# TREATMENT OF CARDIOMYOPATHY NOT ASSOCIATED WITH LAMINOPATHY

This application claims priority from GB 2312938.0 filed 24 August 2023 and GB 2404416.6 filed 27 March 2024, the contents and elements of which are herein incorporated by reference for all purposes.

## Technical Field

The present disclosure relates to the treatment and prevention of diseases and conditions through LINC complex inhibition.

## Background

Mutations in *LMNA*, which encodes lamins A and C, result in a plethora of diseases known as laminopathies. Disruption of Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes has recently been proposed as a therapeutic strategy for the treatment of laminopathies – see *e.g.* Chai *et al.*, Nature Communications (2021) 12: 4722.

Desmoplakin is an essential component of desmosomes, which form intercellular junctions between adjacent cells. Desmosomes (also known as macular adherens) are specialised structures mediating lateral cell-to-cell adhesion between cells particularly of tissue subjected to considerable mechanical stress, such as cardiac muscle tissue (also bladder tissue, gastrointestinal mucosa and epithelia). The structure and function of desmosomes is reviewed *e.g.* in Delva *et al.*, Cold Spring Harb Perspect Biol. (2009) 1(2): a002543 and Johnson *et al.*, Cold Spring Harb Perspect Med. (2014) 4(11): a015297, both of which are hereby incorporated by reference in their entirety.

The N-terminal globular domain of human desmoplakin comprises a 584 amino acid region which is required for interaction with plakophilin and plakoglobin (see *e.g.* Stappenbeck *et al.*, J Cell Biol. (1993) 123(3):691-705 and Kowalczyk *et al.*, J Cell Biol. (1997) 139(3):773-84). Desmoplakin binds to intermediate filaments via the C-terminus (see *e.g.* Stappenbeck and Green, J Cell Biol. (1992) 116(5):1197-209 and Stappenbeck *et al.*, J Cell Biol. (1993) 123(3):691-705), and this may be mediated by a glycine-serine-arginine repeat region at the C-terminus (see *e.g.* Fogl *et al.*, Nat Commun. (2016) 7:10827).

Mutations in desmoplakin (which is encoded by the *DSP* gene) cause cardiomyopathies, such as dilated cardiomyopathy (DCM) and arrhythmogenic cardiomyopathy (ACM). Mutations in *DSP* can also result in skin diseases, such as epidermolysis bullosa, and pleiotropic disorders such as dilated cardiomyopathy with woolly hair and keratoderma. These diseases are thought to arise as a consequence of a loss or reduction in the level of expression of desmoplakin, which reduces the number and/or inhibits the function of desmosomes.

The intercalated disc (ICD) between neighbouring cardiomyocytes is fundamental to cellular function and regulation, specifically facilitating the arrangement of cells into organised layers thereby maintaining tissue integrity and efficient electromechanical coupling. The basic components of the intercellular junction can be broadly divided into those involved in mechanical coupling maintaining cell–cell adhesion, and those involved in electrical coupling facilitating the rapid transmission of ions between cells allowing

rapid transmission of depolarising wave fronts (D.J. Abrams, J.E. Saffitz, in Cardioskeletal Myopathies in Children and Young Adults, 2017). Mechanical coupling and intercellular adhesion are provided by the adherens junctions and desmosomes. Electrical coupling between myocardial cells occurs primarily via gap junctions.

The desmosomes of ICDs are composed of desmosomal cadherins, which make intercellular connections, and are connected intracellularly via the adaptor proteins plakoglobin and plakophilin 2 to desmoplakin, which in turn recruits desmin intermediate filaments.

The sarcomere is the single contractile unit of striated muscle cells consisting of actin, myosin, and associated regulatory and scaffolding proteins, which repeat to form myofibrils—the basic rod-like organelle unit of a cardiomyocyte (Warner E. et al. Circulation Research. 2022;130:1723–1741). The actin filaments tether at the Z disc. Z disc–associated proteins include α-actinin, titin, nebulin, and intermediate filaments, such as desmin intermediate filaments.

Microtubules extend between Z discs and appear to stabilise at the Z line, whereby growth can pause. Intermediate filaments, such as desmin, anchor detyrosinated microtubules to the Z disc and cross-link the microtubules to the intermediate filament network, which increases resistance and forces microtubules to be compressed and display a buckling conformation when cardiomyocyte contracts (Warner E. et al., 2022). Desmin is thus proposed to function as a mechanical anchor for microtubules, confining their distribution to specific spatial regions (Uchida K. et al., Annu. Rev. Physiol. 2022. 84:257–83).

**Summary**

In a first aspect, the present disclosure provides a LINC complex inhibitor for use in a method of treating or preventing cardiomyopathy, wherein the cardiomyopathy is not cardiomyopathy associated with a laminopathy.

The present disclosure also provides the use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing cardiomyopathy, wherein the cardiomyopathy is not cardiomyopathy associated with a laminopathy.

The present disclosure also provides a method of treating or preventing cardiomyopathy, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject, wherein the cardiomyopathy is not cardiomyopathy associated with a laminopathy.

In some embodiments, in accordance with the various aspects of the present disclosure, the cardiomyopathy is selected from dilated cardiomyopathy, arrhythmogenic cardiomyopathy, hypertrophic cardiomyopathy and restrictive cardiomyopathy.

In some embodiments, the cardiomyopathy is associated with desmosome deficiency/insufficiency or dysfunction. In some embodiments, the cardiomyopathy is associated with mutation to a gene encoding a desmosome protein.

In some embodiments, the cardiomyopathy is associated with mutation to a gene selected from: *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*.

In some embodiments, the cardiomyopathy is characterised by microtubule dysfunction.

In some embodiments, the cardiomyopathy is selected from dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic cardiomyopathy, diabetic cardiomyopathy and ischaemic cardiomyopathy.

The present disclosure also provides a LINC complex inhibitor for use in a method of treating or preventing a disease or condition characterised by desmosome deficiency or dysfunction.

The present disclosure also provides the use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing disease or condition characterised by desmosome deficiency or dysfunction.

The present disclosure also provides a method of treating or preventing disease or condition characterised by desmosome deficiency or dysfunction, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject.

In some embodiments, in accordance with the various aspects of the present disclosure, the disease or condition characterised by desmosome deficiency or dysfunction is associated with mutation to a gene encoding a desmosome protein.

In some embodiments, the disease or condition characterised by desmosome deficiency or dysfunction is associated with mutation to a gene selected from: *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*.

In some embodiments, the disease or condition characterised by desmosome deficiency or dysfunction is characterised by cardiomyopathy.

In some embodiments, the disease or condition characterised by desmosome deficiency or dysfunction is characterised by microtubule dysfunction.

The present disclosure also provides a LINC complex inhibitor for use in a method of treating or preventing a disease or condition characterised by microtubule dysfunction.

The present disclosure also provides the use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing a disease or condition characterised by microtubule dysfunction.

The present disclosure also provides a method of treating or preventing disease or condition characterised by microtubule dysfunction, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject.

In some embodiments, the disease or condition characterised by microtubule dysfunction is or is characterised by cardiomyopathy.

The present disclosure also provides a LINC complex inhibitor for use in a method of treating or preventing a disease or condition characterised by sarcomere deficiency/insufficiency or dysfunction.

The present disclosure also provides the use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing a disease or condition characterised by sarcomere deficiency/insufficiency or dysfunction.

The present disclosure also provides a method of treating or preventing disease or condition characterised by sarcomere deficiency/insufficiency or dysfunction, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject.

In some embodiments, the disease or condition characterised by sarcomere deficiency or dysfunction is associated with mutation to a gene encoding a sarcomere protein.

In some embodiments, the disease or condition characterised by sarcomere deficiency or dysfunction is associated with mutation to a gene selected from: *TTN, MYH7, TNNT2, TNNI3, TNNC1, TPM1, MYBPC3, MYL2, MYL3, CSRP3, RBM20, ACTN2, TCAP and ACTC1.*

In some embodiments, the disease or condition characterised by sarcomere deficiency/insufficiency or dysfunction is or is characterised by cardiomyopathy.

In some embodiments, the disease or condition characterised by sarcomere deficiency or dysfunction is characterised by microtubule dysfunction.

In some embodiments, in accordance with the various aspects of the present disclosure, the cardiomyopathy is selected from dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic cardiomyopathy, diabetic cardiomyopathy and ischaemic cardiomyopathy.

In some embodiments, in accordance with the various aspects of the present disclosure, the treating or preventing comprises administering nucleic acid comprising or encoding a LINC complex inhibitor to a subject.

In some embodiments, the treating or preventing comprises administering nucleic acid encoding a LINC complex inhibiting polypeptide to a subject.

In some embodiments, the LINC complex inhibiting polypeptide comprises: (i) an inhibitory region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein, and (ii) an endoplasmic reticulum retention motif.

In some embodiments, the LINC complex inhibiting polypeptide comprises, or consists essentially of an amino acid sequence having at least 80% amino acid sequence identity to the amino acid sequence of SEQ ID NO:99, 72, 95, 71, 69, 70 or 73.

In some embodiments, the nucleic acid encoding a LINC complex inhibiting polypeptide to a subject is comprised in an adeno-associated virus (AAV) vector.

**Description**

The present disclosure is based on the inventors' unexpected finding that LINC complex inhibition is useful for the treatment/prevention of diseases/conditions other than laminopathies, in particular cardiomyopathy having a cause other than mutation to *LMNA*.

The present disclosure is also based on the inventors' unexpected finding that LINC complex inhibition is useful for the treatment/prevention of diseases/conditions in which aberrant microtubule function is pathologically implicated.

The inventors demonstrate herein that LINC complex inhibition is useful for the treatment/prevention of diseases associated with desmosome deficiency/dysfunction, *e.g.* arising as a consequence of mutation to desmosome proteins. In particular, the inventors demonstrate that the pathology of cardiomyopathy associated with *DSP* mutation can be ameliorated through LINC complex inhibition.

The inventors demonstrate herein that LINC complex inhibition is useful for the treatment/prevention of diseases associated with sarcomere dysfunction, *e.g.* arising as a consequence of mutation to sarcomere proteins. In particular, the inventors demonstrate that the pathology of cardiomyopathy associated with *TTN* mutation can be ameliorated through LINC complex inhibition.

The particular models of cardiomyopathy employed in the experimental examples arise as a consequence of different factors (*i.e.* mutations in *LMNA, DSP or TTN*). Cardiomyopathy associated with mutations but also acquired cardiopathy (*i.e.* non-genetic cardiomyopathy), and other cardiac disease, are characterised by aberrant organisation of microtubules which leads to alterations in microtubule load-bearing and force transmission. Thus, the inventors unexpectedly demonstrate that inhibition of the LINC

complex can reverse dysfunctional microtubule organisation. Without wishing to be bound by any particular theory, the inventors reason that uncoupling of the nuclear envelope from the microtubule network by disrupting the LINC complex can diminish the anchoring sites of the perinuclear microtubule population and reverse the aberrant load-beading and force transmission from the microtubule network to the nuclear envelope, and thereby ameliorate the pathology of cardiomyopathy and other disease.

The present disclosure provides a LINC complex inhibitor for use in a method of treating or preventing cardiac disease. The present disclosure also provides the use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing cardiac disease. The present disclosure also provides a method of treating or preventing cardiac disease, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject. In some embodiments, the cardiac disease is not associated with a laminopathy.

**LINC complex structure and function**

Linker of nucleoskeleton and cytoskeleton (LINC) complexes are polypeptide complexes comprising SUN domain-containing proteins and KASH domain-containing proteins. LINC complex structure is reviewed in *e.g.* in Sosa *et al.*, Curr Opin Struct Biol. (2013) 23(2):285-91 and Hieda, Cells (2017) 6(1):3, both of which are hereby incorporated by reference in their entirety.

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 translumenal tethers for KASH domain proteins in the ONM.

Herein, a 'SUN domain-containing protein' refers to any polypeptide comprising a SUN domain. SUN (Sad1 and UNC-84) domain proteins are important INM components comprising conserved, carboxy terminal SUN domains which localise to the perinuclear space. SUN domains comprise ~175 residues and are provided at the end of helical stalk regions. The nucleoplasmic domains of SUN domain-containing proteins interact with structural components of the nucleoskeleton.

A SUN domain may comprise or consist of the amino acid sequence shown in SEQ ID NO:8, 18, 27, 28, 29 or 30, or an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO:8, 18, 27, 28, 29 or 30.

In some embodiments, a SUN domain-containing protein is selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO. In some embodiments, a SUN domain-containing protein is SUN1 or SUN2.

In some embodiments, a SUN domain-containing protein is capable of forming a LINC complex. In some embodiments, a SUN domain-containing protein is capable of interacting with a KASH domain and/or a KASH domain-containing protein.

Human SUN1 is the polypeptide identified by UniProtKB O94901, the amino acid sequence of which is shown in SEQ ID NO:1. Human SUN2 is the polypeptide identified by UniProtKB Q9UH99, the amino acid sequence of which is shown in SEQ ID NO:13. Human SUN3 is the polypeptide identified by UniProtKB Q8TAQ9, the amino acid sequence of which is shown in SEQ ID NO:23. Human SUN5 is the polypeptide identified by UniProtKB A9Z1W8, the amino acid sequence of which is shown in SEQ ID NO:24. Human SPAG4 is the polypeptide identified by UniProtKB Q9NPE6, the amino acid sequence of which is shown in SEQ ID NO:25. Human SUCO is the polypeptide identified by UniProtKB Q9UBS9, the amino acid sequence of which is shown in SEQ ID NO:26.

In this specification 'SUN1', 'SUN2, 'SUN3', 'SUN5' 'SPAG4' and 'SUCO' respectively refer to SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO from any species and include isoforms, fragments, variants or homologues thereof.

As used herein, a 'fragment', 'variant' or 'homologue' of a protein may optionally be characterised as having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of the reference protein (*e.g.* a reference isoform of the reference protein). In some embodiments fragments/variants/ isoforms/homologues may be characterised by ability to perform a function performed by the reference protein.

A 'fragment' generally refers to a fraction of the reference protein. A 'variant' generally refers to a protein having an amino acid sequence comprising one or more amino acid substitutions, insertions, deletions or other modifications relative to the amino acid sequence of the reference protein, but retaining a considerable degree of sequence identity (*e.g.* at least 60%) to the amino acid sequence of the reference protein. An 'isoform' generally refers to a variant of the reference protein expressed by the same species as the species of the reference protein. A 'homologue' generally refers to a variant of the reference protein produced by a different species as compared to the species of the reference protein. Homologues include orthologues.

A 'fragment' may be of any length (by number of amino acids), although may optionally be at least 20% of the length of the reference protein (that is, the protein from which the fragment is derived) and may have a maximum length of one of 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the length of the reference protein.

Isoforms, fragments, variants or homologues may optionally be functional isoforms, fragments, variants or homologues, *e.g.* having a functional property/activity of the reference protein, as determined by analysis by a suitable assay for the functional property/activity.

In this specification, reference to 'SUN1' refers to the protein having the amino acid sequence shown in SEQ ID NO:1, and fragments, variants or homologues thereof. In some embodiments SUN1 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:1.

In this specification, reference to 'SUN2' refers to the protein having the amino acid sequence shown in SEQ ID NO:13, and fragments, variants or homologues thereof. In some embodiments SUN2 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:13.

In this specification, reference to 'SUN3' refers to the protein having the amino acid sequence shown in SEQ ID NO:23, and fragments, variants or homologues thereof. In some embodiments SUN3 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:23.

In this specification, reference to 'SUN5' refers to the protein having the amino acid sequence shown in SEQ ID NO:24, and fragments, variants or homologues thereof. In some embodiments SUN5 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:24.

In this specification, reference to 'SPAG4' refers to the protein having the amino acid sequence shown in SEQ ID NO:25, and fragments, variants or homologues thereof. In some embodiments SPAG4 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:25.

In this specification, reference to 'SUCO' refers to the protein having the amino acid sequence shown in SEQ ID NO:26, and fragments, variants or homologues thereof. In some embodiments SUCO comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:26.

Herein, a 'KASH domain-containing protein' refers to any polypeptide comprising a KASH domain. KASH (Klarsicht, ANC-1, Syne homology) domain proteins are carboxy terminal-anchored membrane proteins which are targeted to the nuclear envelope. The 50-60 amino acid KASH domain is found at the C-terminus. KASH domains are hydrophobic, and comprise a single-membrane spanning helix which spans the ONM, and a ~30 amino acid region which extends into the perinuclear space.

A KASH domain may comprise or consist of the amino acid sequence shown in SEQ ID NO:37, 38, 39, 40, 41 or 42, or an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO:37, 38, 39, 40, 41 or 42.

In some embodiments, a KASH domain-containing protein is selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 (also known as SYNE1, SYNE2, SYNE3 and SYNE4, respectively), KASH5 and LRMP. In some embodiments, a KASH domain-containing protein is Nesprin-1, Nesprin-2 or Nesprin-3.

In some embodiments, a KASH domain-containing protein is capable of forming a LINC complex. In some embodiments, a KASH domain-containing protein is capable of interacting with a SUN domain and/or a SUN domain-containing protein.

Human Nesprin-1 is the polypeptide identified by UniProtKB Q8NF91, the amino acid sequence of which is shown in SEQ ID NO:31. Human Nesprin-2 is the polypeptide identified by UniProtKB Q8WXH0, the amino acid sequence of which is shown in SEQ ID NO:32. Human Nesprin-3 is the polypeptide identified by UniProtKB Q6ZMZ3, the amino acid sequence of which is shown in SEQ ID NO:33. Human Nesprin-4 is the polypeptide identified by UniProtKB Q8N205, the amino acid sequence of which is shown in SEQ ID NO:34. Human KASH5 is the polypeptide identified by UniProtKB Q8N6L0, the amino acid sequence of which is shown in SEQ ID NO:35. Human LRMP is the polypeptide identified by UniProtKB Q12912, the amino acid sequence of which is shown in SEQ ID NO:36.

In this specification ‘Nesprin-1’, ‘Nesprin-2’, ‘Nesprin-3’, ‘Nesprin-4’, ‘KASH5’ and ‘LRMP’ respectively refer to Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP from any species and include isoforms, fragments, variants or homologues thereof.

In this specification, reference to ‘Nesprin-1’ refers to the protein having the amino acid sequence shown in SEQ ID NO:31, and fragments, variants or homologues thereof. In some embodiments Nesprin-1 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:31.

In this specification, reference to ‘Nesprin-2’ refers to the protein having the amino acid sequence shown in SEQ ID NO:32, and fragments, variants or homologues thereof. In some embodiments Nesprin-2 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:32.

In this specification, reference to ‘Nesprin-3’ refers to the protein having the amino acid sequence shown in SEQ ID NO:33, and fragments, variants or homologues thereof. In some embodiments Nesprin-3 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%,

75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:33.

In this specification, reference to 'Nesprin-4' refers to the protein having the amino acid sequence shown in SEQ ID NO:34, and fragments, variants or homologues thereof. In some embodiments Nesprin-4 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:34.

In this specification, reference to 'KASH5' refers to the protein having the amino acid sequence shown in SEQ ID NO:35, and fragments, variants or homologues thereof. In some embodiments KASH5 comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:35.

In this specification, reference to 'LRMP' refers to the protein having the amino acid sequence shown in SEQ ID NO:36, and fragments, variants or homologues thereof. In some embodiments LRMP comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:36.

As used herein, a 'LINC complex' refers to a polypeptide complex comprising a SUN domain-containing protein and a KASH domain-containing protein.

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. Non-covalent interactions include *e.g.* hydrogen bonds, ionic interaction, Van der Waals forces and hydrophobic bonds. Covalent interactions include *e.g.* disulphide bonds.

SUN domain proteins are thought to oligomerise to form trimers via interaction between their stalk regions to form coiled-coil triple helix (Zhou *et al.*, J. Biol. Chem. (2012) 287: 5317-5326). Deletion of SUN domain protein stalk regions has been shown to disrupt LINC complex formation. SUN domains assume a β sandwich structure and SUN domains of the trimer interact extensively with one another in part through protruding β sheets known as KASH lids; the KASH lid of one SUN domain partially overlaps the β sandwich of the adjacent SUN domain (Sosa *et al.*, Cell (2012) 149:1035-1047).

KASH domain proteins can also oligomerise, which may involve protein-protein interactions between the transmembrane helices. A single KASH domain interacts with two adjacent SUN domains along the groove formed between the KASH lid of one SUN domain and the upper region of the β sandwich of the adjacent SUN domain. In this way, SUN and KASH domains are thought to interact to form a 3:3 hexameric heterocomplex. The 2-3 proline residues immediately prior to the C-terminus of the KASH

domain are thought to be accommodated in a deep pocket within the surface of a SUN domain. This region of KASH is important for SUN-KASH interactions; extension of the C-terminus by only a single amino acid disrupts LINC complex formation. Conserved cysteine residues of SUN and KASH domains form disulphide bonds, further stabilising the SUN-KASH complex. The disulphide bonds may be important for force transmission through the LINC complex (Jahed *et al.*, Biophys. J. (2015) 109:501-509).

As explained hereinabove, LINC complexes are thought to form through the interaction of the SUN domains of three SUN domain-containing proteins and the KASH domains of three KASH domain-containing proteins. Interaction between the SUN and KASH domain proteins is thought to be promiscuous; SUN1 and SUN2 have been shown to interact with Nesprin-1, Nesprin-2 and Nesprin-3.

A LINC complex according to the present disclosure may comprise any SUN domain-containing protein and any KASH domain-containing protein. The SUN domain-containing proteins of the LINC complex may be identical or non-identical. The KASH domain-containing proteins of the LINC complex may be identical or non-identical.

LINC complex function is reviewed *e.g.* in Hieda, Cells (2017) 6(1):3 (incorporated by reference hereinabove), and Stroud, Biophys Rev. (2018) 10(4):1033–1051, hereby incorporated by reference its entirety.

The LINC complex performs diverse functions, including providing structural support to the nucleus, shaping and positioning the nucleus, maintaining connection between the centrosome and the nucleus and spacing of the nuclear membrane, DNA repair, cell migration and moving chromosomes within the nucleus during meiosis.

The LINC complex has a mechanosensory role to translate mechanical stimuli and changes in the extracellular matrix into signals allowing the cell to adapt to its environment by modulation of cytoskeleton organization, gene expression, nuclear organisation, and structure. The microtubule cytoskeleton and the LINC complex play important roles in nucleus protection and in transmitting forces from the cell body/cytoplasm to the nucleus (Becker R. Cells 2020. 9(6): 1395).

Integrins mediate the transduction of forces from the external microenvironment to the intracellular cytoskeleton, and nucleo-cytoskeletal molecular connections transmit the forces to chromosomal organisations in the nucleus. The nuclear lamina triggers the deformation of nuclear structures, and initiates changes in gene regulation.

The nuclear envelope is a key structure in such processes. On the nucleoplasmic side of the INM, the nuclear lamina (composed of A-type and B-type lamins) forms a lattice structure which contributes to the mechanical stress resistance of the nucleus, and which is essential to the structural integrity of the nuclear envelope. Nuclear lamins are involved in processes critical to cell function and viability, including

maintenance of nuclear integrity, regulation of cell cycle, mechanotransduction, cell signalling and DNA repair.

Deviations from normal expression and/or function of nuclear envelope proteins, and deviations from normal expression and/or function of factors directly or indirectly associated with the nuclear envelope, are implicated in a variety of diseases including muscular dystrophies, cardiomyopathies, lipodystrophy, progeria, cancer, and neurological diseases.

**Microtubules in cardiomyocytes**

Terminally differentiated cells, such as cardiomyocytes and skeletal muscle cells have highly organised microtubule networks (MTNs).

In view of the physiological demand of rhythmic pumping, cardiomyocytes have a natural mechanical stretch sensor ability. Microtubules, together with myofilaments and intermediate filaments, form the composite cytoskeleton that controls cell mechanics (Warner E. et al 2022). The cross-linking between microtubule and other cytoskeletal components (such as desmin and actin) may significantly amplify the mechanical force transduced within the cell (Warner E. et al 2022). The microtubule cytoskeleton has high inherent stiffness, can bear compressional stress and can exhibit resistance to motion.

Cardiomyocytes are equipped with machinery for the rapid generation of force and movement, with microtubule buckling estimated to provide about 20% of the cardiomyocyte contractile force. Factors influencing the mechanical force include microtubule growth rate, microtubule length, microtubule post-translational modifications, and microtubule network (MTN) density (Warner E. et al 2022). Microtubules link the extracellular space and the contractile apparatus to the nucleus via the LINC complex, which allows transmission of mechanical force to regulate gene expression (Becker R. Cells 2020. 9(6): 1395).

Microtubules are made of heterodimers of α- and β-tubulin. These tubulin subunits form a higher order assembly containing protofilaments that arrange into a hollow polymer. Microtubules are nucleated from microtubule organizing centres (MTOCs) that contain γ-tubulin, which serves as a structural template for high efficiency nucleation (Uchida K. et al., 2022).

In mitotic cells, centrosomal MTOCs continually play a role in nucleating microtubules for the cell's entire lifetime. Yet when cardiomyocytes transform to postmitotic cells shortly after birth, they undergo a concomitant loss of centrosome integrity. Adult cardiomyocytes rely solely on non-centrosomal MTOCs decorating the nuclear envelope and associated Golgi for nucleation (Figure 5A, and Uchida K. et al., 2022). Thus, the entire cardiomyocyte nuclear envelope can be thought of as the cellular MTOC.

Dysfunction of microtubules associated with various heart disorders, where variations have been observed in MTN density (Cheng G. et al., Am J Physiol Heart Circ Physiol. 2008;294:H2231–H2241), in microtubule post-translational modification (Schuldt M. et al., Circ Heart Fail. 2021;14:e007022) and association with microtubule-associated proteins (MAPs) (Li L. et al., Front Physiol. 2020;11:1044. Doi: 10.3389/fphys.2020.01044).

The most well-studied post-translational modifications in cardiomyocytes are detyrosination and acetylation (Warner E. et al. 2022). Detyrosination enhances the biophysical rigidity of the microtubules. Detyrosination by TCP (tubulin carboxypeptidase) occurs preferentially on microtubule polymers rather than tubulin dimers. Upon detyrosination, the C-terminal tyrosine of α-tubulin is removed.

Recent studies have demonstrated that enhanced microtubule detyrosination in the myocyte cells, through crosslinking of detyrosinated microtubules with desmin at the Z discs, is linked with increased mechanical resistance and compromised cell contraction at various pathophysiological conditions. Detyrosinated tubulin is significantly increased in patients with clinically diagnosed hypertrophic and dilated cardiomyopathies (HCM and DCM, respectively), along with a modest increase in total tubulin content (Robison P., *et al*. Science. 352, aaf0659 (2016)).

In the cardiomyocyte, microtubules enriched in acetylated α-tubulin increase cytoskeletal stiffness and viscoelastic resistance independently of alterations in MTN density or in levels of detyrosinated microtubules (Coleman A. et al. J Gen Physiol. 2021; 153(7): e202012743). Macquart C. et al. Human Molecular Genetics, 2019, Vol. 28, No. 24, 4043-4052 describe that the levels of acetylation of α-tubulin are decreased in *LMNA* cardiomyopathy. The activity of HDAC6, the histone deacetylase modulating tubulin deacetylation, is increased in cardiac stress, and HDAC inhibitors have been shown to ameliorate pressure overload hypertrophy and atrial fibrillation (Warner E. et al. 2022).

Microtubule-targeting therapies are currently available in the clinic for the treatment of cancer and neurodegenerative disorders; however, therapeutic interventions targeting microtubules for the treatment of cardiovascular disease have been restricted to colchicine treatment, and this is predominantly related to colchicine's anti-inflammatory effects at lower doses, such as in the treatment of gout (Warner E. et al. 2022).

**LINC complex inhibition**

The present disclosure is concerned with LINC complex inhibition. As used herein 'LINC complex inhibition' encompass inhibition of formation of a LINC complex (*i.e.* inhibition of LINC complex assembly), disruption/degradation of a LINC complex, and inhibition of LINC complex activity/function.

In some embodiments, formation of a LINC complex may be inhibited by inhibiting the 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. For conciseness, in the present disclosure 'a constituent protein of a LINC complex' may be referred to simply as 'a LINC complex protein'.

In some embodiments, inhibiting formation of a LINC complex comprises one or more of: inhibiting the gene or protein expression of encoding a LINC complex protein; modifying a gene encoding a LINC complex protein to reduce/prevent its expression; reducing the level of RNA encoding a LINC complex protein; inhibiting transcription of nucleic acid encoding a LINC complex protein; increasing degradation of

RNA encoding a LINC complex protein; reducing the level of a LINC complex protein; disrupting normal post-transcriptional processing (*e.g.* splicing, translation, post-translational processing) of RNA encoding a LINC complex protein; and increasing degradation of a LINC complex protein.

Gene expression can be determined by means well known to the skilled person. The level of RNA encoding constituent proteins of LINC complexes can be determined *e.g.* by techniques such as RT-qPCR, northern blot, *etc.* A reduction in the level of RNA encoding a constituent protein of a LINC complex may *e.g.* be the result of reduced transcription of nucleic acid encoding the LINC complex protein, or increased degradation of RNA encoding the LINC complex protein.

Reduced transcription of nucleic acid encoding a LINC complex protein may be a consequence of inhibition of assembly and/or activity of factors required for transcription of the DNA encoding the LINC complex protein. Increased degradation of RNA encoding a LINC complex protein may be a consequence of increased enzymatic degradation of RNA encoding the LINC complex protein, *e.g.* as a consequence of RNA interference (RNAi), and/or reduced stability of RNA encoding the LINC complex protein.

Protein expression can be determined by means well known to the skilled person. The levels of constituent proteins of LINC complexes can be determined *e.g.* by antibody-based methods including western blot, immunohisto/cytochemistry, flow cytometry, ELISA, or by reporter-based methods.

Protein degradation can be evaluated *e.g.* by detection of, or analysis of the level/proportion of, ubiquitinated protein, association with ubiquitin ligase and/or proteosomal localisation.

A reduction in the level of a LINC complex protein may *e.g.* be the result of a reduced level of RNA encoding the LINC complex protein, reduced post-transcriptional processing of RNA encoding the LINC complex protein, or increased degradation of the LINC complex protein.

Disruption to normal post-transcriptional processing of a LINC complex protein may *e.g.* be reduced/altered splicing of pre-mRNA to mature mRNA encoding the LINC complex protein, reduced translation of mRNA encoding the LINC complex protein, or reduced/altered post-translational processing of the LINC complex protein.

Reduced/altered splicing of pre-mRNA to mature mRNA encoding the LINC complex protein may be a consequence of inhibition of assembly and/or activity of factors required for normal splicing. Reduced translation of mRNA encoding the LINC complex protein may be a consequence of inhibition of assembly and/or activity of factors required for translation. Reduced/altered post-translational processing (*e.g.* enzymatic processing, folding) may be a consequence of inhibition of assembly and/or activity of factors required for normal post-translational processing of the LINC complex protein. Increased degradation of the LINC complex protein may be a consequence of increased enzymatic (*e.g.* protease-mediated) degradation of the protein, which may *e.g.* be associated with misfolding.

In some embodiments, formation of a LINC complex may be inhibited by inhibiting trafficking of, and/or disrupting normal subcellular localisation of, constituent proteins of a LINC complex (*e.g.* SUN domain-containing proteins and/or KASH domain-containing proteins). In some embodiments, inhibiting formation of a LINC complex comprises one or more of: reducing the level/proportion of a LINC complex protein localised to the nuclear envelope; reducing the level/proportion of SUN domain-containing protein associated with inner nuclear membrane; reducing the level/proportion of KASH domain-containing protein associated with outer nuclear membrane; increasing retention of a LINC complex protein in the endoplasmic reticulum; and increasing the level/proportion of a LINC complex protein localised to the endoplasmic reticulum.

The subcellular localisation of constituent proteins of LINC complexes within cells can be analysed using techniques known to the person skilled in the art. Such techniques include *e.g.* analysis by immunocytochemistry and reporter-based methods. For example, Boni *et al.*, J. Cell Biology (2015) 209(5):705-720 and Smoyer *et al.*, J. Cell Biology (2016) 215(4):575-590 describe a reporter system permitting imaging of proteins in the ER, INM and ONM. Such methods can be employed to analyse the levels/proportions of constituent proteins of LINC complexes in the nuclear envelope, inner nuclear membrane and an outer nuclear membrane.

In some embodiments, formation of a LINC complex may be inhibited by inhibiting interaction between constituent proteins of LINC complexes (*e.g.* SUN domain-containing proteins and KASH domain-containing proteins). In some embodiments, formation of a LINC complex may be inhibited by inhibiting interaction between constituent proteins of a LINC complex and interaction partners for constituent proteins of a LINC complex.

In some embodiments, inhibiting formation of a LINC complex comprises one or more of: inhibiting interaction between a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein; inhibiting interaction between a KASH domain-containing protein and an interaction partner for a KASH domain-containing protein; inhibiting interaction between a SUN domain-containing protein and a KASH domain-containing protein; inhibiting interaction between a SUN domain-containing protein and a lamin; inhibiting interaction between a SUN domain-containing protein and a chromatin-binding protein; inhibiting interaction between a KASH domain-containing protein and a SUN domain-containing protein; and inhibiting interaction between a KASH domain-containing protein and a cytoskeletal component (*e.g.* a microfilament or a constituent thereof (*e.g.* actin), a microtubule or a constituent thereof (*e.g.* tubulin) or an intermediate filament or a constituent thereof).

In some embodiments, LINC complex inhibition comprises disruption/degradation of a LINC complex.

In some embodiments, LINC complex disruption/degradation comprises one or more of: inhibiting interaction between a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein; inhibiting interaction between a KASH domain-containing protein and an interaction partner for a KASH domain-containing protein; inhibiting interaction between a SUN domain-containing protein and a KASH domain-containing protein; inhibiting interaction between a SUN domain-containing

protein and a lamin; inhibiting interaction between a SUN domain-containing protein and a chromatin-binding protein; inhibiting interaction between a KASH domain-containing protein and a SUN domain-containing protein; and inhibiting interaction between a KASH domain-containing protein and a cytoskeletal component (*e.g.* a microfilament or a constituent thereof (*e.g.* actin), a microtubule or a constituent thereof (*e.g.* tubulin) or an intermediate filament or a constituent thereof); increasing LINC complex disassembly; increasing LINC complex degradation; displacing a LINC complex protein from a LINC complex; and reducing the level of a LINC complex.

Herein, an interaction partner for a SUN domain-containing protein may be any molecule (*e.g.* protein/nucleic acid) with which SUN domain-containing protein interacts. An interaction partner for a SUN domain-containing protein may be a protein capable of forming a complex with a SUN domain-containing protein through protein-protein interaction. In some embodiments an interaction partner for a SUN domain-containing protein may be a KASH domain-containing protein (*e.g.* Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4 or KASH5), a SUN domain-containing protein (*e.g.* SUN1, SUN2, SUN3, SUN5, SPAG4 or SUCO), a nucleoplasmic protein, a lamin (*e.g.* lamin A, lamin C, lamin B1 or lamin B2) or a chromatin-binding protein.

Herein, an interaction partner for a KASH domain-containing protein may be any molecule (*e.g.* protein/nucleic acid) with which KASH domain-containing protein interacts. An interaction partner for a KASH domain-containing protein may be a protein capable of forming a complex with a KASH domain-containing protein through protein-protein interaction. In some embodiments an interaction partner for a KASH domain-containing protein may be a SUN domain-containing protein (*e.g.* SUN1, SUN2, SUN3, SUN5, SPAG4 or SUCO), a KASH domain-containing protein (*e.g.* Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 or LRMP), a cytoplasmic protein, a cytoskeletal protein, a microfilament, actin, a microtubule, tubulin, a microtubule motor, or an intermediate filament protein.

Interaction between constituent proteins of LINC complexes and interaction partners for such proteins can be analysed using techniques well known to the skilled person such as co-immunoprecipitation, and resonance energy transfer (RET) assays using appropriately labelled species. Inhibition of interaction can be determined in such assays by detection of a reduction in the level of interaction as compared to a control, uninhibited condition.

LINC complexes and constituent proteins thereof may be detected *e.g.* using methods well known to the skilled person such as antibody-based methods including western blot, immunohisto/cytochemistry, flow cytometry, ELISA, or by reporter-based methods.

LINC complex inhibition may be characterised by a reduced level of a function of a LINC complex. In some embodiments, LINC complex inhibition may be determined by detection of a reduced level of a correlate of LINC complex function.

In particular embodiments contemplated herein, LINC complex inhibition is achieved by one or more of: modifying a gene encoding a SUN domain-containing protein to reduce/prevent its expression; modifying

a gene encoding a KASH domain-containing protein to reduce/prevent its expression; inhibition of expression of a SUN domain-containing protein by RNAi; inhibition of expression of a KASH domain-containing protein by RNAi; or inhibiting interaction between a SUN domain-containing protein and a KASH domain-containing protein.

In some embodiments modifying a gene encoding a KASH domain-containing protein or a SUN domain-containing protein to reduce/prevent its expression is achieved using a site-specific nuclease (SSN) system (*e.g.* a CRISPR-based system) targeting the relevant gene. In some embodiments inhibition of expression of a KASH domain-containing protein or a SUN domain-containing protein by RNAi is achieved using siRNA, miRNA or shRNA targeting the relevant protein.

In some embodiments inhibiting interaction between a SUN domain-containing protein and a KASH domain-containing protein is achieved using a dominant-negative SUN domain-containing protein, a dominant-negative KASH domain-containing protein, a small molecule inhibitor of interaction between a SUN domain-containing protein and a KASH domain-containing protein, a peptidomimetic of a KASH domain or a peptidomimetic of a SUN domain. It will be appreciated that inhibition of interaction is between an endogenous SUN domain-containing protein and an endogenous KASH domain-containing protein.

**LINC complex inhibitors**

Aspects of the present disclosure relate to LINC complex inhibition using a LINC complex inhibitor. A 'LINC complex inhibitor' refers to any agent capable of achieving LINC complex inhibition. LINC complex inhibitors include agents capable of inhibiting formation of a LINC complex (*i.e.* inhibiting LINC complex assembly), disrupting/degrading a LINC complex, or inhibiting LINC complex function. Such agents may be effectors of (*i.e.* may directly or indirectly cause) LINC complex inhibition as described hereinabove. LINC complex inhibitors may also be referred to herein as LINC complex antagonists.

LINC complex inhibitors are described *e.g.* in WO 2019/143300 A1, WO 2021/010898 A1 and WO 2023/101607 A2. In some embodiments, a LINC complex inhibitor according to the present disclosure is a LINC complex inhibitor described in WO 2019/143300 A1, WO 2021/010898 A1 and WO 2023/101607 A2, which are hereby incorporated by reference in its entirety.

In some embodiments, a LINC complex inhibitor may: inhibit formation of a LINC complex; disrupt/degrade a LINC complex; inhibit LINC complex activity; inhibit the gene and/or protein expression of a LINC complex protein; modify a gene encoding a LINC complex protein to reduce/prevent its expression; reduce the level of RNA encoding a LINC complex protein; inhibit transcription of nucleic acid encoding a LINC complex protein; increase degradation of RNA encoding a LINC complex protein; reduce the level of a LINC complex protein; disrupt normal post-transcriptional processing (*e.g.* splicing, translation, post-translational processing) of RNA encoding a LINC complex protein; increase degradation of a LINC complex protein; inhibit trafficking of, and/or disrupt normal subcellular localisation of, a LINC complex protein; reduce the level/proportion of a LINC complex protein localised to the nuclear envelope; reduce the level/proportion of SUN domain-containing protein associated with inner nuclear membrane;

reduce the level/proportion of KASH domain-containing protein associated with outer nuclear membrane; increase retention of a LINC complex protein in the endoplasmic reticulum; increase the level/proportion of a LINC complex protein localised to the endoplasmic reticulum; inhibit interaction between constituent proteins of a LINC complex; inhibit interaction between a LINC complex protein and an interaction partner for a LINC complex protein; inhibit interaction between a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein; inhibit interaction between a KASH domain-containing protein and an interaction partner for a KASH domain-containing protein; inhibit interaction between a SUN domain-containing protein and a KASH domain-containing protein; inhibit interaction between a SUN domain-containing protein and a lamin; inhibit interaction between a SUN domain-containing protein and a chromatin-binding protein; inhibit interaction between a KASH domain-containing protein and a SUN domain-containing protein; inhibit interaction between a KASH domain-containing protein and a cytoskeletal component (*e.g.* a microfilament or a constituent thereof (*e.g.* actin), a microtubule or a constituent thereof (*e.g.* tubulin) or an intermediate filament or a constituent thereof); increase disassembly of a LINC complex; increase degradation of a LINC complex; displace a LINC complex protein from a LINC complex; and/or reduce the level of a LINC complex.

It will be appreciated that a given LINC complex inhibitor may display more than one of the properties recited in the preceding paragraph. A given agent may be evaluated for the properties recited in the preceding paragraph using suitable assays. The assays may be *e.g. in vitro* assays, optionally cell-based assays or cell-free assays. Where assays are cell-based assays, they may comprise treating cells with the test agent in order to determine whether the agent displays one or more of the recited properties. Assays may employ endogenously- or recombinantly-expressed proteins, and may use species labelled with detectable entities in order to facilitate their detection.

Agents capable of reducing gene expression of a LINC complex protein (*e.g.* reducing the level of RNA encoding a LINC complex protein, reducing transcription of nucleic acid encoding a LINC complex protein and/or increasing degradation of RNA encoding a LINC complex protein) may be identified using assays comprising detecting the level of RNA encoding the relevant protein, *e.g.* by RT-qPCR. Such assays may comprise treating cells/tissue with the agent, and subsequently comparing the level of RNA encoding the relevant protein in such cells/tissue to the level of RNA encoding the relevant protein in cells/tissue of an appropriate control condition (*e.g.* untreated/vehicle-treated cells/tissue). Assays for detecting reduced/altered splicing of pre-mRNA of a given protein may comprise detecting and/or quantifying one or more isoforms of the relevant protein, or RNA encoding one or more of said isoforms.

Agents capable of reducing protein expression of a LINC complex protein (*e.g.* reducing the level of a LINC complex protein, increasing degradation of a LINC complex protein) may be identified using assays comprising detecting the level of the relevant protein, *e.g.* using antibody/reporter-based methods (western blot, ELISA, immunohisto/cytochemistry, *etc.*). Such assays may comprise treating cells/tissue with the agent, and subsequently comparing the level of the relevant protein in such cells/tissue to the level of the relevant protein in cells/tissue of an appropriate control condition (*e.g.* untreated/vehicle-treated cells/tissue). Assays of protein degradation may comprise evaluating *e.g.* ubiquitination or

proteosomal localisation of the relevant protein, and/or the proportion of the relevant protein that is ubiquitinated or localised to the proteasome.

Agents capable of inhibiting trafficking and/or disrupting normal subcellular localisation of a LINC complex protein may be identified using assays comprising detecting the presence of, or determining the proportion of, the relevant protein in a given subcellular location, *e.g.* using antibody/reporter-based methods (western blot, ELISA, immunohisto/cytochemistry, *etc.*). Subcellular localisation may be analysed *e.g.* by immunocytochemistry, or western blot of extracts prepared from different cellular fractions, and may employ organelle markers and/or labelled proteins of known subcellular localisation. Assays may comprise treating cells/tissue with the agent, subsequently comparing the subcellular localisation of the relevant protein in such cells to the subcellular localisation of the relevant protein in cells/tissue of an appropriate control condition (*e.g.* untreated/vehicle-treated cells/tissue).

Agents capable of inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein may be identified using assays comprising detecting the level of interaction between a LINC complex protein and an interaction partner for a LINC complex protein, *e.g.* using antibody/reporter-based methods. The level of interaction between a LINC complex protein and an interaction partner for a LINC complex protein can be analysed *e.g.* using resonance energy transfer techniques (*e.g.* FRET, BRET), co-immunoprecipitation or methods analysing a correlate of interaction (*e.g.* a function of a LINC complex). Assays may comprise treating cells/tissue with the agent, and subsequently comparing the level of interaction in such cells/tissue to the level of interaction in cells/tissue of an appropriate control condition (*e.g.* untreated/vehicle-treated cells/tissue). Interaction between a LINC complex protein and an interaction partner for a LINC complex protein can also be analysed *e.g.* using techniques such as ELISA, Surface Plasmon Resonance (SPR; see *e.g.* Hearty *et al.*, Methods Mol Biol (2012) 907:411-442), Bio-Layer Interferometry (see *e.g.* Lad *et al.*, (2015) J Biomol Screen 20(4): 498-507), flow cytometry, or by a radiolabeled antigen-binding assay (RIA) enzyme-linked immunosorbent assay. Assays may comprise comparing the level of interaction in the presence of the agent to the level of interaction in an appropriate control condition (*e.g.* the absence of the agent).

Agents capable of inhibiting a function of a LINC complex may be identified using assays comprising detecting the level of a correlate of LINC complex function.

A LINC complex inhibitor according to the present disclosure may be any agent/plurality of agents achieving the desired inhibitory activity. In some embodiments, a LINC complex inhibitor may be or comprise a peptide/polypeptide, small molecule, nucleic acid or biomolecule.

In some embodiments, a LINC complex inhibitor is capable of binding to: a LINC complex, a LINC complex protein, or an interaction partner for a LINC complex protein.

LINC complex inhibitors may display specific binding to the relevant factor/complex (*i.e.* a LINC complex protein, or an interaction partner for a LINC complex protein). As used herein, 'specific binding' refers to binding which is selective, and which can be discriminated from non-specific binding to non-target

molecules. LINC complex inhibitors that specifically bind to a LINC complex protein or an interaction partner for a LINC complex protein preferably binds to the relevant factor with greater affinity, and/or with greater duration than other, non-target molecules; such LINC complex inhibitors may be described as being 'specific for' the relevant factor.

In some embodiments, a LINC complex inhibitor is capable of inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein. In some embodiments, a LINC complex inhibitor is capable of inhibiting LINC complex function. In some embodiments a LINC complex inhibitor behaves as a competitive inhibitor of interaction between a LINC complex protein and an interaction partner for a LINC complex protein. The LINC complex inhibitor may occupy, or otherwise reduce access to, a region of a LINC complex protein required for binding to an interaction partner for a LINC complex protein, or may occupy, or otherwise reduce access to, a region of an interaction partner for a LINC complex protein required for binding to a LINC complex protein.

In some embodiments a LINC complex inhibitor mimics an interaction partner for a LINC complex protein.

In some embodiments, a LINC complex inhibitor inhibits interaction between a SUN domain and a KASH domain.

In some embodiments, a LINC complex inhibitor inhibits association between the C-terminal region of a KASH domain and the deep pocket on the surface of a SUN domain. In some embodiments a LINC complex inhibitor binds to a SUN domain and inhibits access of a KASH domain to the deep pocket of the SUN domain. In some embodiments a LINC complex inhibitor binds to a KASH domain and inhibits access of the KASH domain to a deep pocket on the surface of a SUN domain.

In some embodiments, a LINC complex inhibitor inhibits the formation of, or disrupts, disulphide bonds between a SUN domain and a KASH domain.

In some embodiments, a LINC complex inhibitor targets the C-terminal, proline-rich region of a KASH domain-containing protein.

In some embodiments, a LINC complex inhibitor inhibits oligomerisation of SUN domain-containing proteins. In some embodiments, a LINC complex inhibitor targets the stalk region of a SUN domain-containing protein.

In some embodiments, a LINC complex inhibitor inhibits protein-protein interaction between a SUN domain and a KASH domain. In some embodiments, a LINC complex inhibitor inhibits protein-protein interaction between: SUN1 and Nesprin-1, SUN2 and Nesprin-1, SUN1 and Nesprin-2, SUN1 and Nesprin-3, SUN2 and Nesprin-2 or SUN2 and Nesprin-3. In some embodiments a LINC complex inhibitor inhibits protein-protein interaction between SUN1 and Nesprin-1.

The ability of a candidate LINC complex inhibitor to inhibit interaction between a LINC complex protein and an interaction partner for a LINC complex protein can be evaluated *e.g.* by analysis of interaction in the presence of, or following incubation of one or both of the interaction partners with, the candidate LINC complex inhibitor. An example of a suitable assay to determine whether a given binding agent is capable of inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein is a competition ELISA.

In some embodiments a molecule which binds to a LINC complex protein or an interaction partner for a LINC complex protein inhibits the ability of a LINC complex protein to bind to an interaction partner for a LINC complex protein.

In some embodiments, a LINC complex inhibitor is capable of binding to a LINC complex protein or an interaction partner for a LINC complex protein, and inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein.

LINC complex inhibitors which are capable of binding to a LINC complex protein or an interaction partner for a LINC complex protein, and inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein can be identified using any suitable assay for detecting binding of a molecule to the relevant factor (*i.e.* the LINC complex protein, or the interaction partner for the LINC complex protein) and inhibition of interaction between a LINC complex protein and an interaction partner for a LINC complex protein. Such assays may comprise *e.g.* detecting the formation of a complex between the relevant factor and the candidate inhibitor molecule, and/or detecting the formation of a complex between the LINC complex protein and the interaction partner for the LINC complex protein.

In some embodiments, LINC complex inhibitors which are capable of binding to a LINC complex protein or an interaction partner for a LINC complex protein, and inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein may *e.g.* be peptide/polypeptides.

A LINC complex inhibitor may *e.g.* be based on an interaction partner for the relevant factor (*i.e.* the LINC complex protein, or the interaction partner for a LINC complex protein) to which the inhibitor binds.

As used herein, a peptide/polypeptide/amino acid sequence which is 'based on' a reference protein comprises or consists of an amino acid sequence having high sequence identity (*e.g.* at least 80%, 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to all or part of the amino acid of the reference protein.

For example, a LINC complex inhibitor which binds to a LINC complex protein may comprise/consist of a peptide/polypeptide fragment of an interaction partner for a LINC complex protein. Similarly, a LINC complex inhibitor which binds to an interaction partner for a LINC complex protein may comprise/consist of a peptide/polypeptide fragment of a LINC complex protein.

Such inhibitors preferably possess the ability to bind to the relevant factor, but lacks or displays a reduced level of one or more other properties of the protein on which they are based. For example, a LINC complex inhibitor may comprise the amino acid sequence(s) required for binding to the relevant factor, and may lack the amino acid sequence(s) required for one or more other properties of the protein on which it is based.

Such peptide/polypeptide LINC complex inhibitors may be referred to as 'decoy', 'dominant-negative' or 'mimetic' versions of the proteins on which they are based, and preferably display competitive inhibition of interaction between a LINC complex protein and an interaction partner for a LINC complex protein. In this way, such peptide/polypeptide LINC complex inhibitors inhibit the formation of LINC complexes and/or disrupt existing LINC complexes via displacement of endogenous interaction partners, forming non-functional complexes/complexes having a reduced level of function.

Crisp *et al.* J Cell Biol. (2006) 172(1): 41–53 describes a dominant-negative version of mouse Sun1 protein, and WO 2019/143300 A1 describes a humanised version thereof. Further dominant-negative versions of SUN1 are described in WO 2023/101607 A2. A dominant-negative SUN2 is described *e.g.* in Stewart-Hutchinson *et al.* Exp Cell Res. (2008) 314(8):1892-905.

Dominant-negative versions of KASH1 are described *e.g.* in Stewart-Hutchinson *et al.* Exp Cell Res. (2008) 314(8):1892-905, Grady *et al.*, Proc. Natl. Acad. Sci. U.S.A. (2005) 102: 4359–4364 and Libotte *et al.*, MBoC (2005) 16: 3411–3424. Dominant-negative versions of KASH2 are described *e.g.* in Stewart-Hutchinson *et al.* Exp Cell Res. (2008) 314(8):1892-905, Libotte *et al.*, MBoC (2005) 16: 3411–3424, Zhen *et al.*, J. Cell. Sci. (2002) 115: 3207–3222 and Kim *et al.* Sci Transl Med (2018) 10. A dominant-negative version of KASH3 is described *e.g.* in Stewart-Hutchinson *et al.* Exp Cell Res. (2008) 314(8):1892-905. A dominant-negative version of KASH4 is described *e.g.* in Roux *et al.* Proc. Natl. Acad. Sci. U.S.A. (2009) 106: 2194–2199. A dominant-negative version of KASH5 is described *e.g.* in Horn *et al.* J Cell Biol (2013) 202: 1023–1039.

In some embodiments, a peptide/polypeptide LINC complex inhibitor is based on a SUN domain-containing protein (*e.g.* a SUN domain containing-protein as described herein). In some embodiments the peptide/polypeptide LINC complex inhibitor is a decoy/dominant-negative version of a SUN domain-containing protein.

LINC complex inhibitors based on a SUN domain-containing protein may display binding to a KASH domain-containing protein (*e.g.* a KASH domain-containing protein as described herein), but lacks or displays a reduced level of one or more other properties of the SUN domain-containing protein on which it is based (*e.g.* binding to a SUN domain-containing protein, nucleoplasmic protein, lamin, and/or chromatin-binding protein).

LINC complex inhibitors based on a SUN domain-containing protein may display binding to a SUN domain-containing protein (*e.g.* a SUN domain-containing protein as described herein), but lacks or displays a reduced level of one or more other properties of the SUN domain-containing protein on which it

is based (*e.g.* binding to a KASH domain-containing protein, nucleoplasmic protein, lamin, and/or chromatin-binding protein).

LINC complex inhibitors based on a SUN domain-containing protein preferably comprise a SUN domain. In some embodiments a LINC complex consists of, or consists essentially of, a SUN domain. The peptide/polypeptide may lack amino acid sequence(s) of a SUN domain-containing protein constituting protein domains other than a SUN domain. The peptide/polypeptide preferably lacks properties of an endogenous SUN domain-containing protein other than properties mediated by the SUN domain. The peptide/polypeptide may behave as a dominant-negative peptide/polypeptide, capable of inhibiting interaction between an endogenous SUN domain-containing protein and an endogenous interaction partner for a SUN domain-containing protein.

In some embodiments, a peptide/polypeptide LINC complex inhibitor is based on a KASH domain-containing protein (*e.g.* a KASH domain containing-protein as described herein). In some embodiments the peptide/polypeptide LINC complex inhibitor is a decoy/dominant-negative version of a KASH domain-containing protein, for example of KASH1, KASH2, KASH3, KASH4 or KASH5.

LINC complex inhibitors based on a KASH domain-containing protein may display binding to a SUN domain-containing protein (*e.g.* a SUN domain-containing protein as described herein), but lacks or displays a reduced level of one or more other properties of the KASH domain-containing protein on which it is based (*e.g.* binding to a KASH domain-containing protein, cytoplasmic protein, cytoskeletal protein, microfilament, actin, microtubule, tubulin, microtubule motor, or intermediate filament protein).

LINC complex inhibitors based on a KASH domain-containing protein may display binding to a KASH domain-containing protein (*e.g.* a KASH domain-containing protein as described herein), but lacks or displays a reduced level of one or more other properties of the KASH domain-containing protein on which it is based (*e.g.* binding to a SUN domain-containing protein, cytoplasmic protein, cytoskeletal protein, microfilament, actin, microtubule, tubulin, microtubule motor, or intermediate filament protein).

LINC complex inhibitors based on a KASH domain-containing protein preferably comprise a KASH domain. In some embodiments a LINC complex consists of, or consists essentially of, a KASH domain. The peptide/polypeptide may lack amino acid sequence(s) of a KASH domain-containing protein constituting protein domains other than a KASH domain. The peptide/polypeptide preferably lacks properties of an endogenous KASH domain-containing protein other than properties mediated by the KASH domain. The peptide/polypeptide may behave as a dominant-negative peptide/polypeptide, capable of inhibiting interaction between an endogenous KASH domain-containing protein and an endogenous interaction partner for a KASH domain-containing protein.

In some embodiments, peptide/polypeptide LINC complex inhibitors capable of binding to a LINC complex/LINC complex protein/interaction partner for a LINC complex protein and inhibiting interaction between a LINC complex protein and an interaction partner for a LINC complex protein and/or LINC complex function include *e.g.* peptide aptamers, thioredoxins, monobodies, anticalin, Kunitz domains,

avimers, knottins, fynomers, atrimers, DARPins, affibodies, nanobodies (*i.e.* single-domain antibodies (sdAbs)) affilins, armadillo repeat proteins (ArmRPs), OBodies and fibronectin – reviewed *e.g.* in Reverdatto *et al.*, Curr Top Med Chem. 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also *e.g.* Boersma *et al.*, J Biol Chem (2011) 286:41273-85 and Emanuel *et al.*, Mabs (2011) 3:38-48). Further peptide/polypeptide LINC complex inhibitors contemplated in connection with the present disclosure include antibodies (immunoglobulins) such as monoclonal antibodies, polyclonal antibodies, monospecific antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and fragments and derivatives thereof (*e.g.* Fv, scFv, Fab, scFab, $F(ab')_2$, $Fab_2$, diabodies, triabodies, scFv-Fc, minibodies, single domain antibodies (*e.g.* VhH), *etc.*).

Such peptide/polypeptide LINC complex inhibitors can be identified by screening of libraries of the relevant peptides/polypeptides for LINC complex inhibition.

LINC complex inhibiting polypeptides are described in further detail hereinbelow.

In some embodiments a LINC complex inhibitor is a small molecule inhibitor of a LINC complex. As used herein, a 'small molecule' refers to a low molecular weight (< 1000 daltons, typically between ~300-700 daltons) organic compound.

A small molecule LINC complex inhibitor may bind to a LINC complex, a LINC complex protein, or an interaction partner for a LINC complex protein. A small molecule inhibitor LINC complex inhibitor may inhibit interaction between a LINC complex protein and an interaction partner for a LINC complex protein. A small molecule inhibitor LINC complex inhibitor may bind to a LINC complex and inhibit LINC complex function.

Suitable small molecule LINC complex inhibitors may be identified *e.g.* by screening of small molecule libraries, *e.g.* as described in Example 7 of WO 2021/010898 A1.

In some embodiments a LINC complex inhibitor is, or comprises, a nucleic acid.

The nucleic acid may bind to a LINC complex, a LINC complex protein, or an interaction partner for a LINC complex protein. The nucleic acid may inhibit interaction between a LINC complex protein and an interaction partner for a LINC complex protein. The nucleic acid may bind to a LINC complex and inhibit LINC complex function.

Nucleic acid aptamers are reviewed *e.g.* in Zhou and Rossi Nat Rev Drug Discov. 2017 16(3):181-202. They may be identified and/or produced by the method of Systematic Evolution of Ligands by EXponential enrichment (SELEX), or by developing SOMAmers (slow off-rate modified aptamers) (Gold L *et al.* (2010) PLoS ONE 5(12):e15004). Aptamers and SELEX are described in Tuerk and Gold, Science (1990) 249(4968):505-10, and in WO 91/19813.

Nucleic acid aptamers may comprise DNA and/or RNA, and may be single-stranded or double-stranded. They may comprise chemically modified nucleic acids, for example in which the sugar and/or phosphate and/or base is chemically modified. Such modifications may improve the stability of the aptamer or make the aptamer more resistant to degradation and may include modification at the 2′ position of ribose.

Nucleic acid aptamers may be chemically synthesised, *e.g.* on a solid support. Solid phase synthesis may use phosphoramidite chemistry. Briefly, a solid supported nucleotide is detritylated, then coupled with a suitably activated nucleoside phosphoramidite to form a phosphite triester linkage. Capping may then occur, followed by oxidation of the phosphite triester with an oxidant, typically iodine. The cycle may then be repeated to assemble the aptamer (*e.g.*, see Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. Nucleic Acids Res. 1984, 12, 4539; and Beaucage, S. L.; Lyer, R. P. (1992). Tetrahedron 48 (12): 2223).

In some embodiments, a LINC complex inhibitor capable of reducing expression (*e.g.* gene and/or protein expression) of a LINC complex protein. In some embodiments the LINC complex inhibitor reduces or prevents the expression of an endogenous LINC complex protein.

Inhibition of expression of a LINC complex protein will result in a decrease in the quantity of a LINC complex protein and/or the quantity of LINC complexes comprising the constituent protein in a cell/tissue/organ/organ system/subject. For example, in a given cell the inhibition of expression of a LINC complex protein will result in a decrease in the level of a LINC complex protein and/or of LINC complexes comprising the constituent protein relative to an untreated cell.

Inhibition may be partial. Preferred degrees of inhibition are at least 50%, more preferably one of at least 60%, 70%, 80%, 85% or 90%. A level of inhibition between 90% and 100% is considered a ‘silencing’ of expression or function. Gene and protein expression may be determined as described herein or by methods in the art that are well known to a skilled person.

In some embodiments a LINC complex inhibitor reduces or prevents the expression of SUN1, SUN2, SUN3, SUN5, SPAG4 or SUCO. In some embodiments a LINC complex inhibitor reduces or prevents the expression of SUN1 or SUN2.

In some embodiments a LINC complex inhibitor reduces or prevents the expression of Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 or LRMP.

In some embodiments, a LINC complex inhibitor may target a particular domain/region of a LINC complex protein. In some embodiments the LINC complex inhibitor reduces or prevents the expression of an isoform of a LINC complex protein comprising one or more domains/regions of interest.

For example, a domain or region of interest may be or comprise: a SUN domain, a KASH domain and/or a domain required for interaction with an interaction partner for the LINC complex protein. For example, a domain or region of interest may be required for interaction with a KASH domain-containing protein, a SUN domain-containing protein, a nucleoplasmic protein, a lamin, a chromatin-binding protein, a

cytoplasmic protein, a cytoskeletal protein, a microfilament, actin, a microtubule, tubulin, a microtubule motor and/or an intermediate filament protein.

In some embodiments, a LINC complex inhibitor may alter splicing of pre-mRNA encoding the LINC complex protein to increase the proportion of mature mRNA encoding isoform(s) lacking the relevant domains/regions, and/or to decrease the proportion of mature mRNA encoding isoform(s) comprising the relevant domains/regions.

In some embodiments, a LINC complex inhibitor may modify nucleic acid encoding the LINC complex protein to increase expression of isoform(s) lacking the relevant domains/regions, and/or may modify the nucleic acid encoding the LINC complex protein to decrease expression of isoform(s) comprising the relevant domains/regions of the protein.

In some embodiments the LINC complex inhibitor is an inhibitory nucleic acid. In some embodiments, the inhibitory nucleic acid is an antisense nucleic acid. In some embodiments the inhibitory nucleic acid is an antisense oligonucleotide (ASO). Antisense oligonucleotides may be single-stranded, and may bind by complementary sequence binding to a target oligonucleotide, *e.g.* mRNA.

ASOs may be designed to inhibit/prevent expression of a LINC complex protein or particular isoforms thereof.

Oligonucleotides designed to inhibit/prevent expression of a LINC complex protein, or particular isoforms thereof, may have substantial sequence identity to a portion of nucleic acid encoding the LINC complex protein/the relevant isoform, or the complementary sequence thereto.

In some embodiments, the inhibitory nucleic acid reduces expression of a LINC complex protein by RNA interference (RNAi). RNAi involves inhibition of gene expression and translation by targeted neutralisation of mRNA molecules. In some embodiments, the inhibitory nucleic acid is a small interfering RNA (siRNA), a short hairpin RNA (shRNA), or a micro RNA (miRNA).

A role for the RNAi machinery and small RNAs in targeting of heterochromatin complexes and epigenetic gene silencing at specific chromosomal loci has been demonstrated. Double-stranded RNA (dsRNA)-dependent post transcriptional silencing, also known as RNA interference (RNAi), is a phenomenon in which dsRNA complexes can target specific genes of homology for silencing in a short period of time. It acts as a signal to promote degradation of mRNA with sequence identity. A 20-nt siRNA is generally long enough to induce gene-specific silencing, but short enough to evade host response. The decrease in expression of targeted gene products can be extensive with 90% silencing induced by a few molecules of siRNA. RNAi based therapeutics have been progressed into Phase I, II and III clinical trials for a number of indications (*Nature* 2009 Jan 22; 457(7228):426-433).

In the art, such RNA sequences are termed ‘short or small interfering RNAs’ (siRNAs) or ‘microRNAs’ (miRNAs) depending on their origin. Both types of sequence may be used to down-regulate gene

expression by binding to complementary RNAs and either triggering mRNA elimination (RNAi) or arresting mRNA translation into protein. siRNAs are derived by processing of long double stranded RNAs and when found in nature are typically of exogenous origin. Micro-interfering RNAs (microRNAs, miRNAs) are endogenously encoded small non-coding RNAs, derived by processing of short hairpins. Both siRNA and miRNA can inhibit the translation of mRNAs bearing partially complimentary target sequences without RNA cleavage and degrade mRNAs bearing fully complementary sequences.

siRNAs are typically double stranded and, in order to optimise the effectiveness of RNA mediated down-regulation of the function of a target gene, it is preferred that the length of the siRNA molecule is chosen to ensure correct recognition of the siRNA by the RISC complex that mediates the recognition by the siRNA of the mRNA target and so that the siRNA is short enough to reduce a host response.

miRNAs are typically single stranded and have regions that are partially complementary enabling the ligands to form a hairpin. miRNAs are RNA genes which are transcribed from DNA, but are not translated into protein. A DNA sequence that codes for a miRNA gene is longer than the miRNA. This DNA sequence includes the miRNA sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a partially double stranded RNA segment. The design of microRNA sequences is discussed *e.g.* in John et al, *PLoS Biology*, 11(2), 1862-1879, 2004.

Typically, the oligonucleotides intended to mimic the effects of siRNA or miRNA have between 10 and 40 ribonucleotides (or synthetic analogues thereof), more preferably between 17 and 30 ribonucleotides, more preferably between 19 and 25 ribonucleotides and most preferably between 21 and 23 ribonucleotides. In some embodiments of the present disclosure employing double-stranded siRNA, the molecule may have symmetric 3' overhangs, *e.g.* of one or two (ribo)nucleotides, typically a UU or dTdT 3' overhang. Based on the disclosure provided herein, the skilled person can readily design suitable siRNA and miRNA sequences, for example using resources such the Ambion siRNA finder. siRNA and miRNA sequences can be synthetically produced and added exogenously to cause gene downregulation or produced using expression systems (*e.g.* vectors). In some embodiments the siRNA is synthesized synthetically.

siRNA-mediated knockdown of LINC complex proteins and siRNAs for achieving the same are described *e.g.* in Östlund *et al.*, Journal of Cell Science (2009) 122:4099-4108, Hatch and Hetzer, J Cell Biol. (2016) 215(1):27-36, Matsumoto *et al.*, Nucleus. (2016) 7(1):68-83, Uzer *et al.*, Stem Cells. (2015) 33(6):2063-76, Thakar *et al.*, Mol Biol Cell. (2017) 28(1): 182-191, Espigat-Georger *et al.*, J Cell Sci. (2016) 129(22):4227-4237, Yang *et al.*, Int J Mol Med. (2013) 32(4):805-12, Rajgor *et al.*, PLoS One. 2012;7(7):e40098, Zhang *et al.*, Exp Cell Res. (2016) 345(2):168-179, Warren *et al.* J Biol Chem. (2010) 285(2):1311-20 and King *et al.*, Cytoskeleton (Hoboken) (2014) 71(7):423-34, which are hereby incorporated by reference in their entirety.

Longer double stranded RNAs may be processed in the cell to produce siRNAs (see for example Myers (2003) Nature Biotechnology 21:324-328). The longer dsRNA molecule may have symmetric 3' or 5'

overhangs, *e.g.* of one or two (ribo)nucleotides, or may have blunt ends. The longer dsRNA molecules may be 25 nucleotides or longer. Preferably, the longer dsRNA molecules are between 25 and 30 nucleotides long. More preferably, the longer dsRNA molecules are between 25 and 27 nucleotides long. Most preferably, the longer dsRNA molecules are 27 nucleotides in length. dsRNAs 30 nucleotides or more in length may be expressed using the vector pDECAP (Shinagawa *et al.*, Genes and Dev., 17, 1340-5, 2003).

Another alternative is the expression of a short hairpin RNA molecule (shRNA) in the cell. shRNAs are more stable than synthetic siRNAs. A shRNA consists of short inverted repeats separated by a small loop sequence. One inverted repeat is complimentary to the gene target. In the cell the shRNA is processed by DICER into a siRNA which degrades the target gene mRNA and suppresses expression. In some embodiments the shRNA is produced within a cell by transcription from a vector. shRNAs may be produced within a cell by transfecting the cell with a vector encoding the shRNA sequence under control of a RNA polymerase III promoter such as the human H1 or 7SK promoter or a RNA polymerase II promoter. Alternatively, the shRNA may be synthesised exogenously (*in vitro*) by transcription from a vector. The shRNA may then be introduced directly into the cell. Preferably, the shRNA molecule comprises a partial sequence of a gene encoding a LINC complex protein. Preferably, the shRNA sequence is between 40 and 100 bases in length, more preferably between 40 and 70 bases in length. The stem of the hairpin is preferably between 19 and 30 base pairs in length. The stem may contain G-U pairings to stabilise the hairpin structure.

shRNA-mediated knockdown of LINC complex proteins and shRNAs for achieving the same are described *e.g.* in Kelkar *et al.*, Nucleus. (2015) 6(6): 479–489, Mroß *et al.*, Nucleus. (2018) 9(1): 503–515, Xing *et al.*, International Journal of Cell Biology (2017) Article ID: 8607532, Arsenovic *et al.*, Biophys J. (2016) 110(1):34-43 and Li *et al.*, Scientific Reports (2017) 7: Article number: 9157, which are hereby incorporated by reference in their entirety.

In some embodiments, the inhibitory nucleic acid is a splice-switching oligonucleotide (SSO). Splice switching oligonucleotides are reviewed *e.g.* in Haves and Hastings, Nucleic Acids Res. (2016) 44(14): 6549–6563, which is hereby incorporated by reference in its entirety. SSOs disrupt the normal splicing of target RNA transcripts by blocking the RNA–RNA base-pairing and/or protein–RNA binding interactions that occur between components of the splicing machinery and pre-mRNA. SSOs may be employed to alter the number/proportion of mature mRNA transcripts encoding a LINC complex protein or particular isoform(s) thereof. SSOs may be designed to target a specific region of the target transcript, *e.g.* to effect skipping of exon(s) of interest, *e.g.* exons encoding domains/regions of interest.

SSOs generally comprise alterations to oligonucleotide sugar-phosphate backbones to prevent RNAse H degradation, and may comprise include *e.g.* phosphorodiamidate morpholino (PMOs), peptide nucleic acid (PNA), locked nucleic acid (LNA), and/or 2′O-methyl (2′OMe) and 2′-O-methoxyethyl (MOE) ribose modifications.

Inhibitory nucleic acids may be made recombinantly by transcription of a nucleic acid sequence, *e.g.* contained within vector. Transcription may be performed in cell-free transcription reactions, or in a cell comprising nucleic acid encoding the inhibitory nucleic acid. In some embodiments inhibitory nucleic acids are produced within a cell, *e.g.* by transcription from a vector. Vectors encoding such molecules may be introduced into cells in any of the ways known in the art. Optionally, expression of the nucleic acid can be regulated using a cell/tissue (*e.g.* heart, muscle, *etc.*) specific promoter.

Inhibitory nucleic acids may also be synthesized using standard solid or solution phase synthesis techniques which are known in the art.

In some embodiments, the LINC complex inhibitor is a molecule/plurality of molecules capable of modifying nucleic acid encoding a LINC complex protein to reduce/prevent expression of a LINC complex protein or particular isoform(s) thereof.

Modifying nucleic acid encoding a LINC complex protein may comprise modifying a gene encoding a LINC complex protein. In some embodiments, modifying nucleic acid encoding a LINC complex protein comprises introducing an insertion, substitution or deletion into a nucleic acid sequence encoding the LINC complex protein.

In some embodiments modifying nucleic acid encoding a LINC complex protein comprises modifying the nucleic acid to introduce a premature stop codon in the sequence transcribed from the nucleic acid. In some embodiments modifying nucleic acid encoding a LINC complex protein comprises modifying the nucleic acid to encode a truncated and/or non-functional version of the LINC complex protein. In some embodiments modifying nucleic acid encoding a LINC complex protein comprises modifying the nucleic acid to encode a version of the LINC complex protein which is misfolded and/or degraded.

The modification may be of nucleic acid comprised in a cell, *e.g.* endogenous nucleic acid encoding a LINC complex protein. The modification causes the cell to have a reduced level of gene and/or protein expression of a LINC complex protein or particular isoform(s) thereof as compared to an equivalent unmodified cell.

In some embodiments, modification may be to a region of the nucleic acid encoding a LINC complex protein involved in (*e.g.* required for) LINC complex formation. For example, in some embodiments modification may target a region of a gene encoding a SUN domain-containing protein encoding a encoding a SUN domain. In some embodiments modification may target a region of a gene encoding a KASH domain-containing protein encoding a KASH domain.

In some embodiments the modification is performed *in vitro* or *ex vivo*. In some embodiments the modification is performed *in vivo*.

Methods for modifying nucleic acids encoding proteins of interest and agents for achieving the same are well known in the art, and include *e.g.* including modification of the target nucleic acid by homologous

recombination, and target nucleic acid editing using site-specific nucleases (SSNs). For example, the inventors demonstrate CRISPR/Cas9-mediated disruption of SUN1 and Nesprin-1 in the experimental examples of WO 2021/010898 A1.

Suitable methods may employ targeting by homologous recombination, which is reviewed, for example, in Mortensen Curr Protoc Neurosci. (2007) Chapter 4:Unit 4.29 and Vasquez *et al.*, PNAS 2001, 98(15): 8403-8410 both of which are hereby incorporated by reference in their entirety. Targeting by homologous recombination involves the exchange of nucleic acid sequence through crossover events guided by homologous sequences.

In some embodiments the methods employ target nucleic acid editing using SSNs. Gene editing using SSNs is reviewed *e.g.* in Eid and Mahfouz, Exp Mol Med. 2016 Oct; 48(10): e265, which is hereby incorporated by reference in its entirety. Enzymes capable of creating site-specific double strand breaks (DSBs) can be engineered to introduce DSBs to target nucleic acid sequence(s) of interest. DSBs may be repaired by either error-prone non-homologous end-joining (NHEJ), in which the two ends of the break are rejoined, often with insertion or deletion of nucleotides. Alternatively DSBs may be repaired by homology-directed repair (HDR), in which a DNA template with ends homologous to the break site is supplied and introduced at the site of the DSB.

SSNs capable of being engineered to generate target nucleic acid sequence-specific DSBs include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced palindromic repeats/CRISPR-associated-9 (CRISPR/Cas9) systems.

ZFN systems are reviewed *e.g.* in Umov *et al.*, Nat Rev Genet. (2010) 11(9):636-46, which is hereby incorporated by reference in its entirety. ZFNs comprise a programmable Zinc Finger DNA-binding domain and a DNA-cleaving domain (*e.g.* a *Fok*I endonuclease domain). The DNA-binding domain may be identified by screening a Zinc Finger array capable of binding to the target nucleic acid sequence.

TALEN systems are reviewed *e.g.* in Mahfouz *et al.*, Plant Biotechnol J. (2014) 12(8):1006-14, which is hereby incorporated by reference in its entirety. TALENs comprise a programmable DNA-binding TALE domain and a DNA-cleaving domain (*e.g.* a *Fok*I endonuclease domain). TALEs comprise repeat domains consisting of repeats of 33-39 amino acids, which are identical except for two residues at positions 12 and 13 of each repeat which are repeat variable di-residues (RVDs). Each RVD determines binding of the repeat to a nucleotide in the target DNA sequence according to the following relationship: 'HD' binds to C, 'NI' binds to A, 'NG' binds to T and 'NN' or 'NK' binds to G (Moscou and Bogdanove, Science (2009) 326(5959):1501.).

CRISPR/Cas9 and related systems *e.g.* CRISPR/Cpf1, CRISPR/C2c1, CRISPR/C2c2 and CRISPR/C2c3 are reviewed *e.g.* in Nakade *et al.*, Bioengineered (2017) 8(3):265-273, which is hereby incorporated by reference in its entirety. These systems comprise an endonuclease (*e.g.* Cas9, Cpf1 *etc.*) and the single-guide RNA (sgRNA) molecule. The sgRNA can be engineered to target endonuclease activity to nucleic acid sequences of interest.

In some embodiments, LINC complex inhibition employs a site-specific nuclease (SSN) system targeting a LINC complex protein. Accordingly in some embodiments the LINC complex inhibitor comprises or consists of SSN system targeting a LINC complex protein. In some embodiments LINC complex inhibition employs nucleic acid(s) encoding a SSN system targeting a LINC complex protein.

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

In particular embodiments, the SSN system may introduce an insertion/deletion in the nucleic acid sequence of a gene encoding a SUN domain-containing protein upstream of the sequence encoding the conserved tyrosine residue.

In some embodiments the SSN system may introduce an insertion/deletion in the nucleic acid sequence of a gene encoding a SUN domain-containing protein, upstream of the sequence encoding the tyrosine at the position corresponding to position 154 of SEQ ID NO:8 (*i.e.* position 153 of SEQ ID NO:18, position 153 of SEQ ID NO:27, position 151 of SEQ ID NO:28, position 152 of SEQ ID NO:29).

In particular embodiments, the SSN system may introduce an insertion/deletion in the nucleic acid sequence of a gene encoding a SUN domain-containing protein upstream of the sequence encoding the conserved tyrosine residue.

In some embodiments the SSN system may introduce an insertion/deletion in the nucleic acid sequence of a gene encoding a KASH domain-containing protein upstream of the sequence encoding the proline-rich region at the C-terminus.

In some embodiments the SSN system may introduce an insertion/deletion in the nucleic acid sequence of a gene encoding a KASH domain-containing protein, upstream of the sequence encoding the proline at the position corresponding to position 57 of SEQ ID NO:37 (*i.e.* position 57 of SEQ ID NO:38, position 56 of SEQ ID NO:39, position 56 of SEQ ID NO:40).

In some embodiments the SSN system is a ZFN system, a TALEN system, CRISPR/Cas9 system, a CRISPR/Cpf1 system, a CRISPR/C2c1 system, a CRISPR/C2c2 system or a CRISPR/C2c3 system.

In some embodiments the SSN system is a CRISPR/Cas9 system. In such embodiments, the LINC complex inhibition may employ nucleic acid(s) encoding a CRISPR RNA (crRNA) targeting nucleic acid encoding a LINC complex protein, and a trans-activating crRNA (tracrRNA) for processing the crRNA to its mature form. 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.

Rather than expressing components of a lumenal domain of a SUN domain-containing protein or a KASH domain 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 the 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.

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 CRISPR/Cas 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 mutated. The truncated protein would be expressed and membrane-localized, but unable to interact with its cognate LINC complex partners.

Accordingly, in some embodiments a LINC complex inhibitor is a CRISPR-Cas or other synthetic nuclease system capable of modifying nucleic acid that encodes the SUN domain or KASH domain of endogenous SUN or Nesprin protein, respectively.

In some embodiments the CRISPR-Cas system modifies the endogenous SUN domain or KASH domain of SUN1 or Nesprin-1 protein, respectively, to disrupt a LINC complex. The respective nucleic acids are Sun1 and Syne1.

In some embodiments, the CRISPR-Cas system comprises a gRNA nucleic acid targeting the SUN domain of a SUN domain-containing protein (*e.g.* SUN1). In some embodiments, the CRISPR-Cas system comprises a gRNA nucleic acid targeting the KASH domain of a KASH domain-containing protein (*e.g.* Nesprin-1).

**LINC complex inhibiting polypeptides**

In some embodiments, a LINC complex inhibitor according to the present disclosure is a LINC complex inhibiting polypeptide.

Herein, a 'LINC complex inhibiting polypeptide' refers to a polypeptide that inhibits the LINC complex formation and/or function. LINC complex inhibiting polypeptides are described *e.g.* in WO 2019/143300 A1, WO 2021/010898 A1 and WO 2023/101607 A2. In some embodiments, a LINC complex inhibitor according to the present disclosure is a LINC complex inhibiting polypeptide according to any embodiment described in WO 2019/143300 A1, WO 2021/010898 A1 or WO 2023/101607 A2, all of which are hereby incorporated by reference in their entirety.

LINC complex inhibiting polypeptides according to the present disclosure preferably bind to a LINC complex protein. The binding may be characterised by non-covalent, protein:protein interaction between

the LINC complex inhibiting polypeptide and the LINC complex protein. The interaction may comprise electrostatic interaction (*e.g.* ionic bonding, hydrogen bonding) and/or Van der Waals forces.

In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) with an affinity in the micromolar range, *i.e.* $K_D$ = 9.9 x $10^{-4}$ to 1 x $10^{-6}$ M. In some embodiments, a LINC complex inhibiting polypeptide binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) with sub-micromolar affinity, *i.e.* $K_D$ < 1 x $10^{-6}$ M. In some embodiments, a LINC complex inhibiting polypeptide binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) with an affinity in the nanomolar range, *i.e.* $K_D$ = 9.9 x $10^{-7}$ to 1 x $10^{-9}$ M. In some embodiments, a LINC complex inhibiting polypeptide binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) with sub-nanomolar affinity, *i.e.* $K_D$ < 1 x $10^{-9}$ M. In some embodiments, a LINC complex inhibiting polypeptide binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) with an affinity in the picomolar range, *i.e.* $K_D$ = 9.9 x $10^{-10}$ to 1 x $10^{-12}$ M. In some embodiments, a LINC complex inhibiting polypeptide binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) with sub-picomolar affinity, *i.e.* $K_D$ < 1 x $10^{-12}$ M.

LINC complex inhibiting polypeptides according to the present disclosure may bind to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) in the manner of a SUN domain-containing protein (*e.g.* a SUN domain-containing protein selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO). In some embodiments, a LINC complex inhibiting polypeptide may bind to the same region, or an overlapping region, of an interaction partner for a SUN domain-containing protein as the region which is bound by a SUN domain-containing protein.

The region of a given target molecule to which a polypeptide binds can be determined by the skilled person using various methods well known in the art, including X-ray co-crystallography analysis of antibody-antigen complexes, peptide scanning, mutagenesis mapping, hydrogen-deuterium exchange analysis by mass spectrometry, phage display, competition ELISA and proteolysis-based 'protection' methods. Such methods are described, for example, in Gershoni *et al.*, BioDrugs, 2007, 21(3):145-156, which is hereby incorporated by reference in its entirety.

In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure inhibits interaction between a SUN domain-containing protein (*e.g.* a SUN domain-containing protein selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) and an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2,

Nesprin-3, Nesprin-4, KASH5 and LRMP). Inhibition of interaction between a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein herein encompasses inhibition of binding between a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein (thereby inhibiting formation of a complex comprising such proteins), and disruption of complexes comprising a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein (*e.g.* via displacement of a constituent protein of such complexes, and consequent disassembly of such complexes).

In some embodiments, a LINC complex inhibiting polypeptide inhibits binding of a SUN domain-containing protein (*e.g.* a SUN domain-containing protein selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP). In some embodiments, a LINC complex inhibiting polypeptide is a competitive inhibitor of binding of a SUN domain-containing protein to an interaction partner for a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide blocks a SUN domain-containing protein from binding to an interaction partner for a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide occupies the region of an interaction partner for a SUN domain-containing protein to which a SUN domain-containing protein binds, thereby inhibiting interaction between the SUN domain-containing protein and the interaction partner therefor. In some embodiments, a LINC complex inhibiting polypeptide displaces a SUN domain-containing protein from a complex comprising a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein.

The ability of a given polypeptide to inhibit interaction between two factors can be determined for example by analysis of a correlate of such interaction in the presence of, or following incubation of one or both of the interaction partners with, the polypeptide.

Analysis may comprise detecting a SUN domain-containing protein/an interaction partner for a SUN domain-containing protein/a complex comprising a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein. Such techniques are well known to the skilled person, and include *e.g.* antibody/reporter-based methods (western blot, ELISA, immunohisto/cytochemistry, *etc.*).

A polypeptide that inhibits a given interaction (*e.g.* between a SUN domain-containing protein and an interaction partner therefor, *e.g.* a KASH domain-containing protein) is identified by the observation of a reduction/decrease in the level of a correlate of interaction between the interaction partners in the presence of – or following incubation of one or both of the interaction partners with – the polypeptide, as compared to the level observed in the absence of the polypeptide (or in the presence of an appropriate control polypeptide known not to inhibit interaction between the interaction partners). Suitable analysis can be performed *in vitro*, *e.g.* using recombinant interaction partners or using cells expressing the interaction partners. Cells expressing interaction partners may do so endogenously, or may do so from nucleic acid introduced into the cell. For the purposes of such assays, one or both of the interaction partners and/or the polypeptide may be labelled or used in conjunction with a detectable entity for the purposes of detecting and/or measuring the level of interaction.

A correlate of interaction between two interaction partners may *e.g.* be the complex formed by association between the interaction partners, a functional property of the complex formed by association between the interaction partners, or a correlate of a downstream activity mediated by the complex formed by association between the interaction partners.

A polypeptide that inhibits interaction between a SUN domain-containing protein (*e.g.* a SUN domain-containing protein selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) and an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) – for example, a polypeptide that inhibits formation of complexes comprising a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein, and/or a polypeptide that disrupts complexes comprising a SUN domain-containing protein and an interaction partner for a SUN domain-containing protein – may be identified by the observation of one or more of the following, in the presence of – or following incubation of one or both of the interaction partners with – the polypeptide, as compared to the level observed in the absence of the polypeptide (or in the presence of an appropriate control polypeptide known not to inhibit interaction between the interaction partners): (i) a decrease in the level of the complex comprising the SUN domain-containing protein and the interaction partner for a SUN domain-containing protein, (ii) a decrease in the level of a functional property of the complex comprising the SUN domain-containing protein and the interaction partner for a SUN domain-containing protein, (iii) a decrease in the level of a correlate of a downstream activity mediated by the complex comprising the SUN domain-containing protein and the interaction partner for a SUN domain-containing protein, (iv) an increase in the level of free (*i.e.* uncomplexed) SUN domain-containing protein, and/or (v) an increase in the level of free (*i.e.* uncomplexed) interaction partner for a SUN domain-containing protein.

LINC complex inhibiting polypeptides according to the present disclosure may disrupt the normal subcellular localisation of a LINC complex protein (*e.g.* a SUN domain-containing protein, or an interaction partner for a SUN domain-containing protein).

In some embodiments, a LINC complex inhibiting polypeptide reduces the level/proportion of a LINC complex protein localised to the nuclear envelope. In some embodiments, a LINC complex inhibiting polypeptide reduces the level/proportion of an interaction partner for the LINC complex inhibiting polypeptide localised to the nuclear envelope. In some embodiments, a LINC complex inhibiting polypeptide reduces the level/proportion of a KASH domain-containing protein associated with outer nuclear membrane. In some embodiments, a LINC complex inhibiting polypeptide reduces the level/proportion of a SUN domain-containing protein associated with inner nuclear membrane.

In some embodiments, a LINC complex inhibiting polypeptide increases the level/proportion of a LINC complex protein not localised to the nuclear envelope. In some embodiments, a LINC complex inhibiting polypeptide increases the level/proportion of a KASH domain-containing protein (*e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) not associated with outer nuclear membrane. In some embodiments, a LINC complex inhibiting polypeptide increases the level/proportion

of a SUN domain-containing protein (*e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) not associated with inner nuclear membrane.

In some embodiments, a LINC complex inhibiting polypeptide increases the level/proportion of a LINC complex protein localised to the endoplasmic reticulum. In some embodiments, a LINC complex inhibiting polypeptide increases the level/proportion of a KASH domain-containing protein (*e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) localised to the endoplasmic reticulum. In some embodiments, a LINC complex inhibiting polypeptide increases the level/proportion of a SUN domain-containing protein (*e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) not associated with inner nuclear membrane.

The subcellular localisation of constituent proteins of LINC complexes within cells can be analysed using techniques known to the person skilled in the art. Such techniques include *e.g.* analysis by immunocytochemistry and reporter-based methods. For example, Boni *et al.*, J. Cell Biology (2015) 209(5):705-720 and Smoyer *et al.*, J. Cell Biology (2016) 215(4):575-590 describe a reporter system permitting imaging of proteins in the ER, INM and ONM. Such methods can be employed to analyse the levels/proportions of constituent proteins of LINC complexes in the nuclear envelope, inner nuclear membrane and an outer nuclear membrane.

LINC complex inhibiting polypeptides that disrupt the normal subcellular localisation of a LINC complex protein may be identified using assays comprising detecting the presence of, or determining the proportion of, the relevant protein(s) in a given subcellular location, *e.g.* using antibody/reporter-based methods (western blot, ELISA, immunohisto/cytochemistry, *etc.*). Subcellular localisation may be analysed *e.g.* by immunocytochemistry, or by western blot of extracts prepared from different cellular fractions, and may employ organelle markers and/or labelled proteins of known subcellular localisation.

Assays may comprise expressing a putative LINC complex inhibiting polypeptide in a cell (*e.g.* from nucleic acid encoding the polypeptide introduced (*e.g.* by transfection/transduction) into the cell), and subsequently comparing the subcellular localisation of the relevant LINC complex protein(s) in such cells to the subcellular localisation observed in cells of an appropriate control condition (*e.g.* non-transfected cells, cells transfected/transduced with empty vector, or cells transfected/transduced with nucleic acid encoding a polypeptide known not to affect subcellular localisation of the relevant LINC complex protein(s)).

In some embodiments, polypeptides may be evaluated for their ability to behave as LINC complex inhibiting polypeptides essentially as described in Example 1 of WO 2023/101607 A2.

Nucleic acid for expressing a putative LINC complex inhibiting polypeptide may be introduced (*e.g.* by transfection) into cells, and the cells may subsequently be evaluated in order to determine the subcellular localisation of one or more LINC complex proteins (*e.g.* a SUN domain-containing protein (*e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) and/or an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* selected from Nesprin-1, Nesprin-2,

Nesprin-3, Nesprin-4, KASH5 and LRMP)). A polypeptide may be identified as a LINC complex inhibiting polypeptide where its expression is determined in such an assay to reduce the level/proportion of a LINC complex protein localised to the nuclear envelope, or to increase the level/proportion of a LINC complex protein not localised to the nuclear envelope (*e.g.* where its expression is determined to reduce the level/proportion of a KASH domain-containing protein associated with outer nuclear membrane, or to increase the level/proportion of a KASH domain-containing protein not associated with outer nuclear membrane, or to increase the level/proportion of a KASH domain-containing protein localised to the endoplasmic reticulum) relative to the level/proportion observed in cells of an appropriate control condition (*e.g.* non-transfected cells, cells transfected/transduced with empty vector, or cells transfected/transduced with nucleic acid encoding a polypeptide known not to affect subcellular localisation of the relevant LINC complex protein(s)).

In some embodiments, cellular expression of a LINC complex inhibiting polypeptide according to the present disclosure: (i) reduces the level of interaction between constituent proteins of a LINC complex (*e.g.* between a SUN domain-containing protein and a KASH domain-containing protein), (ii) reduces the level of a LINC complex (*e.g.* a LINC complex comprising a SUN domain-containing protein and a KASH domain-containing protein), (iii) reduces the level of a correlate of a function/activity of a LINC complex (*e.g.* a LINC complex comprising a SUN domain-containing protein and a KASH domain-containing protein), (iv) reduces the level/proportion of a LINC complex protein (*e.g.* a SUN domain-containing protein or a KASH domain-containing protein) localised to the nuclear envelope, and/or (v) reduces the level/proportion of a KASH domain-containing protein (*e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) localised to the nuclear envelope/outer nuclear membrane to less than 1 times/less than 100%, *e.g.* one of ≤0.99 times/≤99%, ≤0.95 times/≤95%, ≤0.9 times/≤90%, ≤0.85 times/≤85%, ≤0.8 times/≤80%, ≤0.75 times/≤75%, ≤0.7 times/≤70%, ≤0.65 times/≤65%, ≤0.6 times/≤60%, ≤0.55 times/≤55%, ≤0.5 times/≤50%, ≤0.45 times/≤45%, ≤0.4 times/≤40%, ≤0.35 times/≤35%, ≤0.3 times/≤30%, ≤0.25 times/≤25%, ≤0.2 times/≤20%, ≤0.15 times/≤15%, ≤0.1 times/≤10% times, ≤0.05 times/≤5%, or ≤0.01 times/≤1% of the level observed in the absence of the LINC complex inhibiting polypeptide, or in an appropriate control condition, in a given assay.

Preferred levels of reduction in accordance with the preceding paragraph are reduction to less than 0.5 times/≤50%, *e.g.* one of ≤0.4 times/≤40%, ≤0.3 times/≤30%, ≤0.2 times/≤20%, ≤0.15 times/≤15%, or ≤0.1 times/≤10%.

In some embodiments, cellular expression of a LINC complex inhibiting polypeptide according to the present disclosure: (i) increases the level/proportion of a LINC complex protein (*e.g.* a SUN domain-containing protein or a KASH domain-containing protein) not localised to the nuclear envelope, and/or (ii) increases the level/proportion of a LINC complex protein (*e.g.* a SUN domain-containing protein or a KASH domain-containing protein) localised to the endoplasmic reticulum to greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times, ≥10 times, ≥50 times or ≥100 times the level observed in

the absence of the LINC complex inhibiting polypeptide, or in an appropriate control condition, in a given assay.

Polypeptides may be evaluated for certain functional properties in an appropriate *in vivo* model. For example, polypeptides may be evaluated for therapeutic/prophylactic effects *in vivo* in a non-human animal model of a disease/condition described herein. In such assays, the polypeptide may be delivered in the form of nucleic acid encoding the protein, *e.g.* using a viral vector having an appropriate tropism for cells/tissue in which LINC complex inhibition would confer therapeutic/prophylactic benefit.

The lifespan and survival of subjects can be evaluated in such models by monitoring survival over time. Cardiac function and myocardial contractility can be evaluated by measuring correlates thereof, and ejection fraction, fractional shortening, left ventricular posterior wall thickness and/or left ventricular inner diameter can be measured by echocardiography/ultrasound.

It will be appreciated that subjects are preferably evaluated for one or more of the properties recited in the preceding paragraph at a specified time point, *e.g.* after a period of time sufficient for an effect of administration of the polypeptide in the relevant model to be observed. For example, subjects may be evaluated at 7 or more days after administration of viral vector encoding the putative LINC complex inhibiting polypeptide.

In some embodiments, administration of a LINC complex inhibiting polypeptide according to the present disclosure to a subject having a cardiomyopathy (*e.g.* cardiomyopathy associated with mutation to *DSP*; *e.g. via* a viral vector encoding the LINC complex inhibiting polypeptide) increases survival or the lifespan of the subject, and/or increases cardiac function, myocardial contractility, ejection fraction, fractional shortening and/or left ventricular posterior wall thickness in the subject, to greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times, ≥10 times, ≥50 times or ≥100 times the level observed in the absence of such treatment, or following administration of an appropriate control polypeptide (*e.g.* a polypeptide known not to inhibit the LINC complex, *e.g.* delivered using the same viral vector).

In some embodiments, administration of a LINC complex inhibiting polypeptide according to the present disclosure to a subject having a cardiomyopathy (*e.g.* cardiomyopathy associated with mutation to *DSP*; *e.g. via* a viral vector encoding the LINC complex inhibiting polypeptide) reduces left ventricular inner diameter in the subject, to less than 1 times/less than 100%, *e.g.* one of ≤0.99 times/≤99%, ≤0.95 times/≤95%, ≤0.9 times/≤90%, ≤0.85 times/≤85%, ≤0.8 times/≤80%, ≤0.75 times/≤75%, ≤0.7 times/≤70%, ≤0.65 times/≤65%, ≤0.6 times/≤60%, ≤0.55 times/≤55%, ≤0.5 times/≤50%, ≤0.45 times/≤45%, ≤0.4 times/≤40%, ≤0.35 times/≤35%, ≤0.3 times/≤30%, ≤0.25 times/≤25%, ≤0.2 times/≤20%, ≤0.15 times/≤15%, ≤0.1 times/≤10% times, ≤0.05 times/≤5%, or ≤0.01 times/≤1% of the level observed in the absence of such treatment, or following administration of an appropriate control polypeptide (*e.g.* a polypeptide known not to inhibit the LINC complex, *e.g.* delivered using the same viral vector).

In some embodiments, cellular expression of a LINC complex inhibiting polypeptide according to the present disclosure: (i) reduces the level of interaction between constituent proteins of a LINC complex (*e.g.* between a SUN domain-containing protein and a KASH domain-containing protein), (ii) reduces the level of a LINC complex (*e.g.* a LINC complex comprising a SUN domain-containing protein and a KASH domain-containing protein), (iii) reduces the level of a correlate of a function/activity of a LINC complex (*e.g.* a LINC complex comprising a SUN domain-containing protein and a KASH domain-containing protein), (iv) reduces the level/proportion of a LINC complex protein (*e.g.* a SUN domain-containing protein or a KASH domain-containing protein) localised to the nuclear envelope, and/or (v) reduces the level/proportion of a KASH domain-containing protein (*e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP) localised to the nuclear envelope/outer nuclear membrane to less than 1 times/less than 100%, *e.g.* one of ≤0.99 times/≤99%, ≤0.95 times/≤95%, ≤0.9 times/≤90%, ≤0.85 times/≤85%, ≤0.8 times/≤80%, ≤0.75 times/≤75%, ≤0.7 times/≤70%, ≤0.65 times/≤65%, ≤0.6 times/≤60%, ≤0.55 times/≤55%, ≤0.5 times/≤50%, ≤0.45 times/≤45%, ≤0.4 times/≤40%, ≤0.35 times/≤35%, ≤0.3 times/≤30%, ≤0.25 times/≤25%, ≤0.2 times/≤20%, ≤0.15 times/≤15%, ≤0.1 times/≤10% times, ≤0.05 times/≤5%, or ≤0.01 times/≤1% of the level observed in the absence of cellular expression of the LINC complex inhibiting polypeptide (*e.g.* by a cell of the same type).

In some embodiments, cellular expression of a LINC complex inhibiting polypeptide according to the present disclosure: (i) increases the level/proportion of a LINC complex protein (*e.g.* a SUN domain-containing protein or a KASH domain-containing protein) not localised to the nuclear envelope, and/or (ii) increases the level/proportion of a LINC complex protein (*e.g.* a SUN domain-containing protein or a KASH domain-containing protein) localised to the endoplasmic reticulum to greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times, ≥10 times, ≥50 times or ≥100 times the level observed in the absence of cellular expression of the LINC complex inhibiting polypeptide (*e.g.* by a cell of the same type).

In some embodiments, administration of a LINC complex inhibiting polypeptide according to the present disclosure to a subject having a cardiomyopathy (*e.g.* cardiomyopathy associated with mutation to *DSP*; *e.g. via* a viral vector encoding the LINC complex inhibiting polypeptide) increases survival or the lifespan of the subject, and/or increases cardiac function, myocardial contractility, ejection fraction, fractional shortening and/or left ventricular posterior wall thickness in the subject, to greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times, ≥10 times, ≥50 times or ≥100 times the level observed in the absence of such administration.

In some embodiments, administration of a LINC complex inhibiting polypeptide according to the present disclosure to a subject having a cardiomyopathy (*e.g.* cardiomyopathy associated with mutation to *DSP*; *e.g. via* a viral vector encoding the LINC complex inhibiting polypeptide) reduces left ventricular inner diameter in the subject, to less than 1 times/less than 100%, *e.g.* one of ≤0.99 times/≤99%, ≤0.95

times/≤95%, ≤0.9 times/≤90%, ≤0.85 times/≤85%, ≤0.8 times/≤80%, ≤0.75 times/≤75%, ≤0.7 times/≤70%, ≤0.65 times/≤65%, ≤0.6 times/≤60%, ≤0.55 times/≤55%, ≤0.5 times/≤50%, ≤0.45 times/≤45%, ≤0.4 times/≤40%, ≤0.35 times/≤35%, ≤0.3 times/≤30%, ≤0.25 times/≤25%, ≤0.2 times/≤20%, ≤0.15 times/≤15%, ≤0.1 times/≤10% times, ≤0.05 times/≤5%, or ≤0.01 times/≤1% of the level observed in the absence of such administration.

LINC complex inhibiting polypeptides according to the present disclosure may be based on a SUN domain-containing protein, *e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO. In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure comprises an inhibitory region which is based on a SUN domain-containing protein, *e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO.

An 'inhibitory region' of a LINC complex inhibiting polypeptide refers to the region of the polypeptide through which LINC complex inhibition is achieved. An inhibitory region according to the present disclosure typically has a high degree of sequence identity to part of the amino acid sequence of a SUN domain-containing protein. The inhibitory region of a LINC complex inhibiting polypeptide according to the present disclosure may be the region through which the LINC complex inhibiting polypeptide binds to an interaction partner for a SUN domain-containing protein (*e.g.* a KASH domain-containing protein, *e.g.* one or more of Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP). In aspects and embodiments of the present disclosure, an inhibitory region of a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein.

LINC complex inhibiting polypeptides according to the present disclosure may comprise or consist essentially of an amino acid sequence having a high degree of sequence identity (*e.g.* at least 80%, 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to the amino acid sequence of all or part of a SUN domain-containing protein, *e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO. LINC complex inhibiting polypeptides according to the present disclosure may comprise an inhibitory region comprising or consisting of an amino acid sequence having a high degree of sequence identity (*e.g.* at least 80%, 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity) to the amino acid sequence of all or part of a SUN domain-containing protein, *e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO.

Such LINC complex inhibiting polypeptides preferably (i) retain the ability of the SUN domain-containing protein on which they are based to bind to a KASH domain-containing protein (*e.g.* one or more of Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP), but (ii) lack, or display a reduced level of, one or more other properties of the SUN domain-containing protein on which they are based. In preferred embodiments, a LINC complex inhibiting polypeptide lacks or displays a reduced ability to bind to nuclear lamins and/or chromatin-binding proteins, and/or lacks or displays a reduced ability to associate with (*e.g.* localise to) the inner nuclear membrane, relative to the SUN domain-containing protein on which it is based.

For example, a LINC complex inhibiting polypeptide/inhibitory region thereof according to the present disclosure may comprise the amino acid sequence(s) required for binding to a KASH domain-containing protein (*e.g.* one or more of Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP), and may lack the amino acid sequence(s) required for one or more other properties of the SUN domain-containing protein upon which it is based (*e.g.* the amino acid sequence(s) required for binding to nuclear lamins and/or chromatin-binding proteins, and/or the amino acid sequence(s) required for association with the inner nuclear membrane).

As used herein, a polypeptide/amino acid sequence/domain that 'consists essentially of' a reference amino acid sequence either (i) consists of the reference amino acid sequence, or (ii) comprises the reference amino acid sequence, wherein the reference amino acid sequence constitutes at least 80% (*e.g.* one of ≥85% ≥86%, ≥87%, ≥88%, ≥89%, ≥90%, ≥91%, ≥92%, ≥93%, ≥94%, ≥95%, ≥96%, ≥97%, ≥98%, ≥99%) of the polypeptide/amino acid sequence. It will be appreciated that polypeptide or an amino acid sequence that 'consists essentially of' a reference amino acid sequence may comprise the reference amino acid sequence and additional amino acid(s) at one or both of the N-terminal and C-terminal ends of the reference amino acid sequence, provided that the additional amino acid(s) constitute ≤20% of the polypeptide/amino acid sequence.

By way of illustration, a polypeptide consisting of the amino acid sequence of SEQ ID NO:74 consists essentially of the amino acid sequence of SEQ ID NO:44. SEQ ID NO:74 comprises the amino acid sequence of SEQ ID NO:44 (*i.e.* from positions 1 to 197 of SEQ ID NO:74), and the amino acid sequence of SEQ ID NO:44 constitutes ~98% of the polypeptide (*i.e.* 197/201 residues). SEQ ID NO:74 comprises the KDEL retention motif (SEQ ID NO:77) C-terminal to the amino acid sequence of SEQ ID NO:44.

It will be appreciated that in some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure consists essentially of a KASH domain-containing protein-binding fragment of a SUN domain-containing protein. It will similarly be appreciated that in some embodiments, the inhibitory region of a LINC complex inhibiting polypeptide according to the present disclosure consists of a KASH domain-containing protein-binding fragment of a SUN domain-containing protein. Such LINC complex inhibiting polypeptides may be referred to as 'decoy', 'dominant-negative' or 'mimetic' versions of the SUN domain-containing proteins on which they are based. That is, in aspects and embodiments of the present disclosure, a LINC complex inhibiting polypeptide may be a dominant-negative SUN domain-containing polypeptide.

LINC complex inhibiting polypeptides according to the present disclosure preferably display competitive inhibition of interaction between a SUN domain-containing protein (*e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO) and a KASH domain-containing protein (*e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP). Decoy/dominant negative/mimetic LINC complex inhibiting polypeptides preferably bind to a KASH domain-containing protein (*e.g.* selected from Nesprin-1, Nesprin-2, Nesprin-3, Nesprin-4, KASH5 and LRMP), occupying the site required for interaction with,

and thereby in making these species unavailable for binding to, endogenous SUN domain-containing proteins.

Such LINC complex inhibiting polypeptides may inhibit the formation of LINC complexes and/or disrupt existing LINC complexes via inhibition of assembly of the endogenous interaction partners and/or via displacement of the endogenous interaction partners. The decoy/dominant negative/mimetic LINC complex inhibiting polypeptides form non-functional LINC complexes, or LINC complexes having a reduced level of function compared to LINC complexes formed by wildtype, endogenous SUN domain-containing proteins and KASH domain-containing proteins.

In aspects and embodiments according to the present disclosure, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein. It will be appreciated that amino acid sequence(s) of LINC complex inhibiting polypeptides described herein that correspond to one or more regions of a SUN domain-containing protein may be comprised in the inhibitory region of the LINC complex inhibiting polypeptide.

As used herein, an amino acid sequence which 'corresponds' to a specified region of a reference polypeptide or amino acid sequence has at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of the polypeptide/amino acid sequence. An amino acid sequence which 'corresponds' to a specified region of a reference polypeptide or amino acid sequence can be identified by sequence alignment of the subject sequence to the reference sequence, *e.g.* using sequence alignment software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960). By way of illustration, the amino acid sequence from positions 522 to 717 of human SUN2 corresponds to the amino acid sequence from positions 616 to 812 of human SUN1.

In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure consists essentially of a human amino acid sequence. In some embodiments, the inhibitory region of a LINC complex inhibiting polypeptide according to the present disclosure consists essentially of a human amino acid sequence. As used herein, 'a human amino acid sequence' refers to an amino acid sequence that is encoded by nucleic acid of the genome of a human. That is, in some embodiments a LINC complex inhibiting polypeptide or the inhibitory region of a LINC complex inhibiting polypeptide consists essentially of an amino acid sequence having 100% amino acid sequence identity to an amino acid sequence encoded by the genome of a human subject. It will be appreciated that in some embodiments, the amino acid sequence encoded by the genome of a human subject is an amino acid sequence of a human SUN domain-containing protein (*e.g.* selected from SUN1, SUN2, SUN3, SUN5, SPAG4 and SUCO; *e.g.* SUN1 or SUN2). Such embodiments of LINC complex inhibiting polypeptides are contemplated in particular where administration to a human subject is intended, *e.g.* in the context of therapeutic/prophylactic intervention according to the present disclosure.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region of a SUN domain-containing protein. In some

embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:12. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:22.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the SUN domain of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the SUN domain of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the SUN domain of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:8. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:18.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an inhibitory region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:44. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:46.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α2 helix of the CC2 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α2 helix of the CC2 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α2 helix of the CC2 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:11. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:21.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α1 helix of the CC2 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α1 helix of the CC2 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α1 helix of the CC2 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:10. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:20.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC1 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC1 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC1 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:9. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:19.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC1 region and CC2 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC1 region and CC2 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC1 region and CC2 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:88. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:91.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC2 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC2 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the CC2 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:89. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:92.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α2 and α3 helices of the CC2 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α2 and α3 helices of the CC2 region of human SUN1. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to the α2 and α3 helices of the CC2 region of human SUN2. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:90. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:93.

In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to part of the SMART coil-coiled 2 region, the CC1 region and CC2 region of a SUN domain-containing protein. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to positions 483 to 632 of human SUN1 (numbered according to SEQ ID NO:1). In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence corresponding to positions 388 to 538 of human SUN2 (numbered according to SEQ ID NO:13). In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:97. In some embodiments, a LINC complex inhibiting polypeptide comprises an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:98.

In some embodiments, a LINC complex inhibiting polypeptide, or an inhibitory region thereof, comprises or consists essentially of an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59,

60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 94, 95, 101, 102, 103, 104, 105, 106, 107, 108 or 109.

In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure comprises an inhibitory region consisting essentially of an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:43, 66, 94, 65, 63, 64 or 67.

In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure comprises or consists essentially of an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:99, 72, 95, 71, 69, 70 or 73.

LINC complex inhibiting polypeptides according to the present disclosure may comprise one or more additional amino acids or sequences of amino acids. That is, the LINC complex inhibiting polypeptides may comprise one or more amino acids or sequences of amino acids in addition to the inhibitory region of the polypeptide (*i.e.* the region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein).

In accordance with various aspects and embodiments according to the present disclosure, LINC complex inhibiting polypeptides may comprise a sequence preventing secretion of the polypeptide from a cell expressing the polypeptide. In some embodiments the polypeptide comprises an endoplasmic reticulum (ER) retention motif. Such sequences may be provided C-terminal to the inhibitory region of a LINC complex inhibiting polypeptide according to the present disclosure. In some embodiments, a sequence for preventing secretion of the polypeptide from a cell expressing the polypeptide (*e.g.* an ER retention motif) is provided at the C-terminus of the amino acid sequence of the polypeptide. In some embodiments, a sequence for preventing secretion of the polypeptide from a cell expressing the polypeptide (*e.g.* an ER retention motif) is not followed at the C-terminus of the polypeptide by any other amino acids.

Endoplasmic reticulum retention sequences are known in the art. In some embodiments the ER retention motif is a KDEL sequence. In some embodiments, an ER retention motif is a KDEL motif or a variant thereof effective to retain a protein comprising the motif at its C-terminus in the endoplasmic reticulum. KDEL variants may comprise or consist of an amino acid sequence conforming to the prosite motif: [K/R/H/Q/S/A]-[D/E/N/Q]-E-L (described *e.g.* in Hulo *et al.*, 2006, *Nucleic acids research* 34: D227-D230), or a variant described by in Raykhel *et al.*, 2007 (*J. Cell Biol.* 179(6):1193-1204) who proposed an expanded prosite motif definition. Raykhel *et al.* demonstrated endoplasmic retention with variants in which the 4 position (*i.e.* the K position) included F, W Y as an alternative to KRHQSA, a range of 3 position (*i.e.* the D position) residues that extended far beyond DENQ, F or M in the 1 position (the L position), and D in the 2 position (*i.e.* the E position).

For example, a variant of the KDEL motif may *e.g.* be one of CDEL, KCEL or HVEL, as proposed by Raykhel *et al.* Thus, in some aspects disclosed herein the endoplasmic reticulum (ER) retention motif is KDEL or a variant thereof which exhibits ER retention activity.

In some embodiments, the ER retention motif comprises, or consists of, the amino acid sequence of SEQ ID NO:77, or a variant comprising one or more (*e.g.* 1 or 2) substitutions to the amino acid sequence of SEQ ID NO:77.

In some embodiments, LINC complex inhibiting polypeptides may comprise a signal peptide. A signal peptide may be provided N-terminal to the inhibitory region of a LINC complex inhibiting polypeptide according to the present disclosure. In some embodiments, a signal peptide is provided at the N-terminus of the amino acid sequence of the polypeptide. In some embodiments, the signal peptide is not preceded at the N-terminus of the polypeptide by any other amino acids. Signal peptides normally consist of a sequence of 5-30 hydrophobic amino acids, which form a single alpha helix. Secreted proteins and proteins expressed at the cell surface often comprise signal peptides. The signal peptide may be present at the N-terminus of the peptide/polypeptide, and may be present in the newly synthesised peptide/polypeptide. Signal peptides are often removed by cleavage, and thus are not comprised in the mature peptide/polypeptide.

Signal peptides are known for many proteins, and are recorded in databases such as GenBank, UniProt, Swiss-Prot, TrEMBL, Protein Information Resource, Protein Data Bank, Ensembl, and InterPro, and/or can be identified/predicted *e.g.* using amino acid sequence analysis tools such as SignalP (Petersen *et al.*, 2011 Nature Methods 8: 785-786) or Signal-BLAST (Frank and Sippl, 2008 Bioinformatics 24: 2172-2176). In some embodiments, an N-terminal signal sequence is derived from a secretory protein or a type I transmembrane protein. In some embodiments, the secretory protein or type I transmembrane protein is selected from: human serum albumin, proinsulin, transferrin receptor, EGF receptor, pre-pro-opiomelanocortin, a carboxypeptidase, a complement protein, fibrinogen, a cytokine, a chemokine, fibrinogen, a pancreatic digestive enzyme (*e.g.* a protease, amylase or lipase) or an endoplasmic reticulum lumenal protein (*e.g.* a protein disulphide isomerase or GRP94). In some embodiments the N-terminal signal peptide is derived from human serum albumin.

In some embodiments, the signal peptide comprises a signal peptidase cleavage site. The signal peptidase cleavage site provides for removal of the signal peptide from the mature polypeptide. In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure comprises a signal peptidase cleavage site derived from a secretory protein or a type I transmembrane protein, *e.g.* a secretory protein or a type I transmembrane protein described hereinabove.

In some embodiments, the signal peptide comprises, or consists of, an amino acid sequence having at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:76. In some embodiments, the signal peptide comprises, or consists of, an amino acid sequence having at least 60%,

*e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:100.

In some embodiments, LINC complex inhibiting polypeptides may comprise a detectable moiety, *e.g.* a fluorescent label, phosphorescent label, luminescent label, immuno-detectable label (*e.g.* an epitope tag), radiolabel, chemical, nucleic acid or enzymatic label. The LINC complex inhibiting polypeptide may be covalently or non-covalently labelled with the detectable moiety.

In some embodiments, the detectable moiety is provided at the N-terminus of the polypeptide (either before or after processing of the polypeptide by signal peptidase to remove any signal peptide). In some embodiments, the detectable moiety is provided at the C-terminus of the polypeptide, *e.g.* downstream of (after) a sequence for preventing secretion of the polypeptide from a cell expressing the polypeptide (*e.g.* downstream of an ER retention motif).

In some embodiments, the detectable moiety is or comprises an epitope tag. In some embodiments, an epitope tag is selected from: a haemagglutinin A (HA), ALFA, histidine (His; *e.g.* 6XHis), c-Myc, glutathione S-transferase (GST), green fluorescent protein (GFP), maltose-binding protein (MBP), FLAG, E, Biotin, Protein A, Protein G, streptavidin, T7, thioredoxin, V5, or vesicular stomatitis virus glycoprotein (VSV-G) tag. In some embodiments, the detectable moiety is or comprises a moiety having detectable activity, *e.g.* an enzymatic activity on a given substrate. Examples of such moieties include *e.g.* horseradish peroxidase (HRP) and luciferase moieties.

In some embodiments, a LINC complex inhibiting polypeptide according to the present disclosure is provided with one of the following structures:

N-term-[signal peptide]-[inhibitory region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein]-[sequence preventing secretion of the polypeptide from a cell expressing the polypeptide]-C-term

N-term-[inhibitory region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein]-[sequence preventing secretion of the polypeptide from a cell expressing the polypeptide]-C-term

In some embodiments, LINC complex inhibiting polypeptides according to the present disclosure comprise one or more linker sequences between amino acid sequences. In some embodiments, a linker sequence has a length of 1-2, 1-3, 1-4, 1-5 or 1-10 amino acids. In some embodiments, a linker sequence may be provided at one or both ends of one or more of: an inhibitory region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein; signal peptide; sequence preventing secretion of the polypeptide from a cell expressing the polypeptide; and/or detectable entity of the LINC complex inhibiting polypeptide.

Linker sequences are known to the skilled person, and are described *e.g.* in Chen *et al.*, Adv Drug Deliv Rev (2013) 65(10): 1357-1369, which is hereby incorporated by reference in its entirety. In some embodiments, a linker sequence may be a flexible linker sequence. Flexible linker sequences allow for relative movement of the amino acid sequences which are linked by the linker sequence. Flexible linkers are known to the skilled person, and several are identified in Chen *et al.*, Adv Drug Deliv Rev (2013) 65(10): 1357-1369. Flexible linker sequences often comprise high proportions of glycine and/or serine residues. In some embodiments, the linker sequence comprises at least one glycine residue and/or at least one serine residue. In some embodiments, the linker sequence consists of glycine and serine residues.

In preferred embodiments, the LINC complex inhibiting polypeptide is of a size (*i.e.* in terms of the number of amino acids making up the LINC complex inhibiting polypeptide) permitting delivery of the LINC complex inhibiting polypeptide as a gene therapy, *i.e.* in the form of nucleic acid encoding the polypeptide.

In some embodiments, the LINC complex inhibiting polypeptide has a size such that a polynucleotide encoding the polypeptide has a size (*i.e.* in terms of the number of nucleotides making up the polynucleotide) within the packaging limit of a vector for delivering the polynucleotide. In some embodiments, the LINC complex inhibiting polypeptide has a size such that a polynucleotide encoding the polypeptide has a size within the packaging limit of a vector described herein. In some embodiments, the LINC complex inhibiting polypeptide has a size such that a polynucleotide encoding the polypeptide has a size within the packaging limit of an adeno-associated virus (AAV) vector, *e.g.* an AAV vector described herein. In some embodiments, the LINC complex inhibiting polypeptide has a size such that a polynucleotide encoding the polypeptide has a size within the packaging limit of a scAAV vector.

In some embodiments, the LINC complex inhibiting polypeptide consists of an amino acid sequence comprising fewer than 510 amino acids. In some embodiments, the LINC complex inhibiting polypeptide consists of an amino acid sequence comprising fewer than 457 amino acids.

In some embodiments, the LINC complex inhibiting polypeptide consists of an amino acid sequence comprising fewer than 600 amino acids, *e.g.* one of ≤550, ≤500, ≤450, ≤400, ≤350, ≤340, ≤330, ≤320, ≤310, ≤300, ≤290, ≤280, ≤270, ≤260, ≤250, ≤240, ≤230, ≤220 or ≤210 amino acids.

LINC complex inhibiting polypeptides according to the present disclosure may be prepared according to methods for the production of polypeptides known to the skilled person. Polypeptides may be prepared by chemical synthesis, *e.g.* liquid or solid phase synthesis. For example, peptides/polypeptides can be synthesised using the methods described in, for example, Chandrudu *et al.*, Molecules (2013), 18: 4373-4388, which is hereby incorporated by reference in its entirety. Alternatively, antigen-binding molecules and polypeptides may be produced by recombinant expression. Molecular biology techniques suitable for recombinant production of polypeptides are well known in the art, such as those set out in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition), Cold Spring Harbor Press, 2012, and in Nat Methods. (2008); 5(2): 135-146 both of which are hereby incorporated by reference in their entirety.

Methods for the recombinant production of antigen-binding molecules are also described in Frenzel *et al.*, Front Immunol. (2013); 4: 217 and Kunert and Reinhart, Appl Microbiol Biotechnol. (2016) 100: 3451–3461, both of which are hereby incorporated by reference in their entirety.

**Cardiomyopathy**

Aspects and embodiments of the present disclosure relate to the treatment and prevention of cardiomyopathy. The experimental examples of the present disclosure demonstrate that LINC complex inhibition ameliorates pathology in a mouse model of cardiomyopathy.

Herein, 'cardiomyopathy' refers to pathology affecting the structure (*e.g.* the size, shape) and/or function of cardiac muscle tissue (*i.e.* the myocardium), and is characterised by a reduction in cardiac function, *i.e.* a decrease in the ability of the heart to pump blood. Cardiomyopathies are reviewed *e.g.* in Ciarambino *et al.*, Int J Mol Sci. (2021) 22(14): 7722, which is hereby incorporated by reference in its entirety. The underlying causes of cardiomyopathies are diverse, and include cardiovascular disease, genetic variation, infection, autoimmune disease and adverse effects of medication.

Cardiomyopathies can be broadly divided into five main classes: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic cardiomyopathy (ACM; also known as arrhythmogenic right ventricular cardiomyopathy (ARVC) and arrhythmogenic right ventricular dysplasia (AVRD)), and unclassified cardiomyopathy (*i.e.* cardiomyopathy other than DCM, HCM, RCM and ACM).

Dilated cardiomyopathy is characterised by an enlargement (*i.e.* dilation) of one or both ventricles (typically involving the left ventricle), in which the ventricle wall becomes stretched and thin. The thinner ventricle wall is weaker, and therefore can't contract with as much force, and so cardiac function is impaired. Subjects with DCM may develop heart failure, and the most common presenting symptoms are arrhythmia (ventricular/atrial) and sudden cardiac death. The cause of dilated cardiomyopathy is most often genetic, but it is also associated with myocarditis (*e.g.* associated with viral infection), coronary artery disease, infiltrative diseases, pregnancy (peripartum cardiomyopathy), hypertension, connective tissue diseases, drug abuse and doxorubicin treatment.

Genetic variants of the *TTN* gene – which encodes the sarcomeric protein titin (which is identified by UniProt Q8WZ42), that links actin and myosin – account for ~25% of dilated cardiomyopathies, particularly variants that result in truncation of titin, which are thought to be the cause of ~15-20% of dilated cardiomyopathies (Schaefer *et al.*, Nat Genet. (2017) 49(1): 46–53). Mutations to *LMNA* – which encodes lamins A and C – accounts for around ~5% of dilated cardiomyopathies. Mutations to *PLN* (which encodes phospholamban) and mutations to *FLNC* (which encodes filamin C) each account for ~1-5% of dilated cardiomypothies. Other genes in which genetic variation has been causally linked to dilated cardiomyopathy include *DSP* (which encodes desmoplakin), *DMD* (which encodes dystrophin), *ACTC* (which encodes cardiac actin), *DES* (which encodes desmin), *MYH7* (which encodes the cardiac isoforms of beta-myosin-heavy chain), *TNNT2* (which encodes troponin T), *TNNI3* (which encodes troponin I), *SGCD* (which encodes delta-sarcoglycan) and *SCN5A* (which encodes sodium channel, type V).

Ischaemic cardiomyopathy is a common type of dilated cardiomyopathy, and the prevalent cause of systolic heart failure. Ischemic cardiomyopathy (ICM) is a term that refers to the heart's decreased ability to pump blood properly, due to myocardial damage brought upon by ischaemia (reduced blood supply to the heart muscle). Ischaemic cardiomyopathy is associated with a reduced blood supply to the heart muscle, caused by narrowing of the heart arteries, for example due to coronary artery disease or heart attack (known as myocardial infarction). In patients with ischaemic cardiomyopathy, there is a significant increase in the density and stability of microtubules (Chen C. et al. Nature Medicine (2018), 24, 1225–1233, which is herein incorporated by reference in its entirety).

Hypertrophic cardiomyopathy (HCM) is characterised by thickening of the walls of the heart chambers (to a thickness ≥15 mm), and most commonly the walls of the left ventricle. The thickening of the walls of the heart chambers reduces their size such that the volume of blood they are able to contain is reduced, and so cardiac function is impaired. The thicker walls are also often stiff and unable to relax fully, with the result that they are unable to fill with as great a volume of blood. The cause of hypertrophic cardiomyopathy is most often genetic, and is often associated with mutations in genes encoding proteins of the sarcomere. However, there are HCM patients who do not have a genetic link and are sarcomere mutation negative (Warner E. et al. 2022).

Mutations to *MYBPC3*, which encodes the intermediate filament protein cardiac myosin-binding C, are thought to account for up to 40% of cases of hypertrophic cardiomyopathy, while 15-25% of patients with hypertrophic cardiomyopathy comprise mutation to *MYH7*. Mutations to *TNNT2* account for 5-10% of cases, while *TNNI3* mutations are present in 4-8% of cases. Other genes in which genetic variation has been causally linked to hypertrophic cardiomyopathy include *MYL2* and *MYL3* (which encode myosin light chains), *TPM1* (which encodes α-tropomyosin) and *ACTC1* (which encodes α-cardiac actin).

The hallmarks of hypertrophic cardiomyopathy are upregulated protein synthesis and growth of cytoskeletal networks including tubulins (“microtubule network densification”) and MAP4 (Chinnakkannu P. *et al.*, J Biol Chem. 2010;285:21837–21848. doi: 10.1074/jbc.M110.120709). MAP4 is a key microtubule-binding protein that promotes microtubule network stability and is upregulated in hypertrophic cardiomyocytes. A dense, stabilised microtubule network forms that interferes with myocardiocyte contraction and microtubule-based transport.

Reversal of MTN density by the induction of microtubule depolymerisation in pressure overload hypertrophied myocardium has been effective in the reversal of contractile dysfunction in animal models (Warner E. et al. 2022).

Restrictive cardiomyopathy is characterised by stiffening of the ventricle walls leading to diastolic dysfunction, raised end-diastolic pressure and dilated atria. The stiffer ventricles are unable to expand as efficiently to fill with blood, and so cardiac function is impaired. Restrictive cardiomyopathy is relatively rare, and can be idiopathic, or caused by diseases/conditions affecting the heart.

Diseases/conditions that can cause restrictive cardiomyopathy include amyloidosis, sarcoidosis, primary hyperoxaluria, Fabry disease, Gaucher disease, hereditary hemochromatosis, glycogen storage disease, mucopolysaccharidosis type I (Hurler syndrome), mucopolysaccharidosis type II (Hunter syndrome), Niemann-Pick disease, diabetes, scleroderma, myofibrillar myopathies, pseudoxanthoma elasticum, sarcomeric protein disorders, Werner's syndrome, carcinoid heart disease, endomyocardial fibrosis, hypereosinophilic syndrome, cancers (*e.g.* metastatic cancer), chronic eosinophilic leukemia, endocardial fibroelastosis. Restrictive cardiomyopathy can also occur as a side effect of treatment with certain drugs, including anthracyclines, serotonin, methysergide, ergotamine, mercurial agents and busulfan, and also as a side effect of radiotherapy.

Amyloidosis-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *APOA1*, and mutations to *TTR* such as V122I, I68L, L111M, T60A, S23N, P24S, W41L, V30M and V20I. Fabry disease-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *GLA*, while mutations to *GBA* associated with Gaucher disease have also been linked to restrictive cardiomyopathy. Hereditary hemochromatosis-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *HAMP*, *HFE*, *HFE2*, *HJV*, *PNPLA3*, *SLC40A1* and *TfR2*. Mucopolysaccharidosis type I-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *IDUA*, while mutations to *IDS* associated with mucopolysaccharidosis type II have also been linked to restrictive cardiomyopathy. Niemann-Pick disease-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *NPC1, NPC2* and *SMPD1*. Myofibrillar myopathy-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *BAG3, CRYAB, DES, DNAJB6, FHL1, FLNC, LDB3* and *MYOT*. Sarcomeric protein disorder-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *ACTC, β-MHC, TNNT2, TNNI3, TNNC1, DES, MYH, MYL3, CRYAB*. Pseudoxanthoma elasticum-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *ABCC6*, while mutations to *WRN* associated with Werner's syndrome have also been linked to restrictive cardiomyopathy. Endocardial fibroelastosis-associated mutations that have been linked to restrictive cardiomyopathy include mutations to *BMP5, BMP7* and *TAZ*.

Arrhythmogenic cardiomyopathy (ACM) is another rare form of cardiomyopathy, in which muscle tissue of the ventricle(s) is replaced by fat and/or fibrous tissue, often leading to arrhythmia such as ventricular tachycardia. ACM used to be referred to as arrhythmogenic right ventricular cardiomyopathy (ARVC) or arrhythmogenic right ventricular dysplasia (ARVD) as it was thought only to affect the right ventricle, but further research has determined that the right left or both ventricles may be affected. The causes of arrhythmogenic cardiomyopathy are typically genetic.

Mutations to *PKP2* (which encodes plakophilin 2) are thought to account for 34%-74% of ACM cases, while mutations to *DSG2* (which encodes desmoglein 2) account for 5-26% of ACM cases, and mutations to *DSP* account for 2-39% of ACM cases. Other genes in which mutations giving rise to ACM include *DSC2*, *JUP*, *TMEM43*, *DES* and *PLN*.

Diabetic cardiomyopathy is another form of cardiomyopathy, in which diabetes mellitus leads to myocardial remodelling, loss of left ventricular (LV) pump function and ultimately heart failure. Diabetic cardiomyopathy occurs as a result of the dysregulated glucose and lipid metabolism associated with diabetes, which leads to increased oxidative stress and the activation of multiple inflammatory pathways that mediate cellular and extracellular injury, pathological cardiac remodelling, and diastolic and systolic dysfunction (Tan Y. et al., Nature Reviews Cardiology (2020) 17; 585-607). Aberrant increase in microtubule stability contributes to cardiac dysfunction in diabetic cardiomyopathy (Warner E. et al. 2022, and Table A hereinbelow). Microtubule acetylation is also involved in the pathogenesis of diabetic cardiomyopathy, in which hyperglycemia leads to the formation of advanced glycation end products (AGEs). AGEs promote microtubule stabilisation via the suppression of the SIRT2/acetylated α-tubulin signaling pathway (Warner E. et al. 2022).

The most common version of the nucleotide sequence of a given gene may be referred to as the wildtype allele of the gene. A version of the nucleotide sequence of a given gene comprising a 'genetic variant' or 'mutation' relative to the wildtype allele may be referred to as a 'variant' or 'mutant' allele of the gene. As used herein, a 'genetic variant' of, or 'mutation' to, a given gene refers to an insertion, deletion, substitution to, or larger-scale translocation/rearrangement of, the nucleotide sequence of the gene, relative to the nucleotide sequence of a reference allele not comprising the genetic variant/mutation (*e.g.* the 'non-mutated' or 'wildtype' allele). It will be appreciated that the nucleotide sequence of a variant/mutant allele of a given gene has a nucleotide sequence which is non-identical to the nucleotide sequence of the wildtype allele.

Where a given disease/condition is described herein as being 'associated with' a given genetic variant/mutation or disease/condition, the genetic variant/mutation/disease/condition may be positively-associated with the onset, development or progression of the given disease/condition, and/or severity of one or more symptoms of the given disease/condition. The genetic variant/mutation or disease/condition may be a risk factor for the development or progression of the given disease/condition. In some embodiments, a disease/condition that is associated with a given genetic variant/mutation or disease/condition may be caused or exacerbated by the genetic variant/mutation/disease/condition. Where a given disease/condition is described herein as being 'caused by' a given genetic variant/mutation or disease/condition, the genetic variant/mutation/disease/condition may be directly or indirectly implicated in the pathology of the given disease/condition.

The genetic variant/mutation may result in a change in the level of (gene and/or protein) expression of, and/or a change in the activity of, a gene product (*e.g.* RNA and/or a polypeptide) encoded by the wildtype allele of the relevant gene. The genetic variant/mutation may result in a reduction in the level of a gene product encoded by the wildtype allele of the relevant gene. The genetic variant/mutation may result in an increase in the level of a polypeptide encoded by a disease-associated allele of the relevant gene. The genetic variant/mutation may result in the production of a truncated version of a polypeptide encoded by the wildtype allele of the relevant gene. The genetic variant/mutation may result in the production of a version of a polypeptide encoded by the wildtype allele of the relevant gene which is misfolded and/or degraded. The genetic variant/mutation may result in the production of a polypeptide having altered

activity relative to a polypeptide encoded by the wildtype allele of the relevant gene, *e.g.* a reduced or increased level of an activity possessed by a polypeptide encoded by the wildtype allele, and/or an acquired activity which is not possessed by a polypeptide encoded by the wildtype allele.

In some embodiments, the cardiomyopathy according to the present disclosure is dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy or unclassified cardiomyopathy.

In some embodiments, the cardiomyopathy according to the present disclosure is dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic cardiomyopathy, diabetic cardiomyopathy, ischaemic cardiomyopathy or unclassified cardiomyopathy.

In some embodiments, the cardiomyopathy according to the present disclosure is dilated cardiomyopathy. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with: a genetic variant, infectious disease, infection with a virus, myocarditis, coronary artery disease, an infiltrative disease, pregnancy, hypertension, a connective tissue disease, drug abuse or doxorubicin treatment. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to a gene selected from: *TTN*, *LMNA*, *PLN*, *FLNC*, *DSP*, *DMD*, *ACTC*, *DES*, *MYH7*, *TNNT2*, *TNNI3*, *SGCD* and *SCN5A*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to a gene selected from: *TTN*, *PLN*, *FLNC*, *DSP*, *DMD*, *ACTC*, *DES*, *MYH7*, *TNNT2*, *TNNI3*, *SGCD* and *SCN5A*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to *DSP* or *DES*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to *DSP*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to *DMD.*

In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to a gene encoding a sarcomere protein. As used herein, a 'sarcomeric protein' or a 'sarcomere protein' refers to a constituent polypeptide of a sarcomere. Constituent polypeptides of sarcomeres, *i.e.* sarcomeric proteins include titin, troponin T, troponin I, troponin C1, cardiac myosin-binding C, α-tropomyosin, α-cardiac actin, myosin and filamin C. In some embodiments, the cardiomyopathy is cardiomyopathy associated with mutation to a gene selected from: *TTN*, *TNNT2, TNNI3, TNNC1, MYBPC3, MYH7, TPM1, ACTC1, MYL2*, *MYL3* and *FLNC.* In some embodiments, the cardiomyopathy is cardiomyopathy associated with mutation to a gene selected from: *TTN*, *TNNT2, TNNI3, TNNC1, MYBPC3, MYH7, TPM1, ACTC1, MYL2 and MYL3.* In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to *TTN.*

In some embodiments, the cardiomyopathy according to the present disclosure is hypertrophic cardiomyopathy. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* hypertrophic cardiomyopathy) associated with a genetic variant. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* hypertrophic cardiomyopathy) associated with mutation to a gene selected from: *MYBPC3*, *MYH7, TNNT2, TNNI3, MYL2*, *MYL3*, *TPM1* and *ACTC1*.

In some embodiments, the cardiomyopathy according to the present disclosure is restrictive cardiomyopathy. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* restrictive cardiomyopathy) associated with: a genetic variant, amyloidosis, sarcoidosis, primary hyperoxaluria, Fabry disease, Gaucher disease, hereditary hemochromatosis, glycogen storage disease, mucopolysaccharidosis type I (Hurler syndrome), mucopolysaccharidosis type II (Hunter syndrome), Niemann-Pick disease, diabetes, scleroderma, a myofibrillar myopathy, pseudoxanthoma elasticum, a sarcomeric protein disorder, Werner's syndrome, carcinoid heart disease, endomyocardial fibrosis, hypereosinophilic syndrome, a cancer (*e.g.* a metastatic cancer), chronic eosinophilic leukemia, endocardial fibroelastosis, chemotherapy, radiotherapy, or treatment with an anthracycline, serotonin, methysergide, ergotamine, a mercurial agent or busulfan. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* restrictive cardiomyopathy) associated with mutation to a gene selected from: *APOA1*, *TTR* (*e.g.* V122I, I68L, L111M, T60A, S23N, P24S, W41L, V30M or V20I), *GLA*, *GBA* *HAMP*, *HFE*, *HFE2*, *HJV*, *PNPLA3*, *SLC40A1*, *TfR2*, *IDUA*, *IDS*, *NPC1*, *NPC2*, *SMPD1*, *BAG3*, *CRYAB*, *DNAJB6*, *FHL1*, *FLNC*, *LDB3*, *MYOT*, *ACTC*, *β-MHC*, *TNNT2*, *TNNI3*, *TNNC1*, *DES*, *MYH*, *MYL3*, *CRYAB*, *ABCC6*, *WRN*, *BMP5*, *BMP7* and *TAZ*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* restrictive cardiomyopathy) associated with mutation to *DES*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* restrictive cardiomyopathy) associated with mutation to *FLNC.* In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* restrictive cardiomyopathy) not associated with mutation to *FLNC.*

In some embodiments, the cardiomyopathy according to the present disclosure is arrhythmogenic cardiomyopathy. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* arrhythmogenic cardiomyopathy) associated with a genetic variant. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* arrhythmogenic cardiomyopathy) associated with mutation to a gene selected from: *PKP2*, *DSG2*, *DSP*, *DSC2*, *JUP*, *TMEM43*, *DES* and *PLN*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* arrhythmogenic cardiomyopathy) associated with mutation to a gene selected from: *PKP2*, *DSG2*, *DES*, *DSC2*, *JUP* and *DES*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* arrhythmogenic cardiomyopathy) associated with mutation to *DSP*. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* arrhythmogenic cardiomyopathy) associated with mutation to *PKP2*.

In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to a gene selected from: *DSP*, *TTN, FLNC and PKP2.* In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) associated with mutation to a gene selected from: *DSP*, *TTN, and PKP2.*

In some embodiments, the cardiomyopathy is not cardiomyopathy associated with a laminopathy. Cardiomyopathy associated with a laminopathy may be cardiomyopathy caused by mutation to a gene encoding a lamin. In some embodiment, the cardiomyopathy is cardiomyopathy other than cardiomyopathy associated with a laminopathy. As used herein, a 'laminopathy' is a disease/pathological condition associated with mutation to a gene encoding a lamin. Laminopathies are reviewed *e.g.* by Burke

and Stewart, Nat Rev Mol Cell Biol. (2013) 14(1):13-24, and Hah and Kim, Cells (2019) 8(3): 231, which are both hereby incorporated by reference in their entirety. Laminopathies are commonly associated with tissue-specific defects in load bearing at the nuclear level, which can reduce the tolerance of cells to physical forces.

Genes encoding lamins include *LMNA* (which encodes lamins A and C), and *LMNB1*, *LMNB2*, which encode lamins B1 and B2. In some embodiments, the cardiomyopathy is not cardiomyopathy associated with mutation to *LMNA*. In some embodiments, the cardiomyopathy is cardiomyopathy other than cardiomyopathy associated with mutation to *LMNA*. In some embodiments, the cardiomyopathy is not cardiomyopathy associated with mutation to any of *LMNA*, *LMNB1* and *LMNB2* (*i.e.* is not cardiomyopathy caused mutation to *LMNA*, and is not cardiomyopathy associated with mutation to *LMNB1*, and is not cardiomyopathy associated with mutation to *LMNB2*). In some embodiments, the cardiomyopathy other than cardiomyopathy associated with mutation to any of *LMNA*, *LMNB1* and *LMNB2*.

In some embodiments, the cardiomyopathy is not cardiomyopathy associated with a nuclear envelopathy. Nuclear envelopathies are diseases/pathological conditions associated with mutations to genes encoding nuclear envelope proteins (*i.e.* proteins contained in, or directly/indirectly associated with, the ONM, perinuclear space or INM). Nuclear envelopathies are reviewed *e.g.* by Chi *et al.*, Journal of Biomedical Science (2009) 16:96, which is hereby incorporated by reference in its entirety. Nuclear envelopathies include diseases/pathological conditions associated with a genetic variation in *LMNA*, *LMNB1*, *LMNB2*, *EMD*, *LAP2*, *LBR*, *ZMPSTE24*, *SYNE-1* and *NUP62*. In some embodiments, the cardiomyopathy is not cardiomyopathy associated with mutation to any of *LMNA*, *LMNB1*, *LMNB2*, *EMD*, *LAP2*, *LBR*, *ZMPSTE24*, *SYNE-1* and *NUP62*.

In some embodiments, the cardiomyopathy according to the present disclosure is diabetic cardiomyopathy. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* diabetic cardiomyopathy) associated with one or more of: type 1 diabetes, type 2 diabetes, pre-diabetes, metabolic syndrome, pregnancy-associated hyperglycemia (i.e. gestational diabetes), obesity and hyperglycaemia.

In some embodiments, the cardiomyopathy according to the present disclosure is ischaemic cardiomyopathy. In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* ischaemic cardiomyopathy) associated with one or more of: coronary artery disease, myocardial infarction, diabetes mellitus, pre-diabetes, metabolic syndrome, pregnancy-associated hyperglycemia (i.e. gestational diabetes), hyperglycaemia, amyloidosis, hypertension, obesity and hypercholesterolemia.

In some embodiments, the cardiomyopathy is cardiomyopathy (*e.g.* dilated cardiomyopathy) which is not associated with a genetic variant, for example a genetic variant described herein.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with desmosome deficiency/insufficiency or dysfunction.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with mutation to a gene encoding a desmosome protein. As used herein, a 'desmosome protein' refers to a constituent polypeptide of a desmosome. Constituent polypeptides of desmosomes include desmoplakin, desmin, desmogleins (*e.g.* desmogleins 1, 2, 3, 4), desmocollins (*e.g.* desmocolins 1, 2, 3), plakophilins (*e.g.* plakophilins 1, 2, 3) and plakoglobin. In some embodiments, a gene encoding a desmosome protein is selected from *DSP*, *DES*, *DSG1*, *DSG2*, *DSG3*, *DSG4*, *DSC1*, *DSC2, DSC3*, *PKP1*, *PKP2*, *PKP3* and *JUP*.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with mutation to a gene encoding a constituent polypeptide of a desmosome-intermediate filament complex (DIFC).

Desmosomes (also known as macular adherens) are specialised structures mediating lateral cell-to-cell adhesion between cells particularly of tissue subjected to considerable mechanical stress, such as cardiac muscle tissue, bladder tissue, gastrointestinal mucosa and epithelia. The structure and function of desmosomes is reviewed *e.g.* in Delva *et al.*, Cold Spring Harb Perspect Biol. (2009) 1(2): a002543 and Johnson *et al.*, Cold Spring Harb Perspect Med. (2014) 4(11): a015297, both of which are hereby incorporated by reference in their entirety. Desmosomes are comprised of desmosome-intermediate filament complexes (DIFC), which are formed of a network of cadherins, linker proteins, and intermediate filaments. Desmosomes have an extracellular core area known as the desmoglea, which comprises the cadherins (*e.g.* desmogleins, desmocollins, which associate via heterophilic interactions between their N-termini). At their C-termini, the cadherins associate with plakophilins 1 and 2 and plakoglobin, in the outer dense plaque (ODP). Plakophilins 1 and 2 and plakoglobin are linked by desmoplakin to the inner dense plaque (IDP), which comprises intermediate filaments.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with mutation to a gene selected from: *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with desmoplakin deficiency/insufficiency or dysfunction.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with mutation to *DSP*. Cardiomyopathy associated with mutations to *DSP* have even been suggested to form a separate class of cardiomyopathy, so-called 'desmoplakin cardiomyopathies' – see Smith *et al.*, Circulation (2020) 141(23):1872-1884.

Desmoplakin (also known as DSP) is the protein identified by UniProt P15924. The structure and function of desmoplakin is described *e.g.* in Yuan *et al.*, Chin Med J (Engl). (2021) 134(15):1771-1779, Müller *et al.*, Front. Cell Dev. Biol. (2021) 9:745670 and Mohammed and Chidgey, Journal of Structural Biology (2021) 213(3):107749, all of which are hereby incorporated by reference in their entirety.

Desmoplakin is an essential component of desmosomes, which form intercellular junctions between adjacent cells. Desmoplakins bind via their C-terminus to intermediate filaments of the cytoskeleton (see *e.g.* Stappenbeck and Green, J Cell Biol. (1992) 116(5):1197-209 and Stappenbeck *et al.*, J Cell Biol. (1993) 123(3):691-705), which vary by cell type. In cardiomyocytes, desmoplakin binds to the intermediate filament desmin, while in endothelial and epithelial cells desmoplakin binds to cytokeratin type intermediate filaments, and in dendritic cells desmoplakin binds to vimentin intermediate filaments. At the N-terminus, desmoplakin binds to armadillo family proteins such as plakophilins 1 and 2, and plakoglobin, which in turn bind to the desmosomal cadherins, particularly desmocollins and desmogleins. Thus, desmoplakin serves as an adaptor protein, linking intermediate filaments to cadherins of desmosomes.

Mutations in the human *DSP* gene give rise to various cardiomyopathies, including dilated cardiomyopathy and arrhythmogenic cardiomyopathy. Such mutations to *DSP* are described *e.g.* in Mohammed and Chidgey, Journal of Structural Biology (2021) 213(3):107749 (which is hereby incorporated by reference in its entirety; see in particular Figure 4e), and include G2056R, D2757H, K2689T, R2834H, T2595I, R2541K, R2541S, R2639Q, R2366H, R2366C, D230N, S299R, N375I, S442F, I445V, N458Y, K470E, S507F, N287D, N287S, A206T, W207C, E422K, I533T, A556T, N661I, Y787C, R808C, R808H, R1255K, R1255T, L1348R, T1373A, L1654P, R1775I, R1838H, Q198P, D203N, R222L, D241N, E290K, I305F, A306T, R315C, R315P, M316V, A349P, Y350H, T356K, I368T, E384Q, K401N, N408K, R451G, R451H, I461V, C482Y, Y494F, P515L, L540P, T587N, N593S, M599R, R606W, E721K, Y895H, I870M, I870T, R907H, R908H, L933F, G939S, S987P, T1960P, Y1981C, L1995S, A2019S, A2019V, P2061S, H2062R, D2070N, V2107L, R2083C, K2103E, A2148T, T2177N, R2189Q, T2196I, I2263T, T2267S, A2294G, Q2295H, G2338R, E2343K, I2347V, D2579H, I2593S, T2664N, G2666D, A2674T, M2684V, M2707T, A2709T, R2759S, P2777H, I2797T, M2819L, G2844V, I2869V, K1094N, Y1169N, L1178R, L1178V, Y1188H, R1207K, T1217M, D1251N, D1258E, A1274P, K1288Q, T1319I, R1341H, E1357D, R1392Q, R1392W, E1407D, A1435P, R1458G, Y1512C, L1514P, L1535P, K1537C, K1537H, T1557M, K1581E, K1592R, M1601I, S1623C, V1639M, S1658F, R1666Q, R1666W, H1684R, E1721D, R1738Q, E1740K, E1833V, R1852H, R2366H, T2770C, G2832V, S2616L, G2647D, R1838H, T13I, E21K, V30M, R44Q, C81Y, Q90R, R105Q, E109K, M118K, I125F, C174F and A566T. Mutations to *DSP* associated with Erythrokeratodermia-cardiomyopathy syndrome include L622P, Q616P and H618P. Mutations to *DSP* associated with cardiocutaneous syndromes such as Naxos disease and Carvajal syndrome (which are characterised by a combination of dilated cardiomyopathy, keratoderma and woolly hair) include I560F, T564I, L583P, S597L, E2193K, L2329P, Q2371K, G2375R and A2655D.

In some embodiments, the cardiomyopathy according to the present disclosure is cardiomyopathy associated with one or more of the following mutations to *DSP*: G2056R, D2757H, K2689T, R2834H, T2595I, R2541K, R2541S, R2639Q, R2366H, R2366C, D230N, S299R, N375I, S442F, I445V, N458Y, K470E, S507F, N287D, N287S, A206T, W207C, E422K, I533T, A556T, N661I, Y787C, R808C, R808H, R1255K, R1255T, L1348R, T1373A, L1654P, R1775I, R1838H, Q198P, D203N, R222L, D241N, E290K, I305F, A306T, R315C, R315P, M316V, A349P, Y350H, T356K, I368T, E384Q, K401N, N408K, R451G, R451H, I461V, C482Y, Y494F, P515L, L540P, T587N, N593S, M599R, R606W, E721K, Y895H, I870M, I870T, R907H, R908H, L933F, G939S, S987P, T1960P, Y1981C, L1995S, A2019S, A2019V, P2061S,

H2062R, D2070N, V2107L, R2083C, K2103E, A2148T, T2177N, R2189Q, T2196I, I2263T, T2267S, A2294G, Q2295H, G2338R, E2343K, I2347V, D2579H, I2593S, T2664N, G2666D, A2674T, M2684V, M2707T, A2709T, R2759S, P2777H, I2797T, M2819L, G2844V, I2869V, K1094N, Y1169N, L1178R, L1178V, Y1188H, R1207K, T1217M, D1251N, D1258E, A1274P, K1288Q, T1319I, R1341H, E1357D, R1392Q, R1392W, E1407D, A1435P, R1458G, Y1512C, L1514P, L1535P, K1537C, K1537H, T1557M, K1581E, K1592R, M1601I, S1623C, V1639M, S1658F, R1666Q, R1666W, H1684R, E1721D, R1738Q, E1740K, E1833V, R1852H, R2366H, T2770C, G2832V, S2616L, G2647D, R1838H, T13I, E21K, V30M, R44Q, C81Y, Q90R, R105Q, E109K, M118K, I125F, C174F, A566T, L622P, Q616P, H618P, I560F, T564I, L583P, S597L, E2193K, L2329P, Q2371K, G2375R or A2655D.

In some embodiments of the various aspect of the present disclosure, the cardiomyopathy according to the present disclosure is cardiomyopathy characterised by microtubule dysfunction.

**Diseases/conditions characterised by microtubule dysfunction**

Aspects and embodiments of the present disclosure relate to the treatment and prevention of diseases and conditions comprising/characterised by microtubule dysfunction. The experimental examples of the present disclosure demonstrate that LINC complex inhibition ameliorates pathology in mouse models of disease characterised by dysfunction of the microtubule network.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition in which microtubule dysfunction or deficiency/insufficiency is pathologically-implicated, *e.g.* a disease/condition in which microtubule dysfunction or deficiency/insufficiency is positively-associated with the onset, development or progression of the disease/condition, and/or severity of one or more symptoms of the disease/condition. In some embodiments, microtubule dysfunction or deficiency/insufficiency may be a risk factor for the onset, development or progression of the disease/condition.

As explained hereinabove, dysfunction of microtubules is associated with heart/cardiac disease (Warner E. et al., 2022, Table A hereinbelow). In some embodiments, the disease/condition characterised by microtubule dysfunction is heart disease.

Dysfunction of microtubules is often associated with cardiomyopathies. In some embodiments, the disease/condition characterised by microtubule dysfunction is cardiomyopathy.

In some embodiments, the disease or condition characterised by microtubule dysfunction is characterised by cardiomyopathy. That is, in some embodiments, cardiomyopathy is a symptom of the disease or condition characterised by microtubule dysfunction.

In some embodiments, the cardiomyopathy is a cardiomyopathy as described herein. In some embodiments, the cardiomyopathy is non-genetic cardiomyopathy.

Various conditions that are characterised by dysfunction of microtubules are reviewed in Warner E. et al. 2022, Circulation Research. 2022;130:1723–1741, which is hereby incorporated by reference in its entirety. For example, in patients suffering from cardiomyopathy, such as ischaemic cardiomyopathy, hypertrophic cardiomyopathy, diabetic cardiomyopathy, and dilated cardiomyopathy, there is a drastic increase in the density and stability of microtubules. Dystrophic cardiomyopathy, which is cardiomyopathy arising from mutations in *DMD* (which encodes dystrophin) has also been associated with dysfunction of microtubules (see Table A hereinbelow).

In some embodiments, the disease/condition characterised by microtubule dysfunction is or is characterised by ischaemic cardiomyopathy, hypertrophic cardiomyopathy, diabetic cardiomyopathy, and dilated cardiomyopathy. In some embodiments, the disease/condition characterised by microtubule dysfunction is or is characterised by dystrophic cardiomyopathy.

Heart failure can occur when the heart muscle is stiff and unable to relax normally (diastolic failure). In failing cardiomyocytes, an increase in stiffness of the microtubule network (MTN) leads to contractile dysfunction. This increase in MTN stiffness is often induced by increased interaction between detyrosinated microtubules and the intermediate filament Desmin at the sarcomere Z disc (Warner E. et al., 2022). That is, detyrosinated microtubules provide mechanical resistance that can impede the motion of contracting cardiomyocytes (Chen C. et al., Nature Medicine (2018) volume 24, 1225–1233).

Failing cardiomyocytes are also often characterised by an increase in the density of the microtubule network, which is associated with increased myocyte stiffness and impaired contractility (Chen C. et al., Nature Medicine (2018) volume 24, 1225–1233). The increase in MTN density is concomitant with a significant upregulation of total tubulin in ischaemic cardiomyopathy, hypertrophic cardiomyopathy, and dilated cardiomyopathy-affected hearts.

The level of detyrosinated α-tubulin was significantly higher in cardiomyocytes isolated from ischaemic hearts compared with cardiomyocytes isolated from mice that received a sham operation, in contrast to the remaining cell pool tested, *e.g.* immune cells, fibroblasts and endothelial cells (Yu X. et al. 2021 Nature: 594, 560–565, incorporated herein by reference in its entirety).

In some embodiments, the disease/condition characterised by microtubule dysfunction is heart failure. In some embodiments, the heart failure is associated with ischaemic injury. That is, in some embodiments, the heart failure is ischaemic heart failure.

Various models of Heart Failure with preserved Ejection Fraction (HFpEF) and Heart Failure with reduced Ejection Fraction (HFrEF) have been reported to be characterised by microtubule dysfunction, as summarised in Table A hereinbelow.

Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome in which patients have signs and symptoms of heart failure as the result of high left ventricular (LV) filling pressure despite normal or near normal LV ejection fraction (LVEF; ≥50%) (Harper A. et al. Clin Med (Lond). 2018; 18(Suppl 2): s24–

s29). Heart Failure with reduced Ejection Fraction (HFrEF) is heart failure exhibiting a reduced left ventricular EF (LVEF is <40%). In some cases, hypertrophic cardiomyopathy leads to heart failure with preserved (HFpEF) or reduced ejection fraction (HFrEF).

In some embodiments, the disease/condition characterised by microtubule dysfunction is Heart Failure with preserved Ejection Fraction (HFpEF). In some embodiments, the disease/condition characterised by microtubule dysfunction is Heart Failure with reduced Ejection Fraction (HFrEF). In some embodiments, HFpEF and/or HFrEF is associated with hypertrophic cardiomyopathy.

Aortic stenosis is characterised by microtubule dysfunction, as described in *e.g.* Zile MR et al. J Am Coll Cardiol. 2001 Mar 15;37(4):1080-4 (see Table A hereinbelow). In some embodiments, the disease/condition characterised by microtubule dysfunction is aortic stenosis.

Stones R. et al. J Mol Cell Cardiol. 2013; 56: 91–96 describe that microtubule proliferation is a common response to pulmonary hypertension in failing right ventricles (see Table A hereinbelow). Prins K. et al. J Am Heart Assoc. 2017;6(6):e006195 describe that pulmonary arterial hypertension (PAH) is characterised by abnormal microtubular structure in right ventricular (RV) cardiomyocytes which impairs RV function (see Table A hereinbelow). Drug-induced pulmonary arterial hypertension (D-PAH) is a form of pulmonary hypertension (PH) defined by severe small vessel loss and obstructive vasculopathy, which leads to progressive right heart failure and death (Orcholski M et al. Am J Physiol Lung Cell Mol Physiol. 2018; 314(6): L967–L983).

In some embodiments, the disease/condition characterised by microtubule dysfunction is pulmonary arterial hypertension (PAH). In some embodiments, the disease/condition characterised by microtubule dysfunction is pulmonary hypertension (PH). In some embodiments, the disease/condition characterised by microtubule dysfunction is drug-induced pulmonary hypertension or hypoxia-induced pulmonary hypertension (*e.g.* hypoxia-induced neonatal pulmonary hypertension).

Microtubule dysfunction has also been described to characterise right ventricular pressure overload and left ventricular pressure overload (summarised in Table A hereinbelow). In some embodiments, the disease/condition characterised by microtubule dysfunction is right ventricular (RV) pressure overload. In some embodiments, the disease/condition characterised by microtubule dysfunction is left ventricular (LV) pressure overload.

Myocardial infarction (also known as heart attack) is associated with increased microtubule associated protein 4 (MAP4) phosphorylation (see Table A hereinbelow). In some embodiments, the disease/condition characterised by microtubule dysfunction is myocardial infarction, such as acute myocardial infarction.

As used herein ‘microtubule dysfunction’ or ‘microtubule network dysfunction’ refers to a state in which normal function of the microtubule cytoskeleton is impaired/aberrant/insufficient, with the result that a subject exhibiting microtubule dysfunction in a specific tissue (*e.g.* in heart tissue) displays improper

function of the associated tissue. Normal functions of the microtubule network and microtubule dysfunction are reviewed in *e.g.* Warner E. et al. (2022).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by aberrant organisation of the microtubule network (*e.g.* in a cell), *e.g.* as compared to the organisation observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the density of the microtubule network (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the density observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell). In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the density of the perinuclear microtubule network (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the density observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell).

As used herein 'microtubule density' refers to the number of microtubules as a function of cross-sectional area of the cell body. The microtubule density may be determined using immunostaining, as described herein and *e.g.* in Chen C. et al., Nature Medicine (2018) volume 24, 1225–1233, which is incorporated herein by reference in its entirety.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the mechanical resistance of the microtubule network (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the mechanical resistance observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the mechanical force transmitted to the cell nucleus/nuclear envelope (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the mechanical force observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by impaired contraction/contractility of heart tissue or a cardiomyocyte, *e.g.* as compared to the contraction/contractility of heart tissue or a cardiomyocyte in the absence of the disease/condition (*e.g.* in a healthy subject). Chen C. et al., Nature Medicine (2018) volume 24, 1225–1233 describes a method for measuring cell contractility.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the level/expression of tubulin (*e.g.* in a

cell such as a cardiomyocyte), *e.g.* as compared to the level/expression in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell). In some embodiments, the disease/condition is characterised by an increase of the level/expression of α-tubulin. In some embodiments, the disease/condition is characterised by an increase of the level/expression of β-tubulin.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the level/proportion of detyrosinated tubulin (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the level/expression in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell). The proportion of detyrosinated tubulin refers to the ratio of detyrosinated α-tubulin to the total tubulin (*e.g.* in a cell). 'Total tubulin' may refer to the protein level of α-tubulin and/or β-tubulin, which are the main components of microtubules.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the level/proportion of acetylated tubulin (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the level/expression in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell). In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by a decrease in the level/proportion of acetylated tubulin (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the level/expression in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell). The proportion of acetylated tubulin refers to the ratio of acetylated α-tubulin to the total tubulin (*e.g.* in a cell). 'Total tubulin' may refer to the protein level of α-tubulin and/or β-tubulin, which are the main components of microtubules.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the density of detyrosinated microtubules (*e.g.* in a cell such as a cardiomyocyte), *e.g.* as compared to the population of detyrosinated microtubules in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased cell).

In some embodiments, microtubule dysfunction refers to perinuclear microtubule dysfunction. That is, the dysfunction relates to the population/density/function/detyrosination level *etc.*, as described above, of perinuclear microtubules.

A disease/condition in which microtubule dysfunction is pathologically-implicated may be characterised by one or more of the following:

impaired contractility of a cell (*e.g.* a cardiomyocyte), *e.g.* as compared to the contractility observed in the absence of the disease/condition;

aberrant organisation of the microtubule network (*e.g.* in a cell), *e.g.* as compared to the organisation observed in the absence of the disease/condition;

an increase in the mechanical force transmitted to the cell nucleus/nuclear envelope, *e.g.* as compared to the level of mechanical force in the absence of the disease/condition;

aberrant organisation of the sarcomere (*e.g.* in a cell), *e.g.* as compared to the organisation observed in the absence of the disease/condition;

an increase in the mechanical resistance of the microtubule network, *e.g.* as compared to the level in the absence of the disease/condition;

a decrease in contractile magnitude of a cardiomyocyte, *e.g.* as compared to the contractile magnitude in the absence of the disease/condition;

an increase in the density of the microtubule network, for example of perinuclear, interfibrillar and/or cortical microtubules, *e.g.* as compared to the density in the absence of the disease/condition;

an increase in the level/expression of tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* as compared to the level/expression in the absence of the disease/condition,

an increase in the level/proportion of detyrosinated tubulin, *e.g.* as compared to the level/proportion in the absence of the disease/condition;

an increase in the level/proportion of microtubules interacting with an intermediate filament, for example Desmin; *e.g.* as compared to the level/proportion in the absence of the disease/condition; and

an increase in the level/proportion of acetylated tubulin, *e.g.* as compared to the level/proportion in the absence of the disease/condition;

a decrease in the level/proportion of acetylated tubulin, *e.g.* as compared to the level/proportion in the absence of the disease/condition;

aberrant organisation of the perinuclear microtubule network (*e.g.* in a cell), *e.g.* as compared to the organisation observed in the absence of the disease/condition;

an increase in the mechanical force transmitted to the cell nucleus/nuclear envelope by the perinuclear microtubule network, *e.g.* as compared to the level of mechanical force in the absence of the disease/condition;

an increase in the mechanical resistance of the perinuclear microtubule network, *e.g.* as compared to the level in the absence of the disease/condition;

an increase in the density of the perinuclear microtubule network, *e.g.* as compared to the density in the absence of the disease/condition;

an increase in the level/expression of perinuclear tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* as compared to the level/expression in the absence of the disease/condition,

an increase in the level/proportion of detyrosinated perinuclear tubulin, *e.g.* as compared to the level/proportion in the absence of the disease/condition; and

an increase in the level/proportion of perinuclear microtubules interacting with an intermediate filament, for example Desmin; *e.g.* as compared to the level/proportion in the absence of the disease/condition.

*Disease models in which microtubule dysfunction is observed*

Chen C. and co-workers performed functional tests on human myocytes to support a role for modulating microtubule proliferation and dysfunction to treat a range of cardiac diseases. Chen C. et al. noted that earlier studies had demonstrated microtubule proliferation in patients with aortic stenosis and in various animal models of left ventricular pressure overload, right ventricular pressure overload, drug induced

pulmonary hypertension, dystrophic cardiomyopathy and diabetic cardiomyopathy, summarised in Table A below.

Multiple other lines of evidence implicate microtubule dysfunction in heart disease. HDAC6 inhibition, which was shown to increase protein levels of acetylated tubulin, has been proposed as a therapeutic for HFpEF, deficiency of microtubule-affinity regulating kinase 4 (MARK4) reduces left ventricular ejection fraction after acute myocardial infarction in mice, and microtubule associated protein 4 (MAP4) phosphorylation was found to be increased in myocardial infarction (MI) and transverse aortic constriction (TAC) mouse models. Furthermore, in ZSF1 obese rats, a HFpEF model, tubulin and detyrosinated tubulin levels increased.

**Table A.** Disease models in which microtubule dysfunction is observed (Source: Chen C. et al. Nature Medicine (2018), 24, 1225–1233 or as indicated herein below)

| Organism | Disease model | Reference |
|---|---|---|
| Human | Cardiomyocytes from patients with ischemic cardiomyopathy (ICM), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy leading to heart failure with preserved or reduced ejection fraction (HCM with HFpEF and HCM with HFrEF, respectively) | Chen CY, et al.. Suppression of detyrosinated microtubules improves cardiomyocyte function in human heart failure. Nat Med. 2018 Aug;24(8):1225-1233. |
| Human | aortic stenosis | Zile MR, Green GR, Schuyler GT, Aurigemma GP, Miller DC, Cooper G 4th. Cardiocyte cytoskeleton in patients with left ventricular pressure overload hypertrophy. J Am Coll Cardiol. 2001 Mar 15;37(4):1080-4. |
| Dog | Left ventricular pressure overload (HFrEF) | Tagawa H, Koide M, Sato H, Zile MR, Carabello BA, Cooper G 4th. Cytoskeletal role in the transition from compensated to decompensated hypertrophy during adult canine left ventricular pressure overloading. Circ Res. 1998 Apr 20;82(7):751-61 |
| Mouse | Left ventricular pressure overload (HFrEF) | Zhang C, et al. Microtubule-mediated defects in junctophilin-2 trafficking contribute to myocyte transverse-tubule remodeling and Ca2+ handling dysfunction in heart failure. Circulation. 2014 Apr 29;129(17):1742-50. |
| Cat | Right ventricular pressure overload | Tsutsui H, Ishihara K, Cooper G 4th. Cytoskeletal role in the contractile dysfunction of hypertrophied myocardium. Science. 1993 Apr 30;260(5108):682-7. doi: 10.1126/science.8097594. PMID: 8097594. |
| Rat | Drug induced pulmonary hypertension | Stones R, Benoist D, Peckham M, White E. Microtubule proliferation in right ventricular myocytes of rats with monocrotaline-induced pulmonary hypertension. J Mol Cell Cardiol. 2013 Mar;56:91-6<br><br>Prins KW, et al. Colchicine Depolymerizes Microtubules, Increases Junctophilin-2, and Improves Right Ventricular Function in Experimental Pulmonary Arterial Hypertension. J Am Heart Assoc. 2017 May 31;6(6):e006195 |
| Calf | Drug induced pulmonary hypertension | Lemler MS, Bies RD, Frid MG, Sastravaha A, Zisman LS, Bohlmeyer T, Gerdes AM, Reeves JT, |

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|---|---|---|
| | | Stenmark KR. Myocyte cytoskeletal disorganization and right heart failure in hypoxia-induced neonatal pulmonary hypertension. Am J Physiol Heart Circ Physiol. 2000 Sep;279(3):H1365-76. |
| Mouse | Dystrophic cardiomyopathy | Prosser BL, Ward CW & Lederer WJ X-ROS Signaling: Rapid Mechano-Chemo Transduction in Heart. Science 333, 1440–1445 (2011)<br><br>Prins KW, Asp ML, Zhang H, Wang W, & Metzger JM Microtubule-Mediated Misregulation of Junctophilin-2 Underlies T-Tubule Disruptions and Calcium Mishandling in mdx Mice. JACC: Basic to Transl. Sci 1, 122–130 (2016) |
| Rat | Diabetic cardiomyopathy | Shiels H, et al. Stable microtubules contribute to cardiac dysfunction in the streptozotocin-induced model of type 1 diabetes in the rat. Mol Cell Biochem. 2007 Jan;294(1-2):173-80. |
| Mouse | HDAC6 inhibition, which was shown to increase protein levels of acetylated tubulin, has been proposed as a therapeutic for HFpEF | Ranjbarvaziri S, et al. Targeting HDAC6 to treat heart failure with preserved ejection fraction in mice. Nat Commun. 2024 Feb 26;15(1):1352. |
| Mouse | deficiency of microtubule-affinity regulating kinase 4 (MARK4) reduces left ventricular ejection fraction after acute myocardial infarction in mice | Yu X, et al. MARK4 controls ischaemic heart failure through microtubule detyrosination. Nature. 2021 Jun;594(7864):560-565. |
| Mouse | microtubule associated protein 4 (MAP4) phosphorylation was found to be increased in myocardial infarction (MI) and transverse aortic constriction (TAC) mouse models | Li L, et al. Microtubule associated protein 4 phosphorylation leads to pathological cardiac remodeling in mice. EBioMedicine. 2018 Nov;37:221-235. doi: 10.1016/j.ebiom.2018.10.017. Epub 2018 Oct 13. PMID: 30327268; PMCID: PMC6286641. |
| Rats | ZSF1 obese rats, a HFpEF model, tubulin and detyrosinated tubulin levels increased | Schulz L, et al. Tubulin expression and modification in heart failure with preserved ejection fraction (HFpEF). Sci Rep. 2022 Sep 21;12(1):15734. doi: 10.1038/s41598-022-19766-5. PMID: 36131110; PMCID: PMC9492725. |

**Diseases/conditions characterised by desmosome/desmoplakin deficiency/dysfunction**

Aspects and embodiments of the present disclosure relate to the treatment and prevention of diseases and conditions comprising/characterised by desmosome deficiency/insufficiency or dysfunction. The experimental examples of the present disclosure demonstrate that LINC complex inhibition ameliorates pathology in a mouse model of disease characterised by desmosome deficiency/insufficiency.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition in which desmosome dysfunction or deficiency/insufficiency is pathologically-implicated, *e.g.* a disease/condition in which desmosome dysfunction or deficiency/insufficiency is positively-associated with the onset, development or progression of the disease/condition, and/or severity of one or more symptoms of the disease/condition. In some embodiments, desmosome dysfunction or deficiency/insufficiency may be a risk factor for the onset, development or progression of the disease/condition.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by a reduction in the number of desmosomes (*e.g.* formed between adjacent cells of a given tissue), *e.g.* as compared to the number observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased tissue).

Diseases/conditions characterised by desmosome deficiency/insufficiency or dysfunction include diseases/conditions characterised by a reduction in the expression/activity of a desmosome protein (*e.g.* diseases/conditions characterised by a reduction in the expression/activity of a constituent polypeptide of a desmosome-intermediate filament complex (DIFC)).

Diseases/conditions characterised by desmosome deficiency/insufficiency or dysfunction include diseases/conditions associated with mutations to genes encoding desmosome proteins. In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition associated with mutation to a gene encoding a constituent polypeptide of a desmosome-intermediate filament complex (DIFC). In some embodiments, the disease/condition is associated with mutation to a gene selected from *DSP*, *DES*, *DSG1*, *DSG2*, *DSG3*, *DSG4*, *DSC1*, *DSC2, DSC3*, *PKP1*, *PKP2*, *PKP3* and *JUP*. In some embodiments, the disease/condition is associated with mutation to a gene selected from *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*.

Aspects and embodiments of the present disclosure relate to the treatment and prevention of diseases and conditions comprising/characterised by desmoplakin deficiency/insufficiency or dysfunction. The experimental examples of the present disclosure demonstrate that LINC complex inhibition ameliorates pathology in a mouse model of disease caused by desmoplakin deficiency/insufficiency.

In some embodiments, the disease or condition characterised by desmosome/desmoplakin insufficiency/deficiency or dysfunction is characterised by cardiomyopathy. That is, in some embodiments, cardiomyopathy is a symptom of the disease or condition characterised by desmosome/desmoplakin insufficiency/deficiency or dysfunction.

As explained above, mutations to genes encoding desmosome proteins give rise to cardiomyopathies. Mutations to *DSP* are associated with dilated cardiomyopathy and arrhythmogenic cardiomyopathy. Mutations to *DES* are associated with dilated cardiomyopathy, arrhythmogenic cardiomyopathy and restrictive cardiomyopathy. Mutations to *DSG2*, *PKP2* and *JUP* are all associated with arrhythmogenic cardiomyopathy.

Mutations to *DSP* give rise to cardiomyopathies (particularly dilated cardiomyopathy and arrhythmogenic cardiomyopathy) and diseases/conditions characterised by cardiomyopathy such as Erythrokeratodermia-cardiomyopathy syndrome and cardiocutaneous syndromes such as Naxos disease and Carvajal syndrome. Mutations in *DSP* are also associated with various other diseases/conditions, including skin fragility (epidermolysis bullosa) and palmoplantar keratoderma. Autoantibodies to desmoplakin are a hallmark of the autoimmune disease paraneoplastic pemphigus. Decreased desmoplakin expression has

also been observed in oropharyngeal cancer and breast cancer patients; it is thought that its reduced expression may alter cell-cell adhesion properties, and potentiate metastasis.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure may be a disease/condition in which desmoplakin deficiency/insufficiency or dysfunction is pathologically-implicated, *e.g.* a disease/condition in which desmoplakin deficiency/insufficiency or dysfunction is positively-associated with the onset, development or progression of the disease/condition, and/or severity of one or more symptoms of the disease/condition. In some embodiments, desmoplakin deficiency/insufficiency or dysfunction may be a risk factor for the onset, development or progression of the disease/condition.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by a reduction in the expression/activity of desmoplakin, *e.g.* as compared to the level of expression/activity in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased tissue). In some embodiments, the disease/condition is characterised by a reduction in the number of desmosomes (*e.g.* formed between adjacent cells of a given tissue), *e.g.* as compared to the number observed in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased tissue).

Diseases/conditions characterised by desmosome/desmoplakin deficiency/insufficiency or dysfunction include diseases/conditions associated with mutations to *DSP*.

In some embodiments, the mutation to *DSP* is known or predicted to reduce the level of a desmoplakin isoform encoded by the wildtype allele (*e.g.* DPI, DPII and/or DSPIa). In some embodiments, the mutation is a missense mutation. In some embodiments, the mutation is known or predicted to result in the production of a truncated version of a desmoplakin isoform encoded by the wildtype allele. In some embodiments, the mutation is known or predicted to result in the production of a desmoplakin isoform which is dysfunctional and/or non-functional. In some embodiments, the mutation is known or predicted to result in the production of a desmoplakin isoform which displays a reduced level of a desmoplakin-mediated function as compared to the level of the relevant function displayed by a desmoplakin isoform encoded by the wildtype allele (*e.g.* DPI, DPII and/or DSPIa). In some embodiments, the mutation is known or predicted to result in the production of a desmoplakin isoform which is misfolded and/or degraded. In some embodiments the mutation is known or predicted to increase the level of a disease-associated desmoplakin variant. In some embodiments the mutation is known or predicted to increase the level of a desmoplakin isoform encoded by a disease-associated allele of *DSP*.

Diseases/conditions associated with mutations to *DSP* are described *e.g.* in Yuan *et al.*, Chin Med J (Engl) (2021) 134(15):1771-1779, Smith *et al.*, Circulation (2020) 141(23):1872-1884 and Mohammed and Chidgey, Journal of Structural Biology (2021) 213(3):107749, all of which are hereby incorporated by reference in their entirety. Disease-associated missense mutations to *DSP* include G2056R, D2757H, K2689T, R2834H, T2595I, R2541K, R2541S, R2639Q, R2366H, R2366C, D230N, S299R, N375I, S442F, I445V, N458Y, K470E, S507F, N287D, N287S, A206T, W207C, E422K, I533T, A556T, N661I, Y787C,

R808C, R808H, R1255K, R1255T, L1348R, T1373A, L1654P, R1775I, R1838H, Q198P, D203N, R222L, D241N, E290K, I305F, A306T, R315C, R315P, M316V, A349P, Y350H, T356K, I368T, E384Q, K401N, N408K, R451G, R451H, I461V, C482Y, Y494F, P515L, L540P, T587N, N593S, M599R, R606W, E721K, Y895H, I870M, I870T, R907H, R908H, L933F, G939S, S987P, T1960P, Y1981C, L1995S, A2019S, A2019V, P2061S, H2062R, D2070N, V2107L, R2083C, K2103E, A2148T, T2177N, R2189Q, T2196I, I2263T, T2267S, A2294G, Q2295H, G2338R, E2343K, I2347V, D2579H, I2593S, T2664N, G2666D, A2674T, M2684V, M2707T, A2709T, R2759S, P2777H, I2797T, M2819L, G2844V, I2869V, K1094N, Y1169N, L1178R, L1178V, Y1188H, R1207K, T1217M, D1251N, D1258E, A1274P, K1288Q, T1319I, R1341H, E1357D, R1392Q, R1392W, E1407D, A1435P, R1458G, Y1512C, L1514P, L1535P, K1537C, K1537H, T1557M, K1581E, K1592R, M1601I, S1623C, V1639M, S1658F, R1666Q, R1666W, H1684R, E1721D, R1738Q, E1740K, E1833V, R1852H, R2366H, T2770C, G2832V, S2616L, G2647D, R1838H, T13I, E21K, V30M, R44Q, C81Y, Q90R, R105Q, E109K, M118K, I125F, C174F, A566T, I560F, T564I, L583P, S597L, E2193K, L2329P, Q2371K, G2375R, A2655D, N287K, R2366H, R2366C, L622P, Q616P, H618P, P498L, R1184W, S610P, H586P and H586Y.

In some embodiments, the *DSP* mutation is a mutation associated with a heart disease, *e.g.* cardiomyopathy, dilated cardiomyopathy and arrhythmogenic cardiomyopathy. Such mutations to *DSP* include G2056R, D2757H, K2689T, R2834H, T2595I, R2541K, R2541S, R2639Q, R2366H, R2366C, D230N, S299R, N375I, S442F, I445V, N458Y, K470E, S507F, N287D, N287S, A206T, W207C, E422K, I533T, A556T, N661I, Y787C, R808C, R808H, R1255K, R1255T, L1348R, T1373A, L1654P, R1775I, R1838H, Q198P, D203N, R222L, D241N, E290K, I305F, A306T, R315C, R315P, M316V, A349P, Y350H, T356K, I368T, E384Q, K401N, N408K, R451G, R451H, I461V, C482Y, Y494F, P515L, L540P, T587N, N593S, M599R, R606W, E721K, Y895H, I870M, I870T, R907H, R908H, L933F, G939S, S987P, T1960P, Y1981C, L1995S, A2019S, A2019V, P2061S, H2062R, D2070N, V2107L, R2083C, K2103E, A2148T, T2177N, R2189Q, T2196I, I2263T, T2267S, A2294G, Q2295H, G2338R, E2343K, I2347V, D2579H, I2593S, T2664N, G2666D, A2674T, M2684V, M2707T, A2709T, R2759S, P2777H, I2797T, M2819L, G2844V, I2869V, K1094N, Y1169N, L1178R, L1178V, Y1188H, R1207K, T1217M, D1251N, D1258E, A1274P, K1288Q, T1319I, R1341H, E1357D, R1392Q, R1392W, E1407D, A1435P, R1458G, Y1512C, L1514P, L1535P, K1537C, K1537H, T1557M, K1581E, K1592R, M1601I, S1623C, V1639M, S1658F, R1666Q, R1666W, H1684R, E1721D, R1738Q, E1740K, E1833V, R1852H, R2366H, T2770C, G2832V, S2616L, G2647D, R1838H, T13I, E21K, V30M, R44Q, C81Y, Q90R, R105Q, E109K, M118K, I125F, C174F and A566T.

In some embodiments, the *DSP* mutation is a mutation associated with a cardiocutaneous syndrome (CCS). For example, the *DSP* deletion variant c.825_827del has been reported in a subject having cardiocutaneous syndrome characterised by palmoplantar keratoderma and arrhythmogenic cardiomyopathy (Çimen *et al.*, J Clin Med. (2023) 12(3): 913). Cardiocutaneous syndromes include Naxos disease and Carvajal syndrome.

In some embodiments, the *DSP* mutation is a mutation associated with Naxos disease or Carvajal syndrome. Such mutations to *DSP* include I560F, T564I, L583P, S597L, E2193K, L2329P, Q2371K, G2375R and A2655D.

In some embodiments, the *DSP* mutation is a mutation associated with skin fragility-woolly hair syndrome. Such mutations to *DSP* include N287K, R2366H and R2366C.

In some embodiments, the *DSP* mutation is a mutation associated with erythrokeratodermia-cardiomyopathy syndrome. Such mutations to *DSP* include L622P, Q616P and H618P.

In some embodiments, the *DSP* mutation is a mutation associated with non-syndromic alopecia. Such mutations to *DSP* include P498L.

In some embodiments, the *DSP* mutation is a mutation associated with palmoplantar keratoderma. Such mutations to *DSP* include R1184W.

In some embodiments, the *DSP* mutation is a mutation associated with severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome. Such mutations to *DSP* include S610P, H586P and H586Y.

In aspects and embodiments of the present disclosure, the disease/condition to be treated/prevented is selected from: cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, a disease/condition in which desmosome deficiency/insufficiency or dysfunction is pathologically-implicated, a disease/condition in which desmosome deficiency/insufficiency or dysfunction is pathologically-implicated, a disease/condition characterised by a reduction in the number of desmosomes, a disease/condition associated with mutation to a gene encoding a desmosome protein (*e.g.* selected from *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*), a disease/condition characterised by a reduction in the expression/activity of a desmosome protein, a disease/condition in which desmoplakin deficiency/insufficiency or dysfunction is pathologically-implicated, a disease/condition characterised by a reduction in the expression/activity of desmoplakin, a disease/condition associated with mutation to *DSP*, a skin disease, a cardiocutaneous syndrome (CCS), Naxos disease, Carvajal syndrome, keratoderma, palmoplantar keratoderma, epidermolysis bullosa, skin fragility, woolly hair syndrome, erythrokeratodermia-cardiomyopathy syndrome, non-syndromic alopecia, palmoplantar keratoderma and severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome.

**Diseases/conditions characterised by sarcomere/Titin deficiency/dysfunction**

Aspects and embodiments of the present disclosure relate to the treatment and prevention of diseases and conditions comprising/characterised by sarcomere/Titin deficiency/insufficiency or dysfunction. The experimental examples of the present disclosure demonstrate that LINC complex inhibition ameliorates pathology in a mouse model of disease characterised by sarcomere/Titin deficiency/insufficiency.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition in which sarcomere/Titin dysfunction or deficiency/insufficiency is pathologically-implicated, e.g. a disease/condition in which sarcomere/Titin dysfunction or deficiency/insufficiency is positively-associated with the onset, development or progression of the

disease/condition, and/or severity of one or more symptoms of the disease/condition. In some embodiments, sarcomere/Titin dysfunction or deficiency/insufficiency may be a risk factor for the onset, development or progression of the disease/condition.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by disorganisation of the sarcomere, resulting in a reduction of contractile force, e.g. as compared to the organization of the sarcomere observed in the absence of the disease/condition (e.g. in a healthy subject, or in equivalent non-diseased tissue).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by the increase in metabolic stress, e.g. as compared to the cardiac metabolism observed in the absence of the disease/condition (e.g. in a healthy subject, or in equivalent non-diseased tissue).

Diseases/conditions characterised by Titin deficiency/insufficiency or dysfunction include diseases/conditions characterised by a reduction in the expression/activity of the Titin protein (e.g. diseases/conditions characterised by a reduction in the expression/activity of the Titin polypeptide.

Diseases/conditions characterised by sarcomere deficiency/insufficiency or dysfunction include diseases/conditions associated with mutations to genes encoding sarcomere proteins. In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition associated with mutation to a gene encoding a constituent polypeptide of a sarcomere. In some embodiments, the disease/condition is associated with mutation to a gene selected from *TTN, MYH7, TNNT2, TNNI3, TNNC1, TPM1, MYBPC3, MYL2, MYL3, CSRP3, RBM20, ACTN2, TCAP* and *ACTC1*. In some embodiments, the disease/condition is associated with mutation to a gene selected from *TTN, TNNT2, TPM1, MYH7* and *MYBPC3*.

Aspects and embodiments of the present disclosure relate to the treatment and prevention of diseases and conditions comprising/characterised by Titin deficiency/insufficiency or dysfunction. The experimental examples of the present disclosure demonstrate that LINC complex inhibition ameliorates pathology in a mouse model of disease caused by Titin deficiency/insufficiency.

In some embodiments, the disease or condition characterised by sarcomere/Titin insufficiency/deficiency or dysfunction is characterised by cardiomyopathy. That is, in some embodiments, cardiomyopathy is a symptom of the disease or condition characterised by sarcomere/Titin insufficiency/deficiency or dysfunction.

As explained above, mutations to genes encoding sarcomere proteins give rise to cardiomyopathies. Mutations to *TTN* are associated with dilated cardiomyopathy, arrhythmogenic cardiomyopathy, restrictive cardiomyopathy and hypertrophic cardiomyopathy. Mutations to *TNNT2* are associated with dilated cardiomyopathy and hypertrophic cardiomyopathy. Mutations to *TPM1* are associated with dilated cardiomyopathy. Mutations to *MYH7* are associated with dilated cardiomyopathy, hypertrophic

cardiomyopathy and left ventricular noncompaction cardiomyopathy. Mutations to *MYBPC3* are all associated with hypertrophic cardiomyopathy and left ventricular noncompaction cardiomyopathy.

Mutations to *TTN* give rise to cardiomyopathies (particularly dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy and arrhythmogenic cardiomyopathy) and diseases/conditions characterised by cardiomyopathy such as early adult onset recessive distal titinopathy, early-onset myopathy with fatal cardiomyopathy, multi-minicore disease with heart disease and Emery-Dreifuss muscular dystrophy (EDMD). Mutations in Titin are also associated with various other diseases/conditions, including pure skeletal muscle myopathies: limb girdle muscular dystrophy type 2J (LGMD2J), late-onset autosomal dominant tibial muscular dystrophy (TMD), hereditary myopathy with early respiratory failure (HMERF), and congenital centronuclear myopathy (CNM).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure may be a disease/condition in which Titin deficiency/insufficiency or dysfunction is pathologically-implicated, e.g. a disease/condition in which Titin deficiency/insufficiency or dysfunction is positively-associated with the onset, development or progression of the disease/condition, and/or severity of one or more symptoms of the disease/condition. In some embodiments, Titin deficiency/insufficiency or dysfunction may be a risk factor for the onset, development or progression of the disease/condition.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by a reduction in the expression/activity of Titin, e.g. as compared to the level of expression/activity in the absence of the disease/condition (e.g. in a healthy subject, or in equivalent non-diseased tissue). In some embodiments, the disease/condition is characterised by the disorganisation of the sarcomere, resulting in a reduction of contractile force, e.g. as compared to the organization of the sarcomere observed in the absence of the disease/condition (e.g. in a healthy subject, or in equivalent non-diseased tissue). In some embodiments, the disease/condition is characterised by the increase in metabolic stress, e.g. as compared to the cardiac metabolism observed in the absence of the disease/condition (e.g. in a healthy subject, or in equivalent non-diseased tissue).

Diseases/conditions characterised by sarcomere/Titin deficiency/insufficiency or dysfunction include diseases/conditions associated with mutations to *TTN*.

In some embodiments, the mutation to *TTN* is known or predicted to reduce the level of a Titin isoform encoded by the wildtype allele (e.g. inferred complete isoform (IC), N2BA-G, N2BA-N2, N2BA-A1, N2BA-A2, N2B, Novex-1, Novex-2, Novex-3, N2A-soleus and N2A-psoas). In some embodiments, the mutation is a missense mutation. In some embodiments, the mutation is known or predicted to result in the production of a truncated version of a Titin isoform encoded by the wildtype allele. In some embodiments, the mutation is known or predicted to result in the production of a Titin isoform which is dysfunctional and/or non-functional. In some embodiments, the mutation is known or predicted to result in the production of a Titin isoform which displays a reduced level of a Titin-mediated function as compared to the level of the relevant function displayed by a Titin isoform encoded by the wildtype allele (e.g. inferred complete isoform (IC), N2BA-G, N2BA-N2, N2BA-A1, N2BA-A2, N2B, Novex-1, Novex-2, Novex-3, N2A-

soleus and N2A-psoas). In some embodiments, the mutation is known or predicted to result in the production of a Titin isoform which is misfolded and/or degraded. In some embodiments the mutation is known or predicted to increase the level of a disease-associated Titin variant. In some embodiments the mutation is known or predicted to increase the level of a Titin isoform encoded by a disease-associated allele of TTN.

Diseases/conditions associated with mutations to TTN are described e.g. in Laddach, A., M. Gautel and F. Fraternali (2017). Bioinformatics 33(21): 3482-3485 and Wolfgang A. Linke and Nazha Hamdani (2014). Circulation Research; 114:1052–1068, all of which are hereby incorporated by reference in their entirety. Disease-associated missense mutations to TTN (UniProt: Q8WZ42-1) include Val54Met, Arg740Leu, Ala743Val, Trp976Arg, Val1034Met, Arg2083fs, Thr2896Ile, Glu2989GlufsX4, Ser3799Tyr, Gln4053X, Gln4249X, Ser4465Asn, Leu5782Phe, Ser6395fs, Glu7004Lys, Gly7933fs, Asp7971_Ile7976del, Tyr8958Cys, Arg9427His, Arg9531Gln, His9775Tyr, Gly10159, Val11396, 11477InsPro, Val11879, Val11879, Val11879Leu, Ala12675, Ala12873fs, Pro13298_Thr17642dup, Cys13771X, Glu14779fs, Asp14909, Tyr15045Cys, Phe15108fs, Asp16122, Gly16189X, Trp16359X, Lys16782, Arg17295X, Ala17342Thr, Arg17470X, Glu17715fs, Glu17783X, Ile17876Thr, Lys17898fs, Glu17978fs, Glu18141, Asp18235, His18335, Cys18789X, Arg18858X, Arg18985X, Ala19506Thr, Arg19560X, Pro20049fs, Ala20236Ser, Val20591Glu, Gln20809, Arg20858X, Thr21135, Tyr21301, Gly21497, Lys21640fs, Truncation after Ile21923, Glu21956fs, Ala22353fs, Pro22582fs, Asn23824fs, Gln24059fs, Ser24241fs, Gln24281X, Gln25689X, Trp26632X, Arg26949X, Lys27016X, Ser27052IfsX1, Trp27147X, Ser27179fs, Glu27300, Tyr27567X, Tyr28326fs, Glu28386LysfsX10, Trp29318X, Arg29415X, Glu29510X, Thr29725fs, Pro30068Arg, Cys30071Arg, Gln30081X, Trp30088Arg, Trp30088Cys, Trp30088Leu, Pro30091Leu, Asn30145Lys, Gly30150Asp, Thr30165, Asn30348fs, Thr30513fs, Arg30857, Arg31126fs, Arg31195X, Lys31371X, Pro31774Leu, Ser31841X, Asn32379ThrfsX9, Trp32431Arg, Arg32450Trp, Arg32534X, Pro32976fs, Arg33041Gly, His33534fs, Gln33637X, Gly33698fs, Ser33828SerfsX11, Lys33915fs, Met34218Thr, Ser34242GlnfsX10, Glu34286_Trp34289delinsValLysGluLys, His34305Pro, Iso34306Asp, Leu34315Pro, Lys34322AsnfsX9, Lys34322fs, Gln34323X.

In some embodiments, the TTN mutation is a mutation associated with a heart disease, e.g. cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy and arrhythmogenic cardiomyopathy. Such mutations to TTN include Val54Met, Arg740Leu, Ala743Val, Trp976Arg, Val1034Met, Arg2083fs, Thr2896Ile, Glu2989GlufsX4, Ser3799Tyr, Gln4053X, Gln4249X, Ser4465Asn, Leu5782Phe, Ser6395fs, Glu7004Lys, Gly7933fs, Asp7971_Ile7976del, Tyr8958Cys, Arg9427His, Arg9531Gln, His9775Tyr, Gly10159, Val11396, 11477InsPro, Val11879, Val11879, Val11879Leu, Ala12675, Ala12873fs, Pro13298_Thr17642dup, Cys13771X, Glu14779fs, Asp14909, Tyr15045Cys, Phe15108fs, Asp16122, Gly16189X, Trp16359X, Lys16782, Arg17295X, Ala17342Thr, Arg17470X, Glu17715fs, Glu17783X, Ile17876Thr, Lys17898fs, Glu17978fs, Glu18141, Asp18235, His18335, Cys18789X, Arg18858X, Arg18985X, Ala19506Thr, Arg19560X, Pro20049fs, Ala20236Ser, Val20591Glu, Gln20809, Arg20858X, Thr21135, Tyr21301, Gly21497, Lys21640fs, Truncation after Ile21923, Glu21956fs, Ala22353fs, Pro22582fs, Asn23824fs, Gln24059fs, Ser24241fs, Gln24281X, Gln25689X, Trp26632X, Arg26949X, Lys27016X, Ser27052IfsX1, Trp27147X, Ser27179fs, Glu27300,

Tyr27567X, Tyr28326fs, Glu28386LysfsX10, Trp29318X, Arg29415X, Glu29510X, Thr29725fs, Pro30068Arg, Cys30071Arg, Gln30081X, Trp30088Arg, Trp30088Cys, Trp30088Leu, Pro30091Leu, Asn30145Lys, Gly30150Asp, Thr30165, Asn30348fs, Thr30513fs, Arg30857, Arg31126fs, Arg31195X, Lys31371X, Pro31774Leu, Ser31841X, Asn32379ThrfsX9, Trp32431Arg, Arg32450Trp, Arg32534X, Pro32976fs, Arg33041Gly, His33534fs, Gln33637X, Gly33698fs, Ser33828SerfsX11, Lys33915fs, Met34218Thr, Ser34242GlnfsX10, Glu34286_Trp34289delinsValLysGluLys, His34305Pro, Iso34306Asp, Leu34315Pro, Lys34322AsnfsX9, Lys34322fs, Gln34323X.

In some embodiments, the TTN mutation is a mutation associated with a diseases/conditions characterised by cardiomyopathy such as early-onset myopathy with fatal cardiomyopathy (EOMFC), characterized by dilated cardiomyopathy, delayed motor development with generalized muscle weakness predominantly affecting proximal and distal lower limbs. The Titin deletion variants include K35556fs, K26491fs, K26616fs, K26683fs, K33915fs, K32988fs, D26449fs, D26574fs, D26641fs, D32946fs, D33873fs, D35514fs, Q35176fs, Q26303fs, Q32608fs, Q33535fs, Q26111fs, Q26236fs, P12918fs, P13845fs, P15486fs, P6421fs, P6546fs, P6613fs, Y13414fs, P10340fs, P11641fs, P11267fs, E16226Ter, E17867Ter, E8802Ter, E15299Ter, E8927Ter, E8994Ter, T21129fs, T13897fs, T20202fs, T22770fs, T13705fs, T13830fs, R17915Ter, R15347Ter, R9042Ter, R8975Ter, R16274Ter, R8850Ter.

In some embodiments, the TTN mutation is a mutation associated with Autosomal recessive limb-girdle muscular dystrophy type 2J. Such mutations to TTN include Y22311Ter, Y22436Ter, Y22503Ter, Y28808Ter, Y29735Ter, Y31376Ter, K17301Ter, K17426Ter, K17493Ter, K23798Ter, K24725Ter, K26366Ter, E21058Ter, E23626Ter, E21985Ter, E14561Ter, E14686Ter, E14753Ter, E10603Ter, E11530Ter, E13037Ter, E10380Ter, E11681Ter, E11307Ter, D2143fs, D2189fs, K25643fs, K32015fs, K25518fs, K25710fs, K32942fs, K34583fs, M25640fs, M25707fs, M34580fs, M32939fs, M25515fs, M32012fs, E19273fs, E19398fs, E19465fs, E25770fs, E26697fs, E28338fs, E18442fs, E27315fs, E18375fs, E25674fs, E18250fs, E24747fs, I16818fs, I16943fs, I17010fs, I23315fs, I24242fs, I25883fs, P15942fs, P24815fs, P15875fs, P23174fs, P15750fs, P22247fs, D888fs, D842fs, T25284fs, T25476fs, T34349fs, T31781fs, T32708fs, T25409fs, C31712R, C30071R, C29144R, C22647R, C22772R, C22839R, Q13385Ter, Q13510Ter, Q13577Ter, Q19882Ter, Q20809Ter, Q22450Ter, C10433fs, C10500fs, C10308fs, C16805fs, C17732fs, C19373fs, Q24894Ter, Q26535Ter, Q17470Ter, Q17662Ter, Q17595Ter, Q23967Ter, R15103Ter, R12535Ter, R13462Ter, R6163Ter, R6230Ter, R6038Ter, K19784Ter, K26089Ter, K19592Ter, K28657Ter, K19717Ter, K27016Ter, Q32829Ter, Q33756Ter, Q26457Ter, Q26524Ter, Q35397Ter, Q26332Ter, K26507Ter, K32812Ter, K35380Ter, K26440Ter, K26315Ter, K33739Ter, L24957Ter, L26598Ter, L17658Ter, L17725Ter, L17533Ter, L24030Ter, W23785Ter, W25426Ter, W16361Ter, W16486Ter, W22858Ter, W16553Ter, D15446fs, D21751fs, D24319fs, D15254fs, D15379fs, D22678fs, Q21931Ter, Q23572Ter, Q14699Ter, Q21004Ter, Q14507Ter, Q14632Ter, Q13337Ter, Q20569Ter, Q19642Ter, Q22210Ter, Q13145Ter, Q13270Ter, K16846fs, K9614fs, K15919fs, K18487fs, K9422fs, K9547fs, F14486fs, F7254fs, F13559fs, F16127fs, F7187fs, F7062fs, R35252Ter, R32684Ter, R33611Ter, R26312Ter, R26379Ter, R26187Ter, V13897fs, V13964fs, V22837fs, V13772fs, V20269fs, V21196fs, R23351Ter, R14286Ter, R14411Ter, R14478Ter, R20783Ter, R21710Ter, C22206Ter, C19638Ter, C13141Ter, C13266Ter, C20565Ter, C13333Ter, I33379N, I34306N, I35947N, I27007N, I27074N, I26882N, I21018fs, I12145fs, I19377fs, I11953fs,

I12078fs, I18450fs, Y32573Ter, Y35141Ter, Y26268Ter, Y33500Ter, Y26076Ter, Y26201Ter, R26234fs, R35174fs, R33533fs, R26109fs, R26301fs, R32606fs, E12913Ter, Q26794Ter, Q17921Ter, Q24226Ter, Q17854Ter, Q17729Ter, Q25153Ter

In some embodiments, the TTN mutation is a mutation associated with Tibial muscular dystrophy. Such mutations to TTN include I33379N, I34306N, I35947N, I27007N, I27074N, I26882N, I21018fs, I12145fs, I19377fs, I11953fs, I12078fs, I18450fs, Y32573Ter, Y35141Ter, Y26268Ter, Y33500Ter, Y26076Ter, Y26201Ter, Q26794Ter, Q17921Ter, Q24226Ter, Q17854Ter, Q17729Ter, Q25153Ter, K27023fs, K27090fs, K26898fs, K35963fs, K33395fs, K34322fs, R33703Ter, R32062Ter, R24638Ter, R31135Ter, R24763Ter, R24830Ter, P31732L, P29164L, P22792L, P22859L, P30091L, P22667L, R22499Ter, R20858Ter, R19931Ter, R13626Ter, R13434Ter, R13559Ter, R23868Ter, R21300Ter, R22227Ter, R14803Ter, R14995Ter, R14928Ter, L29811fs, L23439fs, L30738fs, L23314fs, L23506fs, L32379fs, R18878Ter, R20519Ter, R11579Ter, R11646Ter, R17951Ter, R11454Ter, P22521fs, P28826fs, P29753fs, P31394fs, P22329fs, P22454fs, Y24539Ter, Y27107Ter, Y25466Ter, Y18042Ter, Y18167Ter, Y18234Ter, N20902fs, N21094fs, N21027fs, N28326fs, N27399fs, N29967fs, R23469Ter, R25110Ter, R16237Ter, R16045Ter, R16170Ter, R22542Ter, R28364Ter, R19299Ter, R25796Ter, R19424Ter, R26723Ter, R19491Ter, E24419Ter, E26060Ter, E23492Ter, E16995Ter, E17120Ter, E17187Ter, R31056Ter, R29415Ter, R21991Ter, R28488Ter, R22116Ter, R22183Ter, R20626Ter, R18058Ter, R18985Ter, R11561Ter, R11686Ter, R11753Ter, R32102Ter, R33743Ter, R24678Ter, R31175Ter, R24803Ter, R24870Ter, R22975Ter, R24616Ter, R15676Ter, R15743Ter, R15551Ter, R22048Ter, R20026Ter, R21667Ter, R12727Ter, R19099Ter, R12602Ter, R12794Ter, R25552Ter, R22984Ter, R16487Ter, R16679Ter, R16612Ter, R23911Ter, G25912Ter, G27553Ter, G24985Ter, G18488Ter, G18613Ter, G18680Ter, R16290Ter, R9918Ter, R17217Ter, R18858Ter, R9793Ter, R9985Ter, R19856fs, R19923fs, R26228fs, R19731fs, R28796fs, A27689fs, A18624fs, A25121fs, A26048fs, A18749fs, A18816fs, W23158Ter, W23283Ter, W23350Ter, W29655Ter, W30582Ter, W32223Ter.

In some embodiments, the TTN mutation is a mutation associated with Myopathy, myofibrillar, 9, with early respiratory failure. Such mutations to TTN include C31712R, C30071R, C29144R, C22647R, C22772R, C22839R, I21018fs, I12145fs, I19377fs, I11953fs, I12078fs, I18450fs, Y32573Ter, Y35141Ter, Y26268Ter, Y33500Ter, Y26076Ter, Y26201Ter, Q26794Ter, Q17921Ter, Q24226Ter, Q17854Ter, Q17729Ter, Q25153Ter, K27023fs, K27090fs, K26898fs, K35963fs, K33395fs, K34322fs, R33703Ter, R32062Ter, R24638Ter, R31135Ter, R24763Ter, R24830Ter, P31732L, P29164L, P22792L, P22859L, P30091L, P22667L, R22499Ter, R20858Ter, R19931Ter, R13626Ter, R13434Ter, R13559Ter, R23868Ter, R21300Ter, R22227Ter, R14803Ter, R14995Ter, R14928Ter, L29811fs, L23439fs, L30738fs, L23314fs, L23506fs, L32379fs, R18878Ter, R20519Ter, R11579Ter, R11646Ter, R17951Ter, R11454Ter, P22521fs, P28826fs, P29753fs, P31394fs, P22329fs, P22454fs, Y24539Ter, Y27107Ter, Y25466Ter, Y18042Ter, Y18167Ter, Y18234Ter, N20902fs, N21094fs, N21027fs, N28326fs, N27399fs, N29967fs, R23469Ter, R25110Ter, R16237Ter, R16045Ter, R16170Ter, R22542Ter, R28364Ter, R19299Ter, R25796Ter, R19424Ter, R26723Ter, R19491Ter, E24419Ter, E26060Ter, E23492Ter, E16995Ter, E17120Ter, E17187Ter, R31056Ter, R29415Ter, R21991Ter, R28488Ter, R22116Ter, R22183Ter, R20626Ter, R18058Ter, R18985Ter, R11561Ter, R11686Ter, R11753Ter, R32102Ter, R33743Ter, R24678Ter, R31175Ter, R24803Ter, R24870Ter, R22975Ter, R24616Ter, R15676Ter,

R15743Ter, R15551Ter, R22048Ter, R20026Ter, R21667Ter, R12727Ter, R19099Ter, R12602Ter, R12794Ter, R25552Ter, R22984Ter, R16487Ter, R16679Ter, R16612Ter, R23911Ter, G25912Ter, G27553Ter, G24985Ter, G18488Ter, G18613Ter, G18680Ter, R16290Ter, R9918Ter, R17217Ter, R18858Ter, R9793Ter, R9985Ter, R19856fs, R19923fs, R26228fs, R19731fs, R28796fs, A27689fs, A18624fs, A25121fs, A26048fs, A18749fs, A18816fs, W23158Ter, W23283Ter, W23350Ter, W29655Ter, W30582Ter, W32223Ter.

In aspects and embodiments of the present disclosure, the disease/condition to be treated/prevented is selected from: cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, a disease/condition in which sarcomere deficiency/insufficiency or dysfunction is pathologically-implicated, a disease/condition in which sarcomere deficiency/insufficiency or dysfunction is pathologically-implicated, a disease/condition characterised by the disorganisation of the sarcomere, resulting in a reduction of contractile force, a disease/condition associated with mutation to a gene encoding a sarcomere protein (e.g. selected from *TTN, TNNT2, TPM1, MYH7* and *MYBPC3*), a disease/condition characterised by a reduction in the expression/activity of a sarcomeric protein, a disease/condition in which titin deficiency/insufficiency or dysfunction is pathologically-implicated, a disease/condition characterised by a reduction in the expression/activity of titin, a disease/condition associated with mutation to *TTN*, a myopathy, an autosomal recessive limb-girdle muscular dystrophy type 2J, a myopathy, myofibrillar, 9, with early respiratory failure, an early-onset myopathy with fatal cardiomyopathy and a Tibial muscular dystrophy.

**Treatment/prevention of diseases/conditions through LINC complex inhibition**

The present disclosure provides methods and articles (agents and compositions) for the treatment and/or prevention of diseases/conditions through LINC complex inhibition. Treatment/prevention of disease is achieved by LINC complex inhibition in *e.g.* a cell, tissue/organ/organ system/subject.

Aspects of the present disclosure are concerned with the treatment/prevention of diseases/conditions described herein, through LINC complex inhibition.

The methods may be aimed at: delaying/preventing the onset of symptoms of the disease/condition; reducing the severity of (*i.e.* alleviating) the symptoms of the disease/condition; and reducing the level of a correlate of the pathology of the disease/condition; reversing the symptoms of the disease/condition; reducing morbidity of subjects having the disease/condition; increasing survival of a subject having the disease/condition; increasing the lifespan of a subject having the disease/condition; reducing mortality of subjects having the disease/condition; and/or delaying/preventing progression of the disease/condition (*e.g.* to a later stage).

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is not a laminopathy. In some embodiments, the disease/condition is not associated with mutation to *LMNA*. In some embodiments, the disease/condition is not associated with mutation to any of *LMNA*, *LMNB1* and *LMNB2*. In some embodiments, the disease/condition is not associated with mutation to any of *LMNA*, *LMNB1* and *LMNB2*.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is not a nuclear envelopathy. In some embodiments, the disease/condition is not associated with mutation to any of *LMNA*, *LMNB1*, *LMNB2*, *EMD*, *LAP2*, *LBR*, *ZMPSTE24*, *SYNE-1* and *NUP62*.

LINC complex inhibitors according to the present disclosure may be provided in the form of nucleic acid comprising or encoding the LINC complex inhibitor. Such nucleic acids comprise or consist of DNA and/or RNA, and may be or comprise a polynucleotide.

Where a LINC complex inhibitor is a nucleic acid, it may be provided in the form of a larger nucleic acid comprising the LINC complex inhibitor. Where a LINC complex inhibitor is a peptide/polypeptide or an SSN system targeting a LINC complex protein, it may be provided in the form of nucleic acid encoding the LINC complex inhibitor.

In some embodiments, a LINC complex inhibitor is administered in the form of nucleic acid encoding the factors required for production of a LINC complex inhibitor (*e.g.* nucleic acid encoding a precursor of the LINC complex inhibitor and/or nucleic acid encoding factors required for production/assembly of the LINC complex inhibitor). For example, the LINC complex inhibitor may be administered in the form of nucleic acid encoding factors required for production of a small molecule or biomolecular LINC complex inhibitor.

The nucleic acid may comprise a LINC complex inhibiting polypeptide-encoding nucleotide sequence, and may additionally comprise one or more non-polypeptide-encoding nucleotide sequence(s). Non-polypeptide-encoding nucleotide sequence(s) may be *e.g.* be 5' cap, 5' UTR, 3' UTR and/or PolyA tail sequences.

The nucleic acid may be, or may be comprised in, a vector. A 'vector' as used herein refers to 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 cell (*i.e.* the vector may be an expression vector). Such vectors may include a promoter sequence operably linked to the nucleotide sequence encoding the sequence to be expressed. A vector may also include a termination codon and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express a peptide or polypeptide from a vector according to the present disclosure.

The term 'operably linked' may include the situation where a selected nucleic acid sequence and regulatory nucleic acid sequence (*e.g.* promoter and/or enhancer) are covalently linked in such a way that the expression of nucleic acid 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. The resulting transcript(s) may then be translated into a polypeptide, *e.g.* a LINC complex inhibiting polypeptide.

Suitable vectors include plasmids, binary vectors, DNA vectors, mRNA vectors, viral vectors (*e.g.* gammaretroviral vectors (*e.g.* murine Leukemia virus (MLV)-derived vectors), lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, vaccinia virus vectors and herpesvirus vectors), transposon-based vectors, and artificial chromosomes (*e.g.* yeast artificial chromosomes), *e.g.* as described in Maus *et al.*, Annu Rev Immunol (2014) 32:189-225 or Morgan and Boyerinas, Biomedicines 2016 4, 9, which are both hereby incorporated by reference in their entirety. In preferred embodiments, the vector is an adeno-associated virus vector or a lentiviral vector.

In some embodiments, a vector is selected based on tropism for a cell type/tissue/organ to which it is desired to deliver the nucleic acid comprising/encoding a LINC complex inhibitor. In some embodiments, a vector is selected based on tropism for a cell type/tissue/organ in which it is desired to express the LINC complex inhibitor. For example, it may be desired to deliver the nucleic acid/express the LINC complex inhibitor in a cell type/tissue/organ affected by a disease to be treated/prevented in accordance with the present disclosure (*e.g.* a cell type/tissue/organ in which the symptoms of the disease manifest).

In some embodiments it is desired to deliver nucleic acid encoding a LINC complex inhibitor to muscle cells/tissue (*e.g.* cardiac and/or skeletal muscle cells/tissue), and vectors having a tropism for such cells/tissue may be employed in such embodiments. In some embodiments, a vector may be cardiotropic. In some embodiments, a vector may be myotropic.

In preferred embodiments, the vector is an adeno-associated virus vector. Adeno-associated virus vectors and their use to vector gene therapy is reviewed *e.g.* in Wang *et al.*, Nat. Rev. Drug Discov. (2019) 18: 358-378 and Li and Samulski, Nat. Rev. Genet. (2020) 12: 255-272, both of which are hereby incorporated by reference in their entirety. In some embodiments, a vector may be an adeno-associated virus vector described in Wang *et al.*, Nat. Rev. Drug Discov. (2019) 18: 358-378. In some embodiments, a vector may be an adeno-associated virus vector described in Li and Samulski, Nat. Rev. Genet. (2020) 12: 255-272.

In some embodiments, the vector is a self-complementary adeno-associated virus (scAAV) vector. Self-complementary adeno-associated virus vectors are described *e.g.* in McCarty, Mol Ther. (2008) 16(10):1648-56, which is hereby incorporated by reference in its entirety. Conventional AAV have a single-stranded DNA genome, and depend on the DNA replication machinery of a transduced cell to synthesise the complementary strand, delaying transgene expression. By contrast, scAAV contain complementary sequences that spontaneously anneal upon infection, eliminating the requirement for DNA synthesis in the transduced host cell. Compared to classical, single-stranded AAV vectors, scAAV vectors have been shown to provide for accelerated onset of transgene expression, and an increased level of transgene expression.

In some embodiments, a vector may be an adeno-associated viral vector of one of the following serotypes: AAV1, AAV2, AAV2i8, AAV5, AAV6, AAV8, AAV9, AAV9.45, AAV10 or AAVrh74. In some embodiments, the vector is an AAV9 vector. In some embodiments, the vector is an AAV6 vector.

In some embodiments, a vector may be a cardiotropic adeno-associated viral vector. In some embodiments, a vector may be an adeno-associated viral vector of one of the following serotypes: AAV1, AAV8, AAV9, AAV9.45.

In some embodiments, a vector may be a skeletal muscle tropic adeno-associated viral vector. In some embodiments, a vector may be an adeno-associated viral vector of one of the following serotypes: AAV1, AAV6, AAV7, AAV8, AAV9, AAV9.45.

In some embodiments a vector comprises modification to increase binding to and/or transduction of a cell-type of interest (*i.e.* as compared to the level of binding/transduction by the unmodified vector). In some embodiments modification is to a capsid protein.

In some embodiments a vector comprises a capsid protein comprising a cell-targeting peptide. In some embodiments the cell-targeting peptide is a cell-targeting peptide described in Büning and Srivastava, Molecular Therapy: Methods & Clinical Development (2019) 12: 248-265, which is hereby incorporated by reference in its entirety, *e.g.* a cell-targeting peptide shown in Table 1, 2, 3 or 4 thereof.

In some embodiments a vector comprises a capsid protein comprising substitution to one or more tyrosine residues, *e.g.* one or more surface-exposed tyrosine residues. In some embodiments, one or more tyrosine residues of the capsid protein are substituted with phenylalanine. In some embodiments a vector comprises a capsid protein in which one or more tyrosine residues are substituted with another amino acid as described in Iida *et al.*, Biomed Res Int. (2013) 2013: 974819, which is hereby incorporated by reference in its entirety.

In some embodiments, a vector may be an adeno-associated virus vector described in Büning and Srivastava, *supra*. In some embodiments, a vector may be an adeno-associated virus vector described in Iida *et al.*, *supra*.

In some embodiments the nucleic acid/vector comprises one or more sequences for controlling expression of the nucleic acid. Accordingly, in some embodiments the nucleic acid/vector comprises a control element for inducible expression of the nucleic acid. A sequence for controlling expression of the nucleic acid may provide for expression of the nucleic acid by cells of a particular type or tissue. For example, expression may be under the control of a cell type- or tissue-specific promoter.

Promoters for cell type- or tissue-specific expression of a nucleic acid in accordance with the present disclosure can be selected in accordance with the disease to be treated/prevented. For example, the promoter may drive expression in a cell type/tissue/an organ affected by the disease (*e.g.* a cell type/tissue/an organ in which the symptoms of the disease manifest).

In some embodiments, a promoter may provide for expression in muscle cells/tissue (*e.g.* cardiac and/or skeletal muscle cells/tissue). In some embodiments, a promoter may be a cardiac or cardiomyocyte-specific promoter (*e.g.* a cTNT, α-MHC or MLC2v promoter). In some embodiments, a promoter may be a

skeletal muscle/striated muscle cell-specific promoter (*e.g.* a MCK, MHCK7 or desmin promoter). In some embodiments, the promoter is cTNT.

In some embodiments, a promoter may be a vascular endothelial cell-specific promoter (*e.g.* a Tie2 promoter). In some embodiments, a promoter may be a vascular smooth muscle cell-specific promoter (*e.g.* a SM22a promoter). In some embodiments, a promoter may be a monocyte/macrophage-specific promoter (*e.g.* a LysM promoter).

A sequence for controlling expression of the nucleic acid may provide for expression of the nucleic acid in response to *e.g.* a given agent/signal. For example, expression may be under the control of inducible promoter. The agent may provide for inducible expression of the nucleic acid *in vivo* by administration of the agent to a subject having been administered with a modified cell according to the disclosure, or *ex vivo/in vitro* by administration of the agent to cells in culture *ex vivo* or *in vitro*.

In some embodiments a nucleic acid or vector according to the present disclosure may employ a conditional expression system for controlling expression of the nucleic acid comprising/encoding a LINC complex inhibitor by cells comprising the nucleic acid/vector. 'Conditional expression' may also be referred to herein as 'inducible expression', and refers to expression contingent on certain conditions, *e.g.* the presence of a particular agent. Conditional expression systems are well known in the art and are reviewed *e.g.* in Ryding *et al.* Journal of Endocrinology (2001) 171, 1-14, which is hereby incorporated by reference in its entirety.

In preferred embodiments, the nucleic acid has a size permitting its delivery as a gene therapy, *i.e.* in a suitable vector.

In some embodiments, the nucleic acid comprising/encoding a LINC complex inhibitor consists of a nucleotide sequence having a size within the packaging limit of a vector for delivering the polynucleotide. In some embodiments, the nucleic acid consists of a nucleotide sequence having a size within the packaging limit of a vector described herein. In some embodiments, the nucleic acid consists of a nucleotide sequence having a size within the packaging limit of an adeno-associated virus (AAV) vector, *e.g.* an AAV vector described herein. In some embodiments, the nucleic acid consists of a nucleotide sequence having a size within the packaging limit of a scAAV vector. In some embodiments, the nucleotide sequence of the nucleic acid consists of fewer than 6,000 nucleotides, *e.g.* one of ≤5,000, ≤4,500, ≤4,000, ≤3,500, ≤3,000, ≤2,500, ≤2,400 or ≤2,300 nucleotides.

A LINC complex inhibitor according to the present disclosure may be produced within a cell. For example, where the LINC complex inhibitor is a LINC complex inhibiting polypeptide, it may be produced within a cell by transcription from nucleic acid encoding the polypeptide, and subsequent translation of the transcribed RNA.

A LINC complex inhibitor according to the present disclosure may be provided in the form of a composition comprising the LINC complex inhibitor, and/or a composition comprising nucleic acid

comprising/encoding the LINC complex inhibitor. Such compositions may comprise the relevant article (*i.e.* the LINC complex inhibiting polypeptide/nucleic acid/vector) in a formulation suitable for clinical use.

The compositions may comprise one or more pharmaceutically-acceptable carriers (*e.g.* liposomes, micelles, microspheres, nanoparticles), diluents/excipients (*e.g.* starch, cellulose, a cellulose derivative, a polyol, dextrose, maltodextrin, magnesium stearate), adjuvants, fillers, buffers, preservatives (*e.g.* vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium, cysteine, methionine, citric acid, sodium citrate, methyl paraben, propyl paraben), anti-oxidants (*e.g.* vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium), lubricants (*e.g.* magnesium stearate, talc, silica, stearic acid, vegetable stearin), binders (*e.g.* sucrose, lactose, starch, cellulose, gelatin, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), xylitol, sorbitol, mannitol), stabilisers, solubilisers, surfactants (*e.g.*, wetting agents), masking agents or colouring agents (*e.g.* titanium oxide).

The term 'pharmaceutically-acceptable' as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, *etc.*, which are, within the scope of sound medical 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, anti-oxidant, lubricant, binder, stabiliser, solubiliser, surfactant, masking agent, colouring agent, flavouring agent or sweetening agent of a composition 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, anti-oxidants, lubricants, binders, stabilisers, solubilisers, surfactants, masking agents, colouring agents, flavouring agents or sweetening agents can be found in standard pharmaceutical texts, for example, Remington's 'The Science and Practice of Pharmacy' (Ed. A. Adejare), 23rd Edition (2020), Academic Press.

The articles of the present disclosure may be formulated for administration to a subject, *e.g.* administration via a route of administration as appropriate for the nature of the therapeutic agent and the disease to be treated/prevented. Administration of the articles of the present disclosure may be parenteral, systemic, topical, intracavitary, intravascular, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, intratumoral, subcutaneous, intradermal, oral or transdermal. In some embodiments, administration may be by injection or infusion, or by ingestion.

In some aspects and embodiments, articles of the present disclosure may be administered to a tissue/organ of interest (*e.g.* a tissue/organ affected by the disease/condition (*e.g.* a tissue/organ in which symptoms of the disease/condition manifest)). In some aspects and embodiments, articles of the present disclosure may be administered to the blood (*i.e.* intravenous/intra-arterial administration) by injection or infusion (*e.g.* via cannula), or may be administered subcutaneously or orally. The particular mode and/or site of administration may be selected in accordance with the location at which the therapeutic/prophylactic effect is required, *e.g.* cardiac muscle cells/tissue.

The pharmaceutical compositions/medicaments may comprise the LINC complex inhibitor/nucleic acid/vector in a sterile or isotonic medium. The pharmaceutical compositions/medicaments may be provided in fluid, including gel, form. Fluid formulations may be formulated for administration by injection or infusion (*e.g.* via cannula) to a blood vessel, or a selected region of the human or animal body. The pharmaceutical compositions/medicaments may be provided in solid form, *e.g.* in lyophilised form.

LINC complex inhibitors, nucleic acids, vectors, cells and compositions according to the present disclosure may be modified and/or formulated to facilitate delivery to, and/or uptake by, a cell/tissue of interest (*e.g.* cardiac muscle cells/tissue). Strategies for targeted delivery of such species are reviewed *e.g.* in Li *et al.*, Int. J. Mol. Sci. (2015) 16: 19518-19536 and Fu *et al.*, Bioconjug Chem. (2014) 25(9): 1602-1608, which are hereby incorporated by reference in their entirety.

In some embodiments, articles of the present disclosure may be encapsulated in a nanoparticle or a liposome. In some embodiments, articles of the present disclosure may be (covalently or non-covalently) associated with a cell-penetrating peptide (*e.g.* a protein transduction domain, trojan peptide, arginine-rich peptide, vectocell peptide), a cationic polymer, a cationic lipid or a viral carrier.

Nanoparticles may be organic, *e.g.* micelles, liposomes, proteins, solid-lipid particles, solid polymer particles, dendrimers, and polymer therapeutics. Nanoparticles may be inorganic, *e.g.* such as nanotubes or metal particles, optionally with organic molecules added. In some embodiments, a nanoparticle is a nanoparticle described in Chen *et al.*, Mol Ther Methods Clin Dev. (2016) 3:16023, which is hereby incorporated by reference in its entirety. In some embodiments, a nanoparticle is a PLGA, polypeptide, poly(β-amino ester), DOPE, β-cyclodextrin-containing polycation, linear PEI, PAMAM dendrimer, branched PEI, chitosan or polyphosphoester nanoparticle.

In some embodiments, LINC complex inhibitors, nucleic acids and vectors according to the present disclosure comprise modification to incorporate one or more moieties facilitating delivery to, and/or uptake by, a cell type or tissue of interest (*e.g.* cardiac and/or skeletal muscle cells/tissue). In some embodiments, LINC complex inhibitors, nucleic acids and vectors according to the present disclosure are linked (*e.g.* chemically conjugated to) one or more moieties facilitating delivery to, and/or uptake by, a cell type or tissue of interest.

Moieties facilitating delivery to, and/or uptake by, cell types or tissues of interest are described *e.g.* in Benizri *et al.*, Bioconjug Chem. (2019) 30(2): 366–383, which is hereby incorporated by reference in its entirety. Such moieties include *e.g.* N-acetylgalactosamine (GalNAc), α-tocopherol, cell-penetrating peptides, nucleic acid aptamers, antibodies and antigen-binding fragments/derivatives thereof, cholesterol, squalene, polyethylene glycol (PEG), fatty acids (*e.g.* palmitic acid) and nucleolipid moieties.

Articles of the present disclosure may be formulated in a sustained release delivery system, in order to release the LINC complex inhibitor, nucleic acid, vector, cell or composition at a predetermined rate. Sustained release delivery systems may maintain a constant drug/therapeutic/prophylactic concentration

for a specified period of time. In some embodiments, articles of the present disclosure are formulated in a liposome, gel, implant, device, or drug-polymer conjugate *e.g.* hydrogel.

Administration of a LINC complex inhibitor to a subject in accordance with the present disclosure is preferably in a 'therapeutically-effective' or 'prophylactically-effective' amount, this being sufficient to show therapeutic/prophylactic benefit to the subject. Administration of a LINC complex inhibitor preferably results in modification of a cell or cells to comprise a LINC complex inhibitor as described herein.

The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease to be treated/prevented, and the nature of the LINC complex inhibitor. Prescription of treatment, *e.g.* decisions on dosage *etc.*, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disease/condition to be treated, the condition of the individual subject, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's 'The Science and Practice of Pharmacy' (Ed. A. Adejare), 23rd Edition (2020), Academic Press.

In preferred embodiments, administration is of a nucleic acid/vector, or of a composition comprising a nucleic acid/vector according to the present disclosure. In preferred embodiments, administration results in modification of a cell or cells to comprise/express a nucleic acid/vector, and/or to comprise/express a polypeptide according to the present disclosure. That is, the nucleic acid/vector/composition is employed as a gene therapy.

In some embodiments, therapeutic or prophylactic intervention according to the present disclosure may further comprise administering another agent for the treatment/prevention of the relevant disease/condition. Administration of LINC complex inhibitors/nucleic acids/vectors/compositions described herein may be alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Simultaneous administration refers to administration with another therapeutic agent together, for example as a pharmaceutical composition containing both agents (combined preparation), or immediately after each other and optionally via the same route of administration (*e.g.* to the same tissue, artery, vein or other blood vessel). Sequential administration refers to administration of one agent followed after a given time interval by separate administration of another agent. It is not required that the two agents are administered by the same route, although this is the case in some embodiments. The time interval may be any time interval.

Multiple doses of the LINC complex inhibitor/nucleic acid/vector/composition may be provided. One or more, or each, of the doses may be accompanied by simultaneous or sequential administration of another therapeutic/prophylactic agent. Multiple doses may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days).

In accordance with various aspects of the present disclosure, a method of treating and/or preventing a disease/condition (*e.g.* a cardiomyopathy) may comprise one or more of the following: promoting normal contraction/contractility of a cell (*e.g.* a cardiomyocyte), promoting normal organisation of the microtubule network (*e.g.* in a cell), promoting normal organisation of the sarcomere (*e.g.* in a cell), decreasing the mechanical force transmitted to the cell nucleus/nuclear envelope, decreasing the mechanical resistance of the microtubule network, increasing the contractile magnitude of a cardiomyocyte, decreasing the density of the microtubule network, decreasing the level of tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decreasing the level/proportion of detyrosinated tubulin, increasing the level/proportion of acetylated tubulin, decreasing the level/proportion of acetylated tubulin, decreasing the level/proportion of microtubules interacting with an intermediate filament, for example Desmin, promoting normal organisation of the perinuclear microtubule network (*e.g.* in a cell), decreasing the mechanical force transmitted to the cell nucleus/nuclear envelope by the perinuclear microtubule network, decreasing the mechanical resistance of the perinuclear microtubule network, decreasing the density of the perinuclear microtubule network, decreasing the level of perinuclear tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decreasing the level/proportion of detyrosinated perinuclear tubulin, and decreasing the level/proportion of microtubules interacting with an intermediate filament, for example Desmin.

The present disclosure further provides the use of LINC complex inhibitor according to the present disclosure to: promote normal contraction/contractility of a cell (*e.g.* a cardiomyocyte), promote normal organisation of the microtubule network (*e.g.* in a cell), promote normal organisation of the sarcomere (*e.g.* in a cell), decrease the mechanical force transmitted to the cell nucleus/nuclear envelope, decrease the mechanical resistance of the microtubule network, increase the contractile magnitude of a cardiomyocyte, decrease the density of the microtubule network, decrease the level of tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decrease the level/proportion of detyrosinated tubulin, increase the level/proportion of acetylated tubulin, decrease the level/proportion of acetylated tubulin, decrease the level/proportion of microtubules interacting with an intermediate filament, for example Desmin, promote normal organisation of the perinuclear microtubule network (*e.g.* in a cell), decrease the mechanical force transmitted to the cell nucleus/nuclear envelope by the perinuclear microtubule network, decrease the mechanical resistance of the perinuclear microtubule network, decrease the density of the perinuclear microtubule network, decrease the level of perinuclear tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decrease the level/proportion of detyrosinated perinuclear tubulin, and/or decrease the level/proportion of microtubules interacting with an intermediate filament, for example Desmin.

The present disclosure further provides methods for: promoting normal contraction/contractility of a cell (*e.g.* a cardiomyocyte), promoting normal organisation of the microtubule network (*e.g.* in a cell), promoting normal organisation of the sarcomere (*e.g.* in a cell), decreasing the mechanical force transmitted to the cell nucleus/nuclear envelope, decreasing the mechanical resistance of the microtubule network, increasing the contractile magnitude of a cardiomyocyte, decreasing the density of the microtubule network, decreasing the level of tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decreasing the level/proportion of detyrosinated tubulin, increasing the level/proportion of acetylated tubulin, decreasing the level/proportion of acetylated tubulin, decreasing the level/proportion of microtubules interacting with an intermediate filament, for example Desmin, promoting normal

organisation of the perinuclear microtubule network (*e.g.* in a cell), decreasing the mechanical force transmitted to the cell nucleus/nuclear envelope by the perinuclear microtubule network, decreasing the mechanical resistance of the perinuclear microtubule network, decreasing the density of the perinuclear microtubule network, decreasing the level of perinuclear tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decreasing the level/proportion of detyrosinated perinuclear tubulin, and/or decreasing the level/proportion of microtubules interacting with an intermediate filament, for example Desmin, using a LINC complex inhibitor according to the present disclosure.

Accordingly, the present disclosure provides methods for: promoting normal contraction/contractility of a cell (*e.g.* a cardiomyocyte), promoting normal organisation of the microtubule network (*e.g.* in a cell), promoting normal organisation of the sarcomere (*e.g.* in a cell), decreasing the mechanical force transmitted to the cell nucleus/nuclear envelope, decreasing the mechanical resistance of the microtubule network, increasing the contractile magnitude of a cardiomyocyte, decreasing the density of the microtubule network, decreasing the level of tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decreasing the level/proportion of detyrosinated tubulin, decreasing the level/proportion of microtubules interacting with an intermediate filament, for example Desmin, promoting normal organisation of the perinuclear microtubule network (*e.g.* in a cell), decreasing the mechanical force transmitted to the cell nucleus/nuclear envelope by the perinuclear microtubule network, decreasing the mechanical resistance of the perinuclear microtubule network, decreasing the density of the perinuclear microtubule network, decreasing the level of perinuclear tubulin (*e.g.* α-tubulin and/or β-tubulin), *e.g.* in a cell, decreasing the level/proportion of detyrosinated perinuclear tubulin, and/or decreasing the level/proportion of microtubules interacting with an intermediate filament, for example Desmin, the methods comprising administering to a subject a LINC complex inhibitor according to the present disclosure.

**Subjects**

A subject in accordance with the various aspects of the present disclosure may be any animal or human. Therapeutic and prophylactic applications may be in humans or animals (veterinary use).

The subject to be administered with an article of the present disclosure (*e.g.* in accordance with therapeutic or prophylactic intervention) may be a subject in need of such intervention. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient.

A subject may have (*e.g.* may have been diagnosed with) a disease or condition described herein (*e.g.* a cardiomyopathy or a disease or condition characterised by desmoplakin dysfunction/deficiency/insufficiency, a disease or condition characterised by titin dysfunction/deficiency/insufficiency, or a disease or condition characterised by microtubule dysfunction), may be suspected of having such a disease/condition, or may be at risk of developing/contracting such a disease/condition. In embodiments according to the present disclosure, a subject may be selected for treatment according to the methods based on characterisation of one or more markers of such a disease/condition, *e.g.* as described hereinabove. A subject may be suspected of having or suffering from a disease/condition described herein based on the presence of other symptoms indicative of the

disease/condition in the subject or in a cell/tissue/organ of the subject. A subject may be considered at risk of developing the disease/condition because of genetic predisposition or other risk factors for the disease.

In some embodiments, a subject comprises a mutation according to any embodiment described herein. In some embodiments, the subject comprises a mutation giving rise to a disease/condition described herein.

In some embodiments, a subject does not comprise a laminopathy. In some embodiments, a subject does not comprise a disease-associated mutation to *LMNA*. In some embodiments, a subject does not comprise mutation to *LMNA* (*i.e.* is homozygous for the wildtype allele of *LMNA*). In some embodiments, a subject does not comprise a disease-associated mutation to any of *LMNA*, *LMNB1* and *LMNB2*. In some embodiments, a subject does not comprise mutation to any of *LMNA*, *LMNB1* and *LMNB2* (*i.e.* is homozygous for the wildtype alleles of *LMNA*, *LMNB1* and *LMNB2*). In some embodiments, a subject does not comprise a nuclear envelopathy. In some embodiments, a subject does not comprise a disease-associated mutation to any of *LMNA*, *LMNB1*, *LMNB2*, *EMD*, *LAP2*, *LBR*, *ZMPSTE24*, *SYNE-1* and *NUP62*. In some embodiments, a subject does not comprise mutation to any of *LMNA*, *LMNB1*, *LMNB2*, *EMD*, *LAP2*, *LBR*, *ZMPSTE24*, *SYNE-1* and *NUP62* (*i.e.* is homozygous for the wildtype alleles of *LMNA*, *LMNB1*, *LMNB2*, *EMD*, *LAP2*, *LBR*, *ZMPSTE24*, *SYNE-1* and *NUP62*).

In some embodiments, methods according to the present disclosure may comprise determining whether a subject has a disease/condition described herein. In some embodiments the methods comprise diagnosing a disease/condition described herein. Determining whether a subject has a disease/condition described herein may comprise analysing a subject for one or more symptoms/correlates of the disease/condition. Genetic factors may be assayed by methods known to those of ordinary skill in the art, including PCR based and sequencing assays. By determining the presence of genetic factors, *e.g.* in a sample obtained from a subject, a diagnosis may be confirmed, and/or a subject may be classified as being at risk of developing a disease/condition described herein, and/or a subject may be identified as being suitable for treatment with a LINC complex inhibitor/nucleic acid/vector/composition described herein.

Assays may be performed *in vitro* on a sample obtained from a subject, or following processing of a sample obtained from a subject. The sample obtained from a subject may be of any kind. A biological sample may be taken from any tissue or bodily fluid, *e.g.* a blood sample, blood-derived sample, serum sample, lymph sample, semen sample, saliva sample, synovial fluid sample. A blood-derived sample may be a selected fraction of a patient's blood, *e.g.* a selected cell-containing fraction or a plasma or serum fraction. A sample may comprise a tissue sample or biopsy; or cells isolated from a subject.

**Sequence identity**

As used herein, 'sequence identity' refers to the percent of nucleotides/amino acid residues in a subject sequence that are identical to nucleotides/amino acid residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum percent sequence identity between the sequences. Pairwise and multiple sequence alignment for the purposes of determining

percent identity between two or more amino acid or nucleic acid sequences can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960), T-coffee (Notredame *et al.* 2000, J. Mol. Biol. (2000) 302, 205-217), Kalign (Lassmann and Sonnhammer 2005, BMC Bioinformatics, 6(298)) and MAFFT (Katoh and Standley 2013, Molecular Biology and Evolution, 30(4) 772–780) software. When using such software, the default parameters, *e.g.* for gap penalty and extension penalty, are preferably used.

**Sequences**

| SEQ ID NO: | DESCRIPTION | SEQUENCE |
|---|---|---|
| 1 | Human SUN1 | MDFSRLHMYSPPQCVPENTGYTYALSSSYSSDALDFETEHKLDPVFDSPRMSR RSLRLATTACTLGDGEAVGADSGTSSAVSLKNRAARTTKQRRSTNKSAFSINHV SRQVTSSGVSHGGTVSLQDAVTRRPPVLDESWIREQTTVDHFWGLDDDGDLKG GNKAAIQGNGDVGAAAATAHNGFSCSNCSMLSERKDVLTAHPAAPGPVSRVYS RDRNQKCDDCKGKRHLDAHPGRAGTLWHIWACAGYFLLQILRRIGAVGQAVSR TAWSALWLAVVAPGKAASGVFWWLGIGWYQFVTLISWLNVFLLTRCLRNICKFL VLLIPLFLLLAGLSLRGQGNFFSFLPVLNWASMHRTQRVDDPQDVFKPTTSRLKQ PLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLRELTTLLQKLQARVDQM EGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMSHLEDILGKLREKSEAIQK ELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQER VDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNV THHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFA LESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFK GSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQ LLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHG EPVK |
| 2 | Human SUN1 nuclear region | MDFSRLHMYSPPQCVPENTGYTYALSSSYSSDALDFETEHKLDPVFDSPRMSR RSLRLATTACTLGDGEAVGADSGTSSAVSLKNRAARTTKQRRSTNKSAFSINHV SRQVTSSGVSHGGTVSLQDAVTRRPPVLDESWIREQTTVDHFWGLDDDGDLKG GNKAAIQGNGDVGAAAATAHNGFSCSNCSMLSERKDVLTAHPAAPGPVSRVYS RDRNQKCDDCKGKRHLDAHPGRAGTLWHIWACAGYFLLQILRRIGAVGQAVSR TAWSALWLAVVAPGKAASGVFWWLGIGWYQFVTLISWLNVFLLTRCLRN |
| 3 | Human SUN1 helical region | ICKFLVLLIPLFLLLAGLSL |
| 4 | Human SUN1 perinuclear space region | RGQGNFFSFLPVLNWASMHRTQRVDDPQDVFKPTTSRLKQPLQGDSEAFPWH WMSGVEQQVASLSGQCHHHGENLRELTTLLQKLQARVDQMEGGAAGPSASVR DAVGQPPRETDFMAFHQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAV GEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLF SEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTS EAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRC SETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSM MIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGE SLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 5 | Human SUN1 LMNA-binding region | MDFSRLHMYSPPQCVPENTGYTYALSSSYSSDALDFETEHKLDPVFDSPRMSR RSLRLATTACTLGDGEAVGADSGTSSAVSLKNRAARTTKQRRSTNKSAFSINHV SRQVTSSGVSHGGTVSLQDAVTRRPPVLDES |
| 6 | Human SUN1 SYNE2-binding region | SRVYSRDRNQKCDDCKGKRHLDAHPGRAGTLWHIWACAGYFLLQILRRIGAVG QAVSRTAWSALWLAVVAPGKAASGVFWWLGIGWYQFVTLIS |
| 7 | Human SUN1 EMD-binding region | CKGKRHLDAHPGRAGTLWHIWACAGYFLLQILRRIGAVGQAVSRTAWSALWLAV VAPGKAASGVFWWLGIGWYQFVTLIS |
| 8 | Human SUN1 SUN domain | GGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQG YLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQF TYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPV |
| 9 | Human SUN1 CC1 | GEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLF SEDQQGGSLEQLLQR |
| 10 | Human SUN1 CC2 α1 | KGDLQTMLRDLQLQILRNVTHHVSV |
| 11 | Human SUN1 CC2 α2 | TSEAVVSAVSEA |

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| 12 | Human SUN1 CC2 α3 | ITEAQARAIVNSALKL |
| 13 | Human SUN2 | MSRRSQRLTRYSQGDDDGSSSSGGSSVAGSQSTLFKDSPLRTLKRKSSNMKRL<br>SPAPQLGPSSDAHTSYYSESLVHESWFPPRSSLEELHGDANWGEDLRVRRRRG<br>TGGSESSRASGLVGRKATEDFLGSSSGYSSEDDYVGYSDVDQQSSSSRLRSAV<br>SRAGSLLWMVATSPGRLFRLLYWWAGTTWYRLTTAASLLDVFVLTRRFSSLKTF<br>LWFLLPLLLLTCLTYGAWYFYPYGLQTFHPALVSWWAAKDSRRPDEGWEARDS<br>SPHFQAEQRVMSRVHSLERRLEALAAEFSSNWQKEAMRLERLELRQGAPGQG<br>GGGGLSHEDTLALLEGLVSRREAALKEDFRRETAARIQEELSALRAEHQQDSED<br>LFKKIVRASQESEARIQQLKSEWQSMTQESFQESSVKELRRLEDQLAGLQQELA<br>ALALKQSSVAEEVGLLPQQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQREE<br>MQAQLRELESKILTHVAEMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQA<br>LQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVI<br>LQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKD<br>FAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMATYQVVELRILTNWGH<br>PEYTCIYRFRVHGEPAH |
| 14 | Human SUN2 nuclear region | MSRRSQRLTRYSQGDDDGSSSSGGSSVAGSQSTLFKDSPLRTLKRKSSNMKRL<br>SPAPQLGPSSDAHTSYYSESLVHESWFPPRSSLEELHGDANWGEDLRVRRRRG<br>TGGSESSRASGLVGRKATEDFLGSSSGYSSEDDYVGYSDVDQQSSSSRLRSAV<br>SRAGSLLWMVATSPGRLFRLLYWWAGTTWYRLTTAASLLDVFVLTRRFSSLKT |
| 15 | Human SUN2 helical region | FLWFLLPLLLLTCLTYGAWYF |
| 16 | Human SUN2 perinuclear space region | YPYGLQTFHPALVSWWAAKDSRRPDEGWEARDSSPHFQAEQRVMSRVHSLER<br>RLEALAAEFSSNWQKEAMRLERLELRQGAPGQGGGGGLSHEDTLALLEGLVSR<br>REAALKEDFRRETAARIQEELSALRAEHQQDSEDLFKKIVRASQESEARIQQLKS<br>EWQSMTQESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGLLPQQIQ<br>AVRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTHVAEMQ<br>GKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGG<br>ASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGF<br>AVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFT<br>YDQDGEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 17 | Human SUN2 LMNA-binding region | MSRRSQRLTRYSQGDDDGSSSSGGSSVAGSQSTLFKDSPLRTLKRKSSNMKRL<br>SPAPQLGPSSDAHTSYYSESLVHESWFPPRSSLEELHGDANWGEDLRVRRRRG<br>TGGSESSRASGLVGRKATEDFLGSSSGYSSEDD |
| 18 | Human SUN2 SUN domain | GASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQ<br>GFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGK<br>FTYDQDGEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPA |
| 19 | Human SUN2 CC1 | QESFQESSVKELRRLED<br>QLAGLQQELAALALKQSSVAEEVGLLPQQIQAVRDDVESQFPAWISQFLA |
| 20 | Human SUN2 CC2 α1 | REEMQAQLRELESKILTHVAEMQGK |
| 21 | Human SUN2 CC2 α2 | AAASLSLTLQKE |
| 22 | Human SUN2 CC2 α3 | VTEEQVHHIVKQALQR |
| 23 | Human SUN3 | MSGKTKARRAAMFFRRCSEDASGSASGNALLSEDENPDANGVTRSWKIILSTML<br>TLTFLLVGLLNHQWLKETDVPQKSRQLYAIIAEYGSRLYKYQARLRMPKEQLELL<br>KKESQNLENNFRQILFLIEQIDVLKALLRDMKDGMDNNHNWNTHGDPVEDPDHT<br>EEVSNLVNYVLKKLREDQVEMADYALKSAGASIIEAGTSESYKNNKAKLYWHGIG<br>FLNHEMPPDIILQPDVYPGKCWAFPGSQGHTLIKLATKIIPTAVTMEHISEKVSPSG<br>NISSAPKEFSVYGITKKCEGEEIFLGQFIYNKTGTTVQTFELQHAVSEYLLCVKLNI<br>FSNWGHPKYTCLYRFRVHGTPGKHI |
| 24 | Human SUN5 | MPRSSRSPGDPGALLEDVAHNPRPRRIAQRGRNTSRMAEDTSPNMTWFTCFAC<br>SLRTQAQQVLFNTCRCKLLCQKLMEKTGILLLCAFGFWMFSIHLPSKMKVWQDD<br>SINGPLQSLRLYQEKVRHHSGEIQDLRGSMNQLIAKLQEMEAMSDEQKMAQKIM<br>KMIHGDYIEKPDFALKSIGASIDFEHTSVTYNHEKAHSYWNWIQLWNYAQPPDVIL<br>EPNVTPGNCWAFEGDRGQVTIQLAQKVYLSNLTLQHIPKTISLSGSLDTAPKDFVI<br>YGMEGSPKEEVFLGAFQFQPENIIQMFPLQNQPARAFSAVKVKISSNWGNPGFT<br>CLYRVRVHGSVAPPREQPHQNPYPKRD |
| 25 | Human SPAG4 | MRRSSRPGSASSSRKHTPNFFSENSSMSITSEDSKGLRSAEPGPGEPEGRRAR<br>GPSCGEPALSAGVPGGTTWAGSSQQKPAPRSHNWQTACGