLLACLLPSSEEDYSCTQANNFARSFYPMMLRYTN
 GPPPT (SEQ ID NO: 6)

30 KASH domain sequence from Nesprin-3

GSLFRRACCVLPLQLLLLLLFLLLFLLPIREEDRSCITLANNFARSFTLMLRYNGPP
 PT (SEQ ID NO: 7)

KASH domain sequence from Nesprin-4

DPASRQPLTFLLILFLLFLLLVGAMFLLPASGGPCCSHARIPRTPYLVLSYVNGLPVP
(SEQ ID NO: 8)

KASH domain sequence from KASH5

5 LIPAPVLGLLLLLLSVLLLGPSPPPTWPHLQLCYLQPPP (SEQ ID NO: 9)

Nucleic acid inhibitors

10 Rather than expressing components of a luminal domain of a SUN domain-containing protein or a KASH domain-containing protein to disrupt a LINC complex by competing for binding with endogenous Nesprins (which comprise a KASH domain) or SUN1 and SUN2 (which comprise a SUN domain), another approach for disrupting the LINC complex is to modify an endogenous SUN domain or KASH domain so that it fails to bind to, or has reduced binding capacity for, its cognate LINC complex binding partner.

15 Accordingly, in some embodiments, the LINC complex inhibitor may be capable of: modifying a gene encoding a SUN domain- or KASH domain-containing protein to reduce or prevent its expression; inhibiting expression of a SUN domain- or KASH domain-containing protein from an encoding RNA (such as by RNA interference (RNAi) or
20 antisense technology); or inhibiting interaction between a SUN domain-containing protein and a KASH domain-containing protein.

In some embodiments, the LINC complex inhibitor is capable of disrupting a gene, or an RNA product thereof, encoding a LINC complex protein. The LINC complex protein may
25 be a SUN domain protein (such as SUN1 or SUN2) or a KASH domain protein (such as Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 or KASH5). In one embodiment, the LINC complex inhibitor is capable of disrupting a gene, or an RNA product thereof, encoding SUN1, SUN2, Nesprin-1 or Nesprin-2.

30 Such inhibitors can be designed to target any region of a target nucleic acid molecule, such as a genomic or mRNA molecule, encoding the LINC complex protein. The inhibitor may hybridise to a nucleic acid molecule, such as a genomic or mRNA molecule, that encodes the LINC complex protein, leading to decreased expression of the LINC complex protein in a cell.

In one embodiment, the LINC complex inhibitor is a nucleic acid inhibitor of SUN1. In one embodiment, the LINC complex inhibitor is a nucleic acid inhibitor of SUN2. In one embodiment, the LINC complex inhibitor is a nucleic acid inhibitor of both SUN1 and SUN2
5 (for example, the nucleic acid inhibitor may target a conserved SUN sequence in SUN1 and SUN2 DNA or mRNA to prevent protein expression). In one embodiment, the LINC complex inhibitor is a combination of a nucleic acid inhibitor of SUN1 and a nucleic acid inhibitor of SUN2. In one embodiment, the LINC complex inhibitor comprises a nucleic acid inhibitor of Nesprin-1. In one embodiment, the LINC complex inhibitor comprises a
10 nucleic acid inhibitor of Nesprin-2. In one embodiment, the LINC complex inhibitor comprises a nucleic acid inhibitor of Nesprin-3. In one embodiment, the LINC complex inhibitor comprises a nucleic acid inhibitor of Nesprin-4. In one embodiment, the LINC complex inhibitor comprises a nucleic acid inhibitor of KASH5.

15 Exemplary LINC complex nucleic acid inhibitors and encoding vectors are described in WO2019143300 and WO2021010898 (which are hereby incorporated by reference in their entirety).

The disruption of the gene (or RNA product thereof) encoding the LINC complex protein
20 may lead to a decrease in the expression of the gene. The decrease may be, for example, a decrease of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%,
25 at least about 95% or up to and including a 100% decrease or any decrease between 5–100% as compared to a reference level (e.g., gene expression level in an untreated cell).

Gene and protein expression can be determined by means well known to the skilled person, e.g., by techniques such as RT-qPCR, and antibody-based methods including western blot,
30 immunocytochemistry, flow cytometry, ELISA, or by reporter-based methods.

Site-specific nuclease (SSN) systems

In one embodiment, the LINC complex inhibitor is capable of modifying a gene encoding a LINC complex protein to reduce its expression. The LINC complex inhibitor may comprise a site-specific nuclease (SSN) targeting a gene encoding a LINC complex protein.

- 5 In one embodiment, the LINC complex inhibitor is a site-specific nuclease (SSN) system. The SSN system will generally comprise a sequence-specific nuclease that recognises a target nucleic acid sequence. The sequence-specific nuclease may act in conjunction with a guide nucleic acid sequence which targets the nuclease to the target sequence. The sequence-specific nuclease may be, for example, a wild-type, engineered or chimeric nuclease. The
- 10 SSN system may target a gene, or RNA product thereof, encoding a LINC complex protein. The SSN system may further comprise a donor template nucleic acid molecule for introducing specific sequence modifications at or adjacent to the target nucleic acid sequence.

The term “donor template nucleic acid”, as used herein, refers to a nucleic acid molecule

15 that can be used by one or more cellular proteins to modify the sequence of a target nucleic acid after a sequence-specific nuclease described herein has altered the target nucleic acid. In some embodiments, the donor template nucleic acid is a double-stranded nucleic acid molecule. In some embodiments, the donor template nucleic acid is a single-stranded nucleic acid molecule. In some embodiments, the donor template nucleic acid is linear molecule. In

20 some embodiments, the donor template nucleic acid is circular (e.g., a plasmid). In some embodiments, the donor template nucleic acid is an exogenous nucleic acid molecule. In some embodiments, the donor template nucleic acid is an endogenous nucleic acid molecule (e.g., a chromosome). In some embodiments the donor template is a DNA molecule. In some embodiments, the donor template is an RNA molecule.

25 SSNs capable of being engineered to generate target nucleic acid sequence-specific double- or single-strand breaks include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced palindromic repeats/CRISPR-associated nuclease (CRISPR/Cas) systems.

30 In some embodiments the SSN system is a ZFN system, a TALEN system, a CRISPR/Cas9 system, a CRISPR/Cas12 system or a CRISPR/Cas13 system.

In some embodiments the SSN system is capable of disrupting a gene encoding a LINC complex protein by modifying the nucleic acid sequence of the gene. Such modifications may include inserting, deleting or substituting one or more nucleotides or bases in the gene. The SSN system may comprise a donor template nucleic acid for introducing the nucleotide
5 modification. In some embodiments, the SSN system is capable of disrupting a gene encoding a LINC complex protein by nucleotide insertion or deletion.

In some embodiments, the SSN system targets a region of the nucleic acid encoding a LINC complex protein involved in LINC complex formation (e.g., a region required for LINC
10 complex formation). For example, the SSN system may disrupt expression of an exon of a gene encoding a SUN domain- or KASH domain-containing protein.

As both the SUN domain and the KASH domain are located at the C-termini of their respective proteins, one way of producing a modified SUN or KASH domain is to use a SSN
15 system to modify the genes encoding SUN or KASH domain proteins to generate a premature stop codon at the 3' end of the respective protein sequences following CRISPR-induced non-homologous end joining. This would result in a truncated protein with its C-terminal SUN or KASH domain deleted. The truncated protein would be expressed and membrane-localised, but unable to interact with its cognate LINC complex partners.

20 Accordingly, in some embodiments, the SSN system modifies the endogenous SUN domain or KASH domain of SUN1 or Nesprin-1 protein, respectively, to disrupt a LINC complex. The respective genes are *SUN1* and *SYNE1*.

25 In some embodiments the SSN system modifies the endogenous SUN domain or KASH domain of SUN2 or Nesprin-2 protein, respectively, to disrupt a LINC complex. The respective nucleic acids are *SUN2* and *SYNE2*.

The targeted sequences may be upstream of C-terminal SUN or KASH domains that are
30 involved in forming LINC complexes. Thus CRISPR-induced deletion upstream of these regions may either result in nonsense-mediated decay of the mRNA or disrupt LINC complex interactions.

- In one embodiment the SSN system is a CRISPR/Cas9 system. The CRISPR/Cas9 system may comprise a gRNA, and the gRNA may comprise a CRISPR RNA (crRNA) containing the guide sequence. The gRNA may also comprise a trans-activating crRNA (tracrRNA) for processing the crRNA to its mature form. Alternatively, the gRNA may be an engineered
- 5 construct comprising both the crRNA and tracrRNA in a single nucleic acid molecule (i.e., a single guide RNA or sgRNA). CRISPR/Cas9 systems for targeted disruption of LINC complex proteins SUN1 and SUN2 are described e.g., in Schaller et al., *J Virol.* (2017) 91(19): pii: e00463-17, which is hereby incorporated by reference in its entirety.
- 10 In one embodiment the SSN system is a CRISPR/Cas12 system, such as a CRISPR/Cas12a (also known as CRISPR/Cpf1) or CRISPR/Cas12b (also known as CRISPR/C2c1) system. In such an embodiment, the CRISPR/Cas system may comprise a single gRNA molecule containing the guide sequence.
- 15 In one embodiment, the CRISPR/Cas system comprises a gRNA that targets a SUN domain-containing protein in the LINC complex. The SUN domain-containing protein may be SUN1 or SUN2. The CRISPR/Cas system may disrupt the SUN domain of the protein, for example, by inserting, deleting and/or substituting nucleotides within the SUN domain, such that expression and/or activity of the SUN domain or SUN domain-
- 20 containing protein is inhibited. For example, the CRISPR/Cas system may introduce a deletion upstream of or within the SUN domain so as to cause an mRNA product of the gene to undergo nonsense-mediated decay, or a protein encoded by the gene to have a deleted or non-functional SUN domain.
- 25 In one embodiment, the CRISPR/Cas system comprises a gRNA that targets a KASH domain-containing protein in the LINC complex. The KASH domain-containing protein may be Nesprin-1 or Nesprin-2. The CRISPR/Cas system may disrupt the KASH domain of the protein, for example, by inserting, deleting and/or substituting nucleotides within the KASH domain, such that expression and/or activity of the KASH domain or
- 30 KASH domain-containing protein is inhibited. For example, the CRISPR/Cas system may introduce a deletion upstream of or within the KASH domain so as to cause an mRNA product of the gene to undergo nonsense-mediated decay, or a protein encoded by the gene to have a deleted or non-functional KASH domain.

In some embodiments, the CRISPR/Cas system comprises a guide RNA (gRNA) targeting SUN1, SUN2, Nesprin-1 and/or Nesprin-2. In some embodiments, the CRISPR/Cas system comprises a guide RNA (gRNA) comprising a nucleic acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%,
5 at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence as set forth in SEQ ID NO: 10–49, or a complement thereof.

In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) targeting
10 SUN1. In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) comprising a nucleic acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence as set forth in SEQ ID NO: 21–31, or a complement
15 thereof.

In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) targeting SUN2. In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) comprising a nucleic acid sequence having at least 70% sequence identity (such as at least
20 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence as set forth in SEQ ID NO: 32–40, or a complement thereof.

25 In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) targeting Nesprin-1. In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) comprising a nucleic acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence
30 identity) to a nucleic acid sequence as set forth in SEQ ID NO: 10–20, or a complement thereof.

In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA) targeting Nesprin-2. In one embodiment, the CRISPR/Cas system comprises a guide RNA (gRNA)

comprising a nucleic acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence as set forth in SEQ ID NO: 41–49, or a complement thereof.

Methods herein may comprise administering one or more nucleic acid vectors encoding components of the SSN system, such as the nuclease or guide RNA of the SSN system. Methods herein may also comprise administering a donor template nucleic acid for modifying a gene encoding a LINC complex protein. Nucleic acids may be delivered in any suitable pharmaceutically acceptable delivery vehicle, including but not limited to a viral vector, liposome, micelle, polymeric nanoparticle, lipid nanoparticle and extracellular vesicle.

Table 1. Exemplary guide RNA (gRNA) sequences for use with CRISPR/Cas systems

Gene	ENSEMBL gene ID	Sequence	PAM	Cas nuclease
SYNE1	ENSG00000131018	UCGUGUAUCUGAGCAUGGGG (SEQ ID NO: 10)	UGGAAU	saCas9
SYNE1	ENSG00000131018	GCCAUUCGUGUAUCUGAGCA (SEQ ID NO: 11)	UGGGGU	saCas9
SYNE1	ENSG00000131018	UCCACCCCAUGCUCAGAUAC (SEQ ID NO: 12)	ACGAAU	saCas9
SYNE1	ENSG00000131018	GAGCAUGGGGUGGAAUGACC (SEQ ID NO: 13)	GGG	spCas9
SYNE1	ENSG00000131018	UGAGCAUGGGGUGGAAUGAC (SEQ ID NO: 14)	CGG	spCas9
SYNE1	ENSG00000131018	UCGUGUAUCUGAGCAUGGGG (SEQ ID NO: 15)	UGG	spCas9
SYNE1	ENSG00000131018	CAUUCGUGUAUCUGAGCAUG (SEQ ID NO: 16)	GGG	spCas9
SYNE1	ENSG00000131018	CCAUUCGUGUAUCUGAGCAU (SEQ ID NO: 17)	GGG	spCas9
SYNE1	ENSG00000131018	GCCAUUCGUGUAUCUGAGCA (SEQ ID NO: 18)	UGG	spCas9

SYNE1	ENSG00000131018	CCCAUGCUCAGAUACACGAA (SEQ ID NO: 19)	UGG	spCas9
SYNE1	ENSG00000131018	CCCGGUCAUUCACCCCAUG (SEQ ID NO: 20)	UUUG	Cpf1
SUN1	ENSG00000164828	UUUUUCUAAACUGGGGCCAUC (SEQ ID NO: 21)	CUGAGU	saCas9
SUN1	ENSG00000164828	CCGAUACAGACAGGUAUACU (SEQ ID NO: 22)	CAGGAU	saCas9
SUN1	ENSG00000164828	AACUUCGGAUUUUUUCUAAAC (SEQ ID NO: 23)	UGG	spCas9
SUN1	ENSG00000164828	ACUUCGGAUUUUUUCUAAACU (SEQ ID NO: 24)	GGG	spCas9
SUN1	ENSG00000164828	CUUCGGAUUUUUUCUAAACUG (SEQ ID NO: 25)	GGG	spCas9
SUN1	ENSG00000164828	ACAGACAGGUAUACUCAGGA (SEQ ID NO: 26)	UGG	spCas0
SUN1	ENSG00000164828	CUGAGUAUACCUGUCUGUAU (SEQ ID NO: 27)	CGG	spCas9
SUN1	ENSG00000164828	UUCUAAACUGGGGCCAUCCUG (SEQ ID NO: 28)	UUUU	Cpf1
SUN1	ENSG00000164828	UCUAAACUGGGGCCAUCCUGA (SEQ ID NO: 29)	UUUU	Cpf1
SUN1	ENSG00000164828	CUAACUGGGGCCAUCCUGAG (SEQ ID NO: 30)	UUUU	Cpf1
SUN1	ENSG00000164828	UAAACUGGGGCCAUCCUGAGU (SEQ ID NO: 31)	UUUC	Cpf1

Table 2. Exemplary guide RNA (gRNA) sequences for use with CRISPR/Cas systems

	Gene	ENSEMBL gene ID	Sequence	PAM
5' of SUN domain encoding region	SUN2	ENSG00000100242	GCCCUGCAUCUCUGCCACAU (SEQ ID NO: 32)	GGG
	SUN2	ENSG00000100242	GAGCAAGAUCUCACCCCAUG (SEQ ID NO: 33)	UGG
	SUN2	ENSG00000100242	AGGUGUGAUUGGAGUGACAG (SEQ ID NO: 34)	AGG

	SUN2	ENSG00000100242	GUGGAAUCUCAGUCCCCGGC (SEQ ID NO: 35)	CUGGA U
	SUN2	ENSG00000100242	CUGCAGAAAGAAGGUGUGAU (SEQ ID NO: 36)	UGGAG U
	SUN2	ENSG00000100242	UGCAUCUCUGCCACAUGGGU (SEQ ID NO: 37)	GAGGA U
	SUN2	ENSG00000100242	CGCAGGAAACUGCUGCUCG (SEQ ID NO: 38)	UUUC
	SUN2	ENSG00000100242	CCACUCUGACUUCAGCUGCU (SEQ ID NO: 39)	UUUG
	SUN2	ENSG00000100242	UGCAGCGUCAGGCUCAGGGA (SEQ ID NO: 40)	UUUC
5' of KASH C- terminal tetra- peptide motif	SYNE2	ENSG00000054654	CCCAUGCUGAGGUACACCAA (SEQ ID NO: 41)	UGG
	SYNE2	ENSG00000054654	GCAGCUGUAGUCUUCUUCGG (SEQ ID NO: 42)	AGG
	SYNE2	ENSG00000054654	CAUUGGUGUACCUCAGCAUG (SEQ ID NO: 43)	GGG
	SYNE2	ENSG00000054654	GACCGGGCAAAGUUGUUGGC (SEQ ID NO: 44)	CUGAG U
	SYNE2	ENSG00000054654	CCCAUUGGUGUACCUCAGCA (SEQ ID NO: 45)	UGGGG U
	SYNE2	ENSG00000054654	GCAGCAGGAGGAGCAGCUGC (SEQ ID NO: 46)	AGGGG U
	SYNE2	ENSG00000054654	CCCGGUCCUUUACCCAUG (SEQ ID NO: 47)	UUUG
	SYNE2	ENSG00000054654	ACCCAUGCUGAGGUACACC (SEQ ID NO: 48)	UUUU
	SYNE2	ENSG00000054654	CCCAUGCUGAGGUACACCA (SEQ ID NO: 49)	UUUA

Inhibitory nucleic acids

In some embodiments, the LINC complex inhibitor is an inhibitory nucleic acid molecule. The inhibitory nucleic acid molecule may be selected from an miRNA, siRNA, shRNA and antisense oligonucleotide (ASO).

- 5 In some embodiments, a nucleic acid inhibitor according to the present disclosure is an antisense nucleic acid. The nucleic acid inhibitor may comprise an antisense nucleic acid as described herein. In some embodiments, a nucleic acid inhibitor may encode an antisense nucleic acid as described herein.
- 10 As used herein, an “antisense nucleic acid” refers to a nucleic acid (e.g. DNA or RNA) that is complementary to at least a portion of a target nucleotide sequence (e.g., of RNA encoding a target gene described herein). Antisense nucleic acids according to the present disclosure are preferably single-stranded nucleic acids, and bind via complementary Watson-Crick base-pairing to a target nucleotide sequence. Complementary base-pairing may involve
- 15 hydrogen bonding between complementary base pairs.

- Complementary base-pairing between the antisense nucleic acid and its target nucleotide sequence may be complete. In such embodiments the antisense nucleic acid comprises, or consists of, the reverse complement of its target nucleotide sequence, and complementary
- 20 base-pairing occurs between each nucleotide of the target nucleotide sequence and complementary nucleotides in the antisense nucleic acid. Alternatively, complementary base-pairing between the antisense nucleic acid and its target nucleotide sequence may be incomplete/partial. In such embodiments complementary base-pairing occurs between some, but not all, nucleotides of the target nucleotide sequence and complementary nucleotides in
- 25 the antisense nucleic acid.

- Such binding between nucleic acids through complementary base pairing may be referred to as “hybridisation”. Through binding to its target nucleotide sequence, an antisense nucleic acid may form a nucleic acid complex comprising (i) the antisense nucleic acid and (ii) a
- 30 target nucleic acid comprising the target nucleotide sequence.

The nucleotide sequence of an antisense nucleic acid is sufficiently complementary to its target nucleotide sequence such that it binds or hybridises to the target nucleotide sequence. It will be appreciated that an antisense nucleic acid preferably has a high degree of sequence

identity to the reverse complement of its target nucleotide sequence. In some embodiments, the antisense nucleic acid comprises or consists of a nucleotide sequence having at least 75% sequence identity (e.g., one of at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%
5 or greater sequence identity) to the reverse complement of its target nucleotide sequence.

In one embodiment, the LINC complex inhibitor is an antisense oligonucleotide (ASO). ASOs are single-stranded nucleic acid molecules comprising or consisting of an antisense nucleic acid capable of hybridising to target nucleotide sequence. An antisense
10 oligonucleotide according to the present disclosure may comprise or consist of an antisense nucleic acid as described herein.

ASOs can modify expression of RNA molecules comprising their target nucleotide sequence by altering splicing, or by recruiting RNase H to degrade RNA comprising the target
15 nucleotide sequence. RNase H recognises nucleic acid complex molecules formed when the ASO binds to RNA comprising its target nucleotide sequence. ASOs according to the present disclosure may comprise or consist of an antisense nucleic acid according to the present disclosure. ASOs may comprise 10 to 40 (e.g., 17 to 30, 20 to 27, 21 to 23) nucleotides in length. Many ASOs are designed as chimeras, comprising a mix of bases with different
20 chemistries, or as gapmers, comprising a central DNA portion surrounded by “wings” of modified nucleotides. ASOs sometimes comprise alterations to the sugar-phosphate backbone in order to increase their stability and/or reduce/prevent RNase H degradation, such as e.g. phosphorothioate linkages, phosphorodiamidate linkages such as phosphorodiamidate morpholino (PMOs), and may comprise e.g., peptide nucleic acids
25 (PNAs), locked nucleic acids (LNAs), methoxyethyl nucleotide modifications, e.g. 2'-O-methyl (2'OMe) and 2'-O-methoxyethyl (MOE) ribose modifications and/or 5'-methylcytosine modifications.

In some embodiments, the nucleic acid inhibitor is an RNA interference (RNAi) agent (e.g.,
30 siRNA, shRNA or miRNA-based shRNA) or a nucleic acid encoding an RNAi agent that reduces expression of an mRNA.

The term “RNAi agent” or “RNAi” is used interchangeably herein to refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an

RNA transcript via an RNA-induced silencing complex (RISC) pathway. RNAi agent directs the site-specific degradation of mRNA through a process known as RNA interference (RNAi). The RNAi agent modulates, e.g., inhibits, the expression of a gene in a cell, e.g., a cell within a subject, such as a mammalian subject. The term “RNAi agent” includes precursor RNAs that are processed by RISC into siRNAs, as well as the siRNAs themselves that inhibit the expression of an endogenous gene.

This disclosure provides for double-stranded RNAi agents capable of inhibiting the expression of a target gene encoding a LINC complex protein. The RNAi agent may comprise a sense strand and an antisense strand. Each strand of the RNAi agent may range from 12–30 nucleotides in length. For example, each strand may be between 14–30 nucleotides in length, 17–30 nucleotides in length, 25–30 nucleotides in length, 27–30 nucleotides in length, 17–23 nucleotides in length, 17–21 nucleotides in length, 17–19 nucleotides in length, 19–25 nucleotides in length, 19–23 nucleotides in length, 19–21 nucleotides in length, 21–25 nucleotides in length, or 21–23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA (“dsRNA”). The duplex region of an RNAi agent may be 12–30 nucleotide pairs in length. For example, the duplex region can be between 14–30 nucleotide pairs in length, 17–30 nucleotide pairs in length, 27–30 nucleotide pairs in length, 17–23 nucleotide pairs in length, 17–21 nucleotide pairs in length, 17–19 nucleotide pairs in length, 19–25 nucleotide pairs in length, 19–23 nucleotide pairs in length, 19–21 nucleotide pairs in length, 21–25 nucleotide pairs in length, or 21–23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In some embodiments, an inhibitory nucleic acid is a micro RNA (miRNA), or a precursor thereof (e.g. a pri-miRNA or a pre-miRNA). miRNA molecules have a similar structure to siRNA molecules, but are encoded endogenously, and derived from processing of short hairpin RNA (shRNA) molecules. They are initially expressed as long primary transcripts (pri-miRNAs), which are processed within the nucleus into 60 to 70 nucleotide hairpins (pre-miRNAs), which are further processed in the cytoplasm into smaller species that interact with RISC and target mRNA. miRNAs comprise “seed sequences” that are essential for

binding to target mRNA. Seed sequences usually comprise six nucleotides and are situated at positions 2 to 7 at the miRNA 5' end.

In some embodiments, an inhibitory nucleic acid is a small interfering RNA (siRNA). As
5 used herein, "siRNA" refers to a double-stranded RNA molecule having a length between 17–30 base pairs (e.g. 20–27 base pairs, e.g. 21–23 base pairs), which is capable of engaging the RNA interference (RNAi) pathway for the targeted degradation of target RNA. Double-stranded siRNA molecules may be formed as a nucleic acid complex of RNA strands having a high degree of complementarity. The strand of the double-stranded siRNA molecule
10 having complementarity to a target nucleotide sequence (i.e., the antisense nucleic acid) may be referred to as the "guide" strand, and the other strand may be referred to as the "passenger" strand.

The siRNA may contain one or more overhang regions and/or capping groups at the 3'-end,
15 5'-end, or both ends of one or both strands e.g. comprising one or two or three nucleotides (e.g. a UU 3' overhang, a TT 3' overhang, or a CCA 5' overhang). The overhang can be 1–6 nucleotides in length, for instance 2–6 nucleotides in length, 1–5 nucleotides in length, 2–5 nucleotides in length, 1–4 nucleotides in length, 2–4 nucleotides in length, 1–3 nucleotides in length, 2–3 nucleotides in length, or 1–2 nucleotides in length. The overhangs can be the
20 result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, e.g., by additional bases to form a hairpin, or by other non-base linkers.

25 In some embodiments, a passenger strand of an siRNA may comprise a CCA modification at the 5' end, i.e., the addition of nucleotides CCA. In some embodiments, a passenger strand of an siRNA according to the present disclosure may comprise a TT modification at the 3' end, e.g., replacing the two 3' nucleotides with a TT sequence.

30 In some embodiments, the guide strand of an siRNA according to the present disclosure may comprise or consist of an antisense nucleic acid according to an embodiment of an antisense nucleic acid described herein.

In some embodiments an siRNA according to the present disclosure may be contained within a longer shRNA sequence that undergoes processing to form the siRNA.

In some embodiments, an inhibitory nucleic acid is a short hairpin RNA (shRNA). shRNA molecules comprise sequences of nucleotides having a high degree of complementarity that associate with one another through complementary base pairing to form the stem region of the hairpin. The sequences of nucleotides having a high degree of complementarity may be linked by one or more nucleotides that form the loop region of the hairpin. shRNA molecules may be processed (e.g., via catalytic cleavage by DICER) to form siRNA or miRNA molecules. shRNA molecules may have a length of between 35–100 (e.g., 40–70) nucleotides. The stem region of the hairpin may have a length between 17 to 30 (e.g., 20–27, e.g., 21–23) base pairs. The stem region may comprise G-U pairings to stabilise the hairpin structure. An shRNA sequence described herein may comprise sequences that will be subsequently processed into shorter siRNA strand(s).

The inhibitory nucleic acid molecule can comprise, for example, nucleotides or non-natural or modified nucleotides, such as nucleotide analogues or nucleotide substitutes. Such nucleotides include a nucleotide that contains a modified base, sugar, or phosphate group, or that incorporates a non-natural moiety in its structure. Examples of non-natural nucleotides include, but are not limited to, dideoxynucleotides, biotinylated, aminated, deaminated, alkylated, benzylated, and fluorophore-labeled nucleotides.

siRNA, miRNAs and shRNAs for the targeted inhibition of gene and/or protein expression may be identified and designed in accordance with principles and/or using tools well known to the skilled person. Parameters and tools for designing siRNA and shRNA molecules are described e.g. in Fakhr et al., *Cancer Gene Therapy* (2016) 23:73-82 (hereby incorporated by reference in its entirety). Software that may be used by the skilled person for the design of such molecules is summarised in Table 1 of Fakhr et al., *Cancer Gene Therapy* (2016) 23:73-82, and includes e.g. siRNA Wizard (InvivoGen). Details for making such molecules can be found in the websites of commercial vendors such as Ambion, Dharmacon, GenScript, Invitrogen and OligoEngine.

In some embodiments, the inhibitory nucleic acid molecule comprises, encodes or hybridises to a nucleic acid sequence having at least 70% sequence identity (such as at least

75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence set forth in SEQ ID NO: 50–141, or a complementary sequence thereof.

5

In some embodiments, the inhibitory nucleic acid molecule is an siRNA or shRNA molecule comprising a nucleic acid sequence which hybridises to a nucleic acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%,
10 at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence set forth in SEQ ID NO: 50–141.

In one embodiment, the inhibitory nucleic acid molecule comprises or encodes a nucleic acid sequence having at least 70% sequence identity (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,
15 at least 96%, at least 97%, at least 98%, or at least 99% sequence identity) to a nucleic acid sequence set forth in SEQ ID NO: 142.

Table 3. Exemplary target human mRNA sequences for antisense inhibition or RNAi

SEQ ID NO.	Gene ID	Gene	Target Seq
50	23353	SUN1	UUCAUGGACGAGGGCAUAUAC
51	23353	SUN1	GCUGUUCUGAAACUUACGAAA
52	23353	SUN1	CAGAUACACUGCAUCAUCUUU
53	23353	SUN1	CCAGAUACACUGCAUCAUCUU
54	23353	SUN1	GCAGAGGUUCUCAUCACAGUU
55	23353	SUN1	GAGCACAAAUUGGACCCUGUA
56	23353	SUN1	GAACUAGAACAGACCAAGCAA
57	23353	SUN1	GCUUUCCAAAUAGUGGAACUU
58	23353	SUN1	CCUCCCAAAGUACUGGGAUUA
59	25777	SUN2	CGAGCCUAUUCAGACGUUUCA
60	25777	SUN2	GUGUAUAUAUGUAGCAUAUCA
61	25777	SUN2	GAGGAAAUCCAGCAACAUGAA
62	25777	SUN2	GCCUAUUCAGACGUUUCACUU
63	25777	SUN2	CUGCAGAAAGAAGGUGUGAUU

64	25777	SUN2	GCAAGACUCAGAAGACCUCUU
65	25777	SUN2	GAAGUCAGAGUGGCAAAGCAU
66	25777	SUN2	GCUCUGACUUCUAGGAGCACA
67	25777	SUN2	UGCUUAGCUCCAGAGCAGGAU
68	23345	SYNE1	GCGUAGUGAUAAAGACUGAUUU
69	23345	SYNE1	CAGAAGUGCUGGUAGCAUAAG
70	23345	SYNE1	GCGUGUGGAAAGAUACCAUAA
71	23345	SYNE1	GGCCAAACCUGGGCAAAUUUA
72	23345	SYNE1	GCGUUCUUGGGAAACCUUAAA
73	23345	SYNE1	AGCCGUGCAGCAGCUUGAUAA
74	23345	SYNE1	CAAUCAGGCAGCAUCACAUUU
75	23345	SYNE1	GAACCAGUCCGUGCUACAAAU
76	23345	SYNE1	GGCGAAUGCAGUAGCAGUAUA
77	23345	SYNE1	GCAAUCCCAAACUACCAACAA
78	23345	SYNE1	GCAGUUUAACUCAGACUUGAA
79	23345	SYNE1	GCUGUGGUACAAUAUGAACAA
80	23345	SYNE1	GAUUCCUGGAGAAACUACAU
81	23224	SYNE2	AUUGAACGAGAGGCAAUUUUU
82	23224	SYNE2	CAUCGACGAUCUCCAUAUUUC
83	23224	SYNE2	GAUGGAAACAAUCAUAUUAU
84	23224	SYNE2	CGGACAUUAGUAUUGAAGAAA
85	23224	SYNE2	GCAGUGAAAUACCUCUUGAAU
86	23224	SYNE2	CCUCAGUUUAUAUCCGACCUAU
87	23224	SYNE2	GCCACCUAUGAGUCUGUCAAU
88	23224	SYNE2	GCGCUAUGAAAGAACGGAGUU
89	23224	SYNE2	GGCAGGAGAAUUGCUUGAAUC
90	23353 & 25777	SUN1 & SUN2	GCAACAUCAGCAGCGCCCCCA
91	23353 & 25777	SUN1 & SUN2	CAACAUCAGCAGCGCCCCCAA
92	23353 & 25777	SUN1 & SUN2	AACAUCAGCAGCGCCCCCAAG
93	23353 & 25777	SUN1 & SUN2	ACAUCAGCAGCGCCCCCAAGG
94	23353 & 25777	SUN1 & SUN2	CAUCAGCAGCGCCCCCAAGGA
95	23353 & 25777	SUN1 & SUN2	AUCAGCAGCGCCCCCAAGGAC
96	23353 & 25777	SUN1 & SUN2	UCAGCAGCGCCCCCAAGGACU
97	23353 & 25777	SUN1 & SUN2	CAGCAGCGCCCCCAAGGACUU

98	23353 & 25777	SUN1 & SUN2	AGCAGCGCCCCCAAGGACUUC
99	23353 & 25777	SUN1 & SUN2	GCAGCGCCCCCAAGGACUUCG
100	23353 & 25777	SUN1 & SUN2	CAGCGCCCCCAAGGACUUCGC
101	23353 & 25777	SUN1 & SUN2	AGCGCCCCCAAGGACUUCGCC
102	23353 & 25777	SUN1 & SUN2	GCGCCCCCAAGGACUUCGCCG
103	23353 & 25777	SUN1 & SUN2	CGCCCCCAAGGACUUCGCCGU
104	23353 & 25777	SUN1 & SUN2	GCCCCCAAGGACUUCGCCGUC
105	23353 & 25777	SUN1 & SUN2	CCCCCAAGGACUUCGCCGUCU
106	23353 & 25777	SUN1 & SUN2	CCCCAAGGACUUCGCCGUCUA
107	23353 & 25777	SUN1 & SUN2	CCCAAGGACUUCGCCGUCUAU
108	23353 & 25777	SUN1 & SUN2	CCAAGGACUUCGCCGUCUAUG
109	23353 & 25777	SUN1 & SUN2	CAAGGACUUCGCCGUCUAUGG
110	23353 & 25777	SUN1 & SUN2	AAGGACUUCGCCGUCUAUGGA
111	23353 & 25777	SUN1 & SUN2	AGGACUUCGCCGUCUAUGGAU
112	23353 & 25777	SUN1 & SUN2	GGACUUCGCCGUCUAUGGAUU
113	23353 & 25777	SUN1 & SUN2	GACUUCGCCGUCUAUGGAUUA
114	23353 & 25777	SUN1 & SUN2	ACUUCGCCGUCUAUGGAUUAG
115	23353 & 25777	SUN1 & SUN2	CUUCGCCGUCUAUGGAUUAGA
116	23353 & 25777	SUN1 & SUN2	GCACUAUCUCCAGUGCCCCCA
117	23353 & 25777	SUN1 & SUN2	CACUAUCUCCAGUGCCCCCAA
118	23353 & 25777	SUN1 & SUN2	ACUAUCUCCAGUGCCCCCAAG
119	23353 & 25777	SUN1 & SUN2	CUAUCUCCAGUGCCCCCAAGG
120	23353 & 25777	SUN1 & SUN2	UAUCUCCAGUGCCCCCAAGGA
121	23353 & 25777	SUN1 & SUN2	AUCUCCAGUGCCCCCAAGGAC
122	23353 & 25777	SUN1 & SUN2	UCUCCAGUGCCCCCAAGGACU
123	23353 & 25777	SUN1 & SUN2	CUCCAGUGCCCCCAAGGACUU
124	23353 & 25777	SUN1 & SUN2	UCCAGUGCCCCCAAGGACUUC
125	23353 & 25777	SUN1 & SUN2	CCAGUGCCCCCAAGGACUUCG
126	23353 & 25777	SUN1 & SUN2	CAGUGCCCCCAAGGACUUCGC
127	23353 & 25777	SUN1 & SUN2	AGUGCCCCCAAGGACUUCGCC
128	23353 & 25777	SUN1 & SUN2	GUGCCCCCAAGGACUUCGCCA
129	23353 & 25777	SUN1 & SUN2	UGCCCCCAAGGACUUCGCCAU
130	23353 & 25777	SUN1 & SUN2	GCCCCCAAGGACUUCGCCAUC
131	23353 & 25777	SUN1 & SUN2	CCCCCAAGGACUUCGCCAUCU
132	23353 & 25777	SUN1 & SUN2	CCCCAAGGACUUCGCCAUCUU
133	23353 & 25777	SUN1 & SUN2	CCCAAGGACUUCGCCAUCUUU

134	23353 & 25777	SUN1 & SUN2	CCAAGGACUUCGCCAUCUUUG
135	23353 & 25777	SUN1 & SUN2	CAAGGACUUCGCCAUCUUUGG
136	23353 & 25777	SUN1 & SUN2	AAGGACUUCGCCAUCUUUGGG
137	23353 & 25777	SUN1 & SUN2	AGGACUUCGCCAUCUUUGGGU
138	23353 & 25777	SUN1 & SUN2	GGACUUCGCCAUCUUUGGGUU
139	23353 & 25777	SUN1 & SUN2	GACUUCGCCAUCUUUGGGUUU
140	23353 & 25777	SUN1 & SUN2	ACUUCGCCAUCUUUGGGUUUG
141	23353 & 25777	SUN1 & SUN2	CUUCGCCAUCUUUGGGUUUGA

Exemplary shRNA targeting human SUN1

GCTGTTCTGAACTTACGAACTCGAGTTTCGTAAGTTTCAGAACAGC (SEQ ID NO: 142)

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Constructs and vectors

Provided herein are nucleic acid constructs comprising or encoding a LINC complex inhibitor as defined herein. For example, the nucleic acid construct may comprise or encode a nucleic acid inhibitor or polypeptide inhibitor, or one or more components of a site-specific nuclease system (e.g., a nuclease and/or a guide RNA).

10

The term “construct” refers to a recombinant genetic molecule including one or more isolated nucleic acid sequences from different sources. Thus, constructs are chimeric molecules in which two or more nucleic acid sequences of different origin are assembled into a single nucleic acid molecule and include any construct that contains (1) nucleic acid sequences, including regulatory and coding sequences that are not found together in nature (i.e., at least one of the nucleotide sequences is heterologous with respect to at least one of its other nucleotide sequences), or (2) sequences encoding parts of functional RNA molecules or proteins not naturally adjoined, or (3) parts of promoters that are not naturally adjoined. Representative constructs include any recombinant nucleic acid molecule such as a plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, or linear or circular single stranded or double stranded DNA or RNA nucleic acid molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a nucleic acid molecule where one or more nucleic acid molecules have been operably linked. Constructs of the present invention will generally include the necessary

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elements to direct expression of a nucleic acid sequence of interest that is also contained in the construct, such as, for example, a target nucleic acid sequence or a modulator nucleic acid sequence. Such elements may include control elements such as a promoter that is operably linked to (so as to direct transcription of) the nucleic acid sequence of interest, and
5 often includes a polyadenylation sequence as well. Within certain embodiments of the invention, the construct may be contained within a vector. In addition to the components of the construct, the vector may include, for example, one or more selectable markers, one or more origins of replication, such as prokaryotic and eukaryotic origins, at least one multiple cloning site, and/or elements to facilitate stable integration of the construct into the genome
10 of a host cell. Two or more constructs can be contained within a single nucleic acid molecule, such as a single vector, or can be containing within two or more separate nucleic acid molecules, such as two or more separate vectors.

The nucleic acid construct may be, or may be comprised in, a vector. A “vector” as used
15 herein is a nucleic acid used as a vehicle to transfer exogenous nucleic acid into a cell. The vector may be a vector for expression of the nucleic acid in the target cell. Such vectors may include a promoter sequence operably linked to the nucleic acid sequence to be expressed. A vector may also include a termination codon and expression enhancers. As used herein, the term “operably linked” may include the situation where a selected nucleic acid sequence
20 and regulatory nucleic acid sequence (e.g., promoter and/or enhancer) are covalently linked in such a way as to place the expression of the nucleotide sequence under the influence or control of the regulatory sequence (thereby forming an expression cassette). Thus, a regulatory sequence is operably linked to the selected nucleic acid sequence if the regulatory sequence is capable of effecting transcription of the nucleic acid sequence. Where
25 appropriate, the resulting transcript may then be translated into a desired polypeptide.

Any suitable vectors, promoters, enhancers and termination codons known in the art may be used. Suitable vectors include, for example, viral vectors and recombinant plasmids. Vector selection and engineering are within those of skill in the art.

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In some embodiments, the vector is a viral vector. In one embodiment, the viral vector is an adeno-associated virus (AAV) vector, including but not limited to AAV vectors derived from one of the following serotypes: AAV-DJ, AAV1, AAV2i8, AAV6, AAV6.2FF (i.e., lung-tropic AAVs), AAV8, AAV9, AAV 9.45, or AAV-LK03 (i.e., liver-tropic AAVs).

Nucleic acid constructs and vectors may be delivered in any suitable pharmaceutically acceptable delivery vehicle, including but not limited to a viral vector, liposome, micelle, polymeric nanoparticle, lipid nanoparticle and extracellular vesicle.

5

Pharmaceutical compositions

Provided in this disclosure are pharmaceutical compositions comprising a LINC complex inhibitor as described herein. The LINC complex inhibitor is preferably formulated as a medicament together with one or more other pharmaceutically acceptable carriers well known to those skilled in the art.

By “pharmaceutically acceptable carrier” is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, colouring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like.

Representative pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antimicrobial agents), isotonic agents, absorption delaying agents, salts, stabilisers, gels, binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient(s), its use in the pharmaceutical compositions is contemplated.

The pharmaceutical composition may comprise a pharmaceutically acceptable delivery vehicle for carrying the LINC complex inhibitor (such as a nucleic acid inhibitor or a construct encoding a nucleic acid or polypeptide inhibitor). Non-limiting examples of pharmaceutically acceptable delivery vehicles include viral vectors, polymer-based carriers, protein-based carriers, lipid-based carriers and extracellular vesicles. The pharmaceutically acceptable delivery vehicle may be a polymer-based carrier system such as a cationic

polymer-nucleic acid complex (i.e., polyplex). The delivery vehicle can be a cyclodextrin-based carrier system such as a cyclodextrin polymer-nucleic acid complex. The delivery vehicle may be a protein-based carrier system such as a cationic peptide-nucleic acid complex. The carrier may be a lipid-based carrier system, including but not limited to
5 micelles, liposomes, cationic lipid-nucleic acid complexes (i.e., lipoplexes), lipid nanoparticles (LNPs), virosomes. The delivery vehicle may be an extracellular vesicle, such as an exosome. Alternatively, the delivery vehicle may be a viral vector.

In some embodiments, the pharmaceutical composition comprises a viral vector for
10 delivering the LINC complex inhibitor (for example, a nucleic acid inhibitor or nucleic acid construct) to a cell. Suitable viral vectors include, e.g., retroviral vectors, lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, vaccinia virus vectors, and herpesvirus vectors.

15 In some embodiments, the delivery vehicle is selected based on tropism for a cell type or tissue to which it is desired to deliver the nucleic acid, e.g., a cell type or tissue affected by the dystrophinopathy. For example, in some embodiments it is desired to deliver nucleic acid encoding a LINC complex inhibitor to muscle cells or tissue (e.g., cardiac and/or skeletal muscle cells or tissue), and vehicles having a tropism for such cells or tissue may be
20 employed. In some embodiments, the vector is cardiotropic. In some embodiments, the vector is myotropic.

In one embodiment, the delivery vehicle is an adeno-associated virus (AAV) vector. In some embodiments, the viral vector is an AAV of one of the following serotypes: AAV-DJ, AAV1,
25 AAV2i8, AAV6, AAV6.2FF (i.e., lung-tropic AAVs), AAV8, AAV9, AAV 9.45, or AAV-LK03 (i.e., liver-tropic AAVs).

In some embodiments, the pharmaceutical composition comprises a lipid nanoparticle (LNP) composition for delivering the LINC complex inhibitor (for example, a nucleic acid inhibitor
30 or nucleic acid construct) to a cell.

As used herein, the term "lipid nanoparticle" refers to a nanoparticle made from lipids (e.g., a cationic or ionisable lipid, a non-cationic lipid, a conjugated lipid and cholesterol), wherein the nucleic acid is fully encapsulated within the lipid. LNPs may contain multiple lipid layers

as well as microdomains of lipids and nucleic acids and may thus be distinguished from lipoplexes (in which the nucleic acid is not encapsulated), micelles (which only contain a lipid monolayer) and liposomes (which only contain a lipid bilayer).

- 5 The pharmaceutical composition may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, gels, creams, powders and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Suitable pharmaceutical compositions may be administered intravenously,
- 10 subcutaneously or intramuscularly. In some embodiments, the compositions are in the form of injectable or infusible solutions. In some embodiments, the administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular, intranasal, topical or transdermal).
- 15 The phrases “parenteral administration” and “administered parenterally” as used herein means modes of administration other than enteral administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural
- 20 and intrasternal injection and infusion.

- Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as
- 25 ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media, such as 0.01–0.1 M and preferably 0.05 M phosphate buffer or 0.8% saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer’s dextrose, dextrose and sodium chloride, lactated Ringer’s, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte
- 30 replenishers, such as those based on Ringer’s dextrose, and the like. Preservatives and other additives can also be present, such as antimicrobials, antioxidants, chelating agents, inert gases, and the like.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic

response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. An LINC complex inhibitor of the present disclosure can be administered on multiple occasions. Intervals between single dosages can
5 be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by monitoring treatment progress in the subject. Alternatively, the LINC complex inhibitor can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the inhibitor in the patient.

10

It may be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired
15 therapeutic effect in association with the required pharmaceutically acceptable carrier. The specification for the dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

20

Methods of treatment

The present disclosure provides methods for treating and/or preventing dystrophinopathies and associated pathologies in cells, tissues, organs and subjects through LINC complex
25 inhibition.

Disclosed herein is a method of treating or preventing a dystrophinopathy, or a myopathy and/or skeletal muscular dystrophy associated with a dystrophinopathy, in a subject, the method comprising administering an effective amount of a LINC complex inhibitor to the
30 subject.

Disclosed herein is a LINC complex inhibitor, for use in treating or preventing a dystrophinopathy, or a myopathy and/or skeletal muscular dystrophy associated with a dystrophinopathy, in a subject.

Disclosed herein is the use of a LINC complex inhibitor in the manufacture of a medicament for treating or preventing a dystrophinopathy, or a myopathy and/or skeletal muscular dystrophy associated with a dystrophinopathy, in a subject.

5

In some embodiments, the dystrophinopathy is selected from Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and DMD-associated dilated cardiomyopathy (DCM).

10 In some embodiments, the myopathy and/or muscular dystrophy associated with the dystrophinopathy is selected from cardiomyopathy, cardiac muscular dystrophy and skeletal muscle dystrophy.

The subject may be suffering from or suspected of suffering from the dystrophinopathy or
15 associated pathology, e.g., based on the presence of symptoms indicative of the disease in a cell or tissue of the subject. Alternatively, the subject may be considered at risk of developing the dystrophinopathy or associated pathology, e.g., because of genetic predisposition or other risk factors for the disease.

20 In some embodiments, methods herein comprise determining whether a subject has a dystrophinopathy or an associated pathology described herein. Such a determination may comprise detecting a mutation in the dystrophin gene (*DMD*) in a sample obtained from the subject. A list of pathological *DMD* mutations may be obtained from clinical or genomic
25 databases available to the skilled person, such as the ClinVar or GenBank databases maintained by the NCBI, USA. The sample obtained from a subject may be of any kind. For example, a biological sample may be taken from any tissue or bodily fluid, e.g. blood, serum sample, lymph, semen, saliva, synovial fluid. The sample may comprise a tissue sample or biopsy, or cells isolated from a subject. In some embodiments, determining whether a subject has a dystrophinopathy may comprise analysing a subject for one or more symptoms or
30 correlates of the disease.

A subject identified as having, likely to have or at risk of developing a dystrophinopathy or associated pathology may be administered a LINC complex inhibitor according to the present disclosure.

In accordance with the present invention, a method of treating and/or preventing a dystrophinopathy or a myopathy and/or skeletal muscular dystrophy associated with a dystrophinopathy may comprise one or more of the following: increasing the lifespan of a
5 subject having the disease; increasing motor, pulmonary and/or cardiac function; delaying the onset of cardiac or motor function decline; increasing myocardial and/or skeletal muscle contractility; decreasing the incidence of bradycardia and/or arrhythmias; increasing cardiac ejection fraction and/or fractional shortening; decreasing cardiac and/or skeletal muscle fibrosis; decreasing cardiac and/or skeletal muscle stiffness; and/or decreasing cardiac
10 and/or skeletal muscle necrosis.

Also disclosed herein is a method of treating a dystrophinopathy in a cell, the method comprising contacting the cell with an effective amount of a LINC complex inhibitor. The cell may be present in a subject suffering from a dystrophinopathy or associated pathology.

15

In some embodiments, the cell is a cardiomyocyte or a skeletal muscle cell.

Dystrophinopathy in a cell may be determined by detecting a mutation in the *DMD* gene in the cell. For muscle cells, a dystrophinopathy may also manifest as a pathological phenotype,
20 including but not limited to reduced contractility, irregular contraction or contraction frequency, dysfunctional calcium ion gating, increased collagen deposition, and cellular necrosis. Accordingly, treating or preventing a dystrophinopathy in a cell may comprise reversing, ameliorating and/or delaying the onset of any of the above pathologies or otherwise restoring normal cellular phenotype or function.

25

As used herein, the term “contacting” is not intended to include the *in vivo* exposure of cells to a LINC complex inhibitor disclosed herein that may occur naturally in a subject (i.e., exposure that may occur as a result of a natural physiological process). The step of contacting a cell with a LINC complex inhibitor as disclosed herein can be conducted in any suitable
30 manner. For example, a cell may be treated in adherent culture, or in suspension culture. It is to be understood that a cell can be contacted with a LINC complex inhibitor and simultaneously or subsequently contacted with another therapeutic agent.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour
5 to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications, which fall within the spirit
10 and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Unless otherwise defined, all technical and scientific terms used herein have the same
15 meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit
20 the scope of the generality hereinbefore described.

EXAMPLES

Example 1: LINC complex disruption alleviates pathophysiology in *in vitro* and mouse
25 models of DMD

Materials and methods

Table 4. Cell lines used in this study.

Fibroblast	Mutation	iPSC	Knock-in DNSUN1	Knock-in GCaMP6s
DMD3429 6yr	Ex45-50del	5 clones	3 subclones	3 subclones

	(cardiomyopathy at 9yr)	(1 clone used in this study)	(2 clones used in this study)	(2 clones used in this study)
DMD5169 9yr	Ex4-43del (cardiomyopathy at 10yr)	5 clones (1 clone used in this study)	3 subclones (2 clones used in this study)	3 subclones (2 clones used in this study)
Ctr5171	Control (father of DMD5169)	5 Clones (1 clone used in this study)	2 subclones (1 clone used in this study)	2 subclones (1 clone used in this study)

Generation of induced pluripotent stem cells (iPSCs) from DMD patient fibroblasts

Dermal fibroblasts were purchased from the Coriell Institute for Medical Research (Camden, New Jersey). Fibroblast line DMD-3429 has a deletion of exons 45-50 (ex.45_50del), and

5 DMD-5169 has a deletion of exons 4-43 (ex.4_43del) in the dystrophin gene. Fibroblast Ctr-5171 is a normal parent control. Induced pluripotent stem cells (iPSCs) were generated from the three fibroblast lines using Sendai virus CytoTune-iPS 2.0 Sendai Reprogramming Kit, #A16517 (Thermo Fisher, Waltham, MA, USA). Five clones from each line were picked and stably maintained feeder-free on Matrigel matrix coated plate using StemMACS iPS-

10 Brew XF medium (Miltenyi Biotec, Germany) containing 1% Penicillin/Streptomycin (Gibco, USA). All experiments were performed with mycoplasma-free cells.

Generation of GCaMP6s and DNSUN1 knock-in iPSC lines

In preparation for transfection, iPSCs were dissociated using Accutase (Nacalai Tesque) at

15 37°C for 3 minutes and 250K cells were seeded overnight on single wells of a 6-well plate. The next day, plasmids were transfected into the seeded hPSCs using Lipofectamine Stem transfection reagent (Invitrogen) at plasmid size-dependent concentrations. For clonal isolation, hPSCs were dissociated using Accutase and resuspended in Phosphate Buffered Saline (PBS) containing 0.5% Fetal Bovine Serum (FBS), 1% Bovine Serum Albumin

20 (BSA), 1% Penicillin/Streptomycin (Gibco, U.S.A.) and 5µM Y27632 (Miltenyi Biotec, Germany) and sorted into single wells using the LSR II (BD Biosciences, USA). GCaMP6s were stably knocked into each iPSC cell line using a Cas9 homology-directed repair protocol described previously with slight modifications (Jiang et al., 2018; Ran et al., 2013). AAVS1 targeting gRNA was cloned into pSPCas9(BB)-2A-GFP (Addgene #48138). GCaMP6s

25 donor plasmid AAVS1-puro-CAG-GcaMP6s (Addgene plasmid #120896) was used. DNSUN1 donor plasmid was generated by inserting the DNSUN1 PCR amplicon into

AAVS1-CAG-hrGFP (Addgene #52344), replacing GFP. The optimised plasmid amounts used were AAVS1 gRNA plasmid containing Cas9 (0.6 µg) and GCaMP6s/DNSUN1 donor plasmid (3.6 µg) transfected with 10 µL of Lipofectamine Stem in 2 mL of iPS-Brew. Two days after transfection, cells were first selected using 1 µg/ml puromycin for two days, and
5 after that, live cells were sorted into single cells for clonal isolation.

Differentiation and purification of iPSC-derived cardiomyocytes (iPSC-CMs)

iPSC-CMs were generated according to the directed differentiation by modulating Wnt/β-catenin signalling. Briefly, iPSCs were cultured on 12-well plates coated with Matrigel (GFR,
10 BD Biosciences, Franklin Lakes, NJ, USA), in StemMACS iPS-Brew XF medium (Miltenyi Biotec) for 5-6 days. To initiate differentiation, cells were seeded onto Matrigel-coated plates at $8.5 \times 10^6/12$ /well plate density in StemMACS iPS-Brew medium supplemented with 5 µM ROCK inhibitor (Cayman Chemical, Ann Arbor, MI, USA). The medium was replaced daily, and after 2 days, the monolayer of cells reached 80%-100% confluence. The culture
15 medium was changed to RPMI supplemented with B27 minus insulin (Invitrogen, Life Technologies, Woburn, MA, USA) containing 8 to 12 µM CHIR99021. The next day (day 2 of differentiation), the medium was changed to RPMI supplemented with B27 minus insulin. On the 4th day, the medium was changed to RPMI supplemented with B27 minus insulin, containing 5-10 µM of IWP4 or IWP2. On the 6th day, the medium was changed to
20 RPMI supplemented with B27 minus insulin. Finally, from the 8th day onwards, the medium was changed to RPMI supplemented with B27 complete supplement (Invitrogen). From differentiation days 10-14, beating cardiomyocytes were dissociated into single cells with dissociating solution (85 mM KCl, 30 mM K₂HPO₄, 5 mM MgSO₄, 1 mM EGTA, 5 mM sodium pyruvate, 5 mM creatine, 20 mM taurine, 20 mM glucose, 2 mM disodium pyruvate,
25 pH=7.4) for 20 min at 37°C. Cells were then plated on Matrigel-coated plates and maintained with RPMI supplemented with B27 complete supplement. From days 14-28, purification of cardiomyocytes was performed by gradually reducing the glucose levels in the media to 0% with RPMI1640, 0% Glucose (HyClone) containing 1X B27 with insulin supplement and 4mM lactate-HEPES solution (Sigma) to facilitate the metabolic maturation and selection of
30 the cardiomyocytes to β-oxidation.

Fluorescent video capture of single cardiomyocyte Ca²⁺ cycles

Ca²⁺ cycles of CMs were imaged at 20× magnification using a Nikon ECLIPSE fluorescent microscope and recorded using an Andor Zyla 4.2 sCMOS camera at 30 frames/s for the 30s.

Ca²⁺ transients are detected as fluorescent emissions using a fluorescein isothiocyanate (FITC) filter cube. Video data were analysed using Nikon's NIS-Elements AR to isolate single CMs as ROIs and obtain fluorescent intensity over time for individual CMs per frame. Fluorescent intensity values were processed using code in R (RStudio version 1.2.1335/Base

5 R version 3.6.3) to identify fluorescent peaks corresponding to single cardiac contractions as previously described (Pang JK et al., 2022). Individual Ca²⁺ transients were decomposed into parameters summarizing the transients' shape mathematically; for parameters describing absolute values, such as beat-to-beat duration, the relative SD was calculated.

10 **Monitoring of the beating frequency of heart organoids**

To generate the heart organoids, 100k to 500k cardiomyocytes from each line were seeded into each well of a 96-well ultra-low attachment plate (CoSTAR) and aggregated by spinning to form organoids. The organoids were then maintained in RPMI medium with B27 supplement for 30 days with medium replacement every two days. Beating parameters were
15 monitored by live image recording with Olympus IX-83 fluorescent microscope. On the day of measurements, heart organoids were incubated in Tyrode's solution (140 mM NaCl, 5 mM HEPES, 4 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 10 mM glucose, pH 7.4) with 3 μM Fluo4-AM (Invitrogen, Carlsbad, CA, USA) for 20 mins at room temperature. After incubation with Fluo4-AM, the solution was replaced with the fresh Tyrode's solution, and
20 the organoids were placed on the stage of an inverted microscope. Ca²⁺ images were recorded with a fluorescent microscope at a frame rate of 10 frames per second for 30 seconds. Image J software analyzed the kinetic properties of intracellular Ca²⁺ variations, including Ca²⁺ transients and diastolic events.

25 **RNA extraction and RT-PCR analysis**

RNA extraction of 10⁶ iPSC-derived cardiomyocytes was performed using an RNeasy mini kit (Qiagen, Hilden, Germany). RNA concentration and purity were determined using NanoDrop (NanoDrop Technologies, Wilmington, USA). Using 0.3 μg of total RNA, cDNA was produced using a High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor
30 (Applied Biosystems). A quantitative PCR experiment was performed using KAPA SYBR Fast qPCR Master Mix (KAPA) and 7900HT Fast Real-Time PCR machine (Applied Biosystems). qPCR reactions were performed following the manufacturer's protocol. In brief, enzyme activation was performed at 95°C for 3 minutes, followed by 40 cycles consisting of denaturation at 95°C for 1 second and annealing/extension at 60°C for 20 seconds. The

PCR primers for amplification of dystrophin spanning exon 12-18 are Forward: 5' AAGTGGAATGTTGCCAAGG (SEQ ID NO: 143), and Reverse: 5' AGGCTCTTCCTCCATTTTCC (SEQ ID NO: 144). Primers for amplification of dystrophin exon 46-49 are Forward: 5' TGGGAACATGCTAAATACAA (SEQ ID NO: 145) and reverse: 5' CCGGTTGTTTAGCTTGAA (SEQ ID NO: 146). mRNA expression levels of the transcript were normalised by GAPDH.

Immunofluorescence analysis of iPSC-derived cardiomyocytes

Differentiated iPSC cardiomyocytes were plated on Matrigel-coated slides, fixed with 4% paraformaldehyde or acetone. The cardiac marker, sarcomeric actinin, was stained using Anti-actinin 1:500 (Abcam, ab137346) and the C-terminus of dystrophin was labelled with an anti-dystrophin antibody (Abcam, ab15277). The cells were permeabilised and blocked with blocking solution (1% bovine serum albumin, BSA, 0.2% Triton X-100 in phosphate buffer saline, PBS, 15 min at RT). Primary antibodies were diluted in PBS/Triton (0.1%) and incubated overnight at 4°C. Samples were extensively washed with PBS/Triton (0.1%) and incubated with appropriate fluorescent secondary antibody for 1hr at room temperature. The following Alexa Fluor-conjugated isotype-specific secondary antibodies (Thermo Fisher, 1:600 dilution) were used: Goat anti-rabbit Alexa 488, Goat anti-mouse Alexa 488, Goat anti-rabbit Alexa 594, Goat anti-mouse Alexa 594. For nuclear counterstain, 4,6-Diamidino-2-phenylindole dihydrochloride (DAPI) was used. iPSC-CMs stained with secondary antibodies were only used as negative controls to distinguish genuine staining from the background and to adjust imaging settings. Fluorescent confocal microscopy recordings were performed using the Confocal Olympus FV3000 microscope.

Statistical Analysis

Statistical evaluation was performed using GraphPad Prism 8.3 software (GraphPad Software, Inc., La Jolla, CA, USA). Available normality tests were performed for the obtained data, and Student's two-tailed *t*-test was used to assess statistically significant differences in normally distributed group pairs.

Mouse Experiments

SUN1^{-/-} mice

SUN1^{-/-} mice were obtained from NIAID and were derived as described in Chi, Y. et al., *Development* 2009, 136, p965). Heterozygous *SUN1* mice were interbred to obtain *SUN1*^{-/-} mice.

- 5 The *mdx/mdx* mice, purchased from the Jackson Laboratory, were euthanised with CO₂ and the quadriceps muscle removed. For histological analyses, quadriceps muscles were cut transversely in the central area, mounted onto tragacanth gum, and frozen in isopentane quenched in liquid nitrogen. These quadriceps blocks were later cryo-sectioned at 10µm for analysis.

10

Histological staining

Muscle blocks were sectioned at 10 µm and stained with H&E. The basic H&E protocol is:

- 1) Stain with Hematoxylin for 30 seconds. 2) Remove excess stain in tap water (until nuclei turns blue). 3) Place in 70% Ethanol for 3 minutes. 4) Stain in Eosin for 15 seconds. 5) Remove excess Eosin and dehydrate in 100% Ethanol for 3 minutes (repeat 3 times). 6) Clear in Xylene for 3 minutes (repeat 3 times). 7) Mount with DPX mountant, coverslip (remove trapped air bubbles) and dried overnight. Images of the sections were taken on a bright field and fluorescence images were captured using the Zeiss AxioImager Z1 upright microscope for analysis.
- 15

20

Muscle necrosis grading/scoring system

- To determine the extent of necrosis in the *mdx/mdx* mice, a grading/scoring system was used by analysing H&E stained sections of quadriceps muscle and diaphragm. The extensor digitorum longus (EDL) muscle, with both tendons, was excised and transplanted onto the tibialis anterior (TA) muscle. The EDL tendons were sutured to the TA, the skin closed and the wound left to heal. At day 9 post-surgery, mice were euthanised. The TA and grafted EDL were excised, mounted in tragacanth gum (Sigma-Aldrich) on cork pieces and snap frozen with isopentane (BDH-AnalaR) for cryosection. Muscle sections at 7 µm thickness were mounted for histological and immunofluorescence analyses. Grafts were harvested at 5, 9 and 14 days post-surgery for gene expression analysis.
- 25
- 30

Immunofluorescence, histology and microscopy

Muscle sections were stained with H&E, and imaged using a Zeiss AxiolmagerZ1 microscope and ZEN2 software. Muscle graft analysis and myofiber quantification was performed with Fiji software. The sections were graded blind.

5 **mdxSUN1^{-/-} mice breeding and sampling**

To obtain mdx *SUN1*^{-/-} double null mice, the following matings were performed. Genotyping was performed on DNA from mouse tail tips.

F0: Male *SUN1*^{+/-} crossed with female mdx mice; all male mice are mdx with 50% *SUN1*^{+/-}.

10 F1: Male mdx *SUN1*^{+/-} crossed with female mdx mice; all mice are mdx with 25% being *SUN1*^{+/-}.

F2: Male mdx *SUN1*^{+/-} crossed with female mdx *SUN1*^{+/-}; 25% of the mice are the desired mdx/mdx *SUN1*^{-/-} genotype.

15 To avoid any influence from genetic background and age, mutant mice were always compared to their wild-type littermates. Mice were sampled at 21 days with the onset of necrosis. All mice were bred and housed in specific-pathogen-free animal care facilities. Mice were maintained in standard cages with free access to water and standard mouse chow.

Results

20 Induced pluripotent stem cells (iPSCs) were generated from fibroblasts derived from two DMD patients and 1 parental (normal) control. The iPSC lines were differentiated to form cardiomyocytes from which 3D heart organoids were derived. DMD cardiomyocyte contractility exhibited significant arrhythmia and bradycardia. A *SUN1* dominant-negative (DNSUN1) mini-protein was delivered to cardiomyocytes to disrupt the LINC complex in
25 DMD-iPSC lines. By expressing DNSUN1, the contraction of the DMD cardiomyocytes and 3D heart organoids were significantly improved to levels seen in normal cardiomyocytes-3D organoids. LINC complex disruption in DMD skeletal muscle cells significantly reduced pro-fibrotic signalling to levels seen in normal skeletal muscle cells. In addition, genetically
30 deleting *SUN1* significantly reduced muscle necrosis in the diaphragm of mdx (DMD) mice.

Referring to Fig. 1A, RT-PCR using primers spanning exons 46-49 and 12-18 of the human dystrophin gene showed reduced dystrophin expression in iPSC-derived cardiomyocytes from DMD patients. Fig. 1B shows representative immunofluorescence images of control

5171, DMD-3429 and DMD-5169 iPSC-derived cardiomyocytes (CMs) stained with antibodies against sarcomeric-actinin, dystrophin and ALFA-tagged DNSUN1. Dystrophin protein expression was detected in control 5171-CM, but not in DMD3429 and DMD5169 cardiomyocytes. DNSUN1-ALFA fusion protein was detected in cell lines with DNSUN1 ALFA knock-in. Scale bar: 50 μ m.

Fig. 2A shows representative video captures showing the time to peak of contraction. The contractions of DMD cardiomyocytes (3429 and 5169) are slower compared to control cardiomyocytes (5171). Referring to Fig. 2B, representative images from videos of DMD-3429 and DMD-5169 subclones (set A) show that slow contractions were rescued by knock-in of DNSUN1. Fig. 2C shows screen captures of fluorescent video imaging of iPSC-derived CM DMD-3429 and DMD-5169 subclones with GCaMP6s knock-in (set B), which also shows that slow contractions can be rescued by DNSUN1 knock-in (Fluo4-AM staining). Fig. 2D is an analysis of the time to the peak of contractions for the cell lines studied. The slow and irregular contractions in the DMD-CM lines (DMD3429, 3429-GCaMP6s, DMD5169, 5169-GCaMP6s) were rescued by DNSUN1 knock-in. (unpaired t-test, $n=20$, $**p<0.01$ $***p<0.001$, $****p<0.0001$).

In Fig. 3, isolated cardiomyocytes were stained with 5 μ M Fluo4-AM. Ca^{2+} cycling was quantified by measuring changes in fluorescence intensity over the duration of the recordings. Data from 20-30 single-cell recordings were summarised and analysed. Referring to Fig. 3A, compared to the control (5171), DMD cardiomyocytes (3429) showed large variations in Ca^{2+} intensity and exhibited significantly increased beat to beat duration (t-test, $p < 0.001$, $n = 20-30$), along with arrhythmia and bradycardia. Knock-in of DNSUN1 led to reduction and improvement in the beating frequency (t-test, $p < 0.001$, $n = 20-30$). Looking at Fig. 3B, where the amplitude of the Ca^{2+} transient indicates CM contractility, a representative 10s live imaging recording shows that the reduced contractility of DMD-CMs was rescued by DNSUN1 knock-in to levels comparable to the control CMs.

Looking now at Fig. 4, 10^5 cardiomyocytes were cultured to form one heart organoid (HO), with 12 organoids being generated from each line. Beating frequencies were recorded and monitored over 28 days. Results show that the DMD-HO (3429) demonstrated slow and arrhythmogenic beating frequency (Fig. 4A), which quickly declined and ceased by day 21. In DNSUN1 knock-in HOs, contractions were significantly rescued starting from day 7 till

day 28 (Fig. 4B). t-test, ***p <0.001, n = 10-12.

Fig. 5 shows that there is no difference in muscle necrosis between the quadriceps of mdx/mdx wild-type and mdx/mdx/SUN1^{-/-} mice, but there is significantly less myofiber
5 necrosis in the mdx/mdxSUN1^{-/-} mice. (P=0.031, Student t-test).

Fig. 7 shows that disrupting the LINC complex through AAV-mediated DNSUN1 expression led to reduced pro-fibrotic signalling and reduced TGFβ collagen I production in DMD skeletal muscle cells.

10

Thus, LINC complex disruption using DNSUN1 is an effective way to arrest cardiomyopathy and skeletal muscular dystrophy in DMD patients, thus improving overall health and lifespan.

15 **Example 2: LINC complex disruption by expression of dominant-negative LINC complex proteins may alleviate pathophysiology in mouse models of DMD**

AAV-mediated delivery of DNSUN1 to DMD patients may be effective at suppressing cardiomyopathy and muscular dystrophy. This has two potential advantages over existing
20 methods of DMD gene therapy: 1) DNSUN1 may not be immunogenic, so avoiding the adverse immune response that has been reported in several patients transduced with a microdystrophin construct; 2) DNSUN1 may be effective against different DMD mutations so avoiding having to develop a tailor-made therapeutic for each mutation.

25 Mouse models of DMD may be administered with nucleic acid encoding dominant-negative SUN1, SUN2, Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 or KASH5.

The mouse models of DMD include the mdx model described in Example 1, as well as more recent models of the disease, the generation and characterization of which are detailed in
30 Fukada et al., Am J Pathol., 2010 May;176(5):2414–2424 and Coley et al., Hum Mol Genet., 2015 Nov 12;25(1):130–145.

The nucleic acids may be delivered using adeno-associated virus (AAV), adenovirus or lentivirus vectors, or nanoparticles.

Mice treated with a LINC complex inhibitor may have improved skeletal muscle and cardiac function, and extended lifespans as compared to untreated mice, or mice treated with vehicle only.

5

Example 3: Gene editing of LINC complex components may alleviate pathophysiology in mouse models of DMD

10 Mouse models of DMD may be administered with nucleic acid encoding gene editing systems (including CRISPR/Cas9 systems) for disrupting LINC complex components. Mouse models of DMD is described in Example 2.

15 The gene editing systems may target an exon of a SUN domain-containing protein encoding a SUN domain, or target an exon of a KASH domain-containing protein encoding a KASH domain. The gene editing system may be delivered using adeno-associated virus, adenovirus or lentivirus vectors, or nanoparticles.

20 Mice treated with a LINC complex inhibitor may have improved skeletal muscle and cardiac function, and extended lifespans as compared to untreated mice, or mice treated with vehicle only.

Example 4: LINC complex disruption using small molecule drugs may alleviate pathophysiology in mouse models of DMD

25 Mouse models of DMD may be administered with small molecule inhibitors of the interaction between SUN and KASH domains. Mouse models of DMD is described in Example 2.

30 The small molecule inhibitors may inhibit association between a KASH domain and a SUN domain.

Mice treated with a LINC complex inhibitor may have improved skeletal muscle and cardiac function, and extended lifespans as compared to untreated mice, or mice treated with vehicle only.

Example 5: RNAi-mediated knockdown of LINC complex component expression may alleviate pathophysiology in mouse models of DMD

- 5 Mouse models of DMD may be administered with nucleic acid encoding shRNA or siRNA targeting LINC complex components. Mouse models of DMD is described in Example 2.

The shRNA/siRNA may target a SUN domain-containing protein or a KASH domain-containing protein.

10

The nucleic acids may be delivered using adeno-associated virus, adenovirus or lentivirus vectors, or nanoparticles.

- Mice treated with a LINC complex inhibitor may have improved skeletal muscle and cardiac
15 function, and extended lifespans as compared to untreated mice, or mice treated with vehicle only.

- It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended
20 to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

CLAIMS

1. A method of treating or preventing a dystrophinopathy in a subject, the method comprising administering an effective amount of a LINC complex inhibitor to the subject.
2. The method of claim 1, wherein the dystrophinopathy is selected from Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), and DMD-associated dilated cardiomyopathy (DCM).
3. The method of claim 1 or 2, wherein the LINC complex inhibitor is a nucleic acid, polypeptide, site-specific nuclease (SSN) system, and/or small molecule.
4. The method of claim 3, wherein the LINC complex inhibitor comprises a polypeptide derived from a LINC complex protein.
5. The method of claim 4, wherein the polypeptide is derived from a SUN domain protein or KASH domain protein.
6. The method of claim 5, wherein the LINC complex inhibitor comprises a dominant negative SUN1 (DNSUN1) polypeptide.
7. The method of claim 6, wherein the DNSUN1 polypeptide comprises an amino acid sequence having at least 70% sequence identity to an amino acid sequence set forth in SEQ ID NO: 2.
8. The method of claim 3, wherein the LINC complex inhibitor is an inhibitory nucleic acid molecule or a site-specific nuclease (SSN) system capable of disrupting a gene, or an RNA product thereof, encoding a LINC complex protein.
9. The method of claim 8, wherein the LINC complex protein is a SUN domain protein or KASH domain protein.
10. The method of claim 9, wherein the SUN domain protein is SUN1 or SUN2.

11. The method of claim 9, wherein the KASH domain protein is Nesprin-1 or Nesprin-2.
12. The method of any one of claims 8 to 11, wherein the LINC complex inhibitor is an inhibitory nucleic acid molecule selected from a miRNA, siRNA, shRNA and antisense oligonucleotide (ASO).
13. The method of any one of claims 8 to 11, wherein the LINC complex inhibitor is an SSN system.
14. The method of claim 13, wherein the SSN system is capable of disrupting the gene encoding the LINC complex protein by nucleotide insertion or deletion.
15. The method of claim 13 or 14, wherein the SSN system is a CRISPR/Cas system.
16. The method of claim 15, wherein the CRISPR/Cas system is a CRISPR/Cas9 or CRISPR/Cas12a system.
17. The method of any one of claims 1 to 16, wherein the LINC complex inhibitor is encoded by a nucleic acid vector.
18. A LINC complex inhibitor, for use in treating or preventing a dystrophinopathy in a subject.
19. Use of a LINC complex inhibitor in the manufacture of a medicament for treating or preventing a dystrophinopathy in a subject.
20. A method of treating or preventing a myopathy and/or muscular dystrophy associated with a dystrophinopathy in a subject, the method comprising administering an effective amount of a LINC complex inhibitor to the subject.
21. The method of claim 20, wherein the myopathy and/or muscular dystrophy is selected from cardiomyopathy, cardiac muscular dystrophy and skeletal muscle dystrophy.

22. A method of treating or preventing a dystrophinopathy in a cell, the method comprising contacting the cell with an effective amount of a LINC complex inhibitor.
23. The method of claim 22, wherein the cell is a cardiomyocyte or a skeletal muscle cell.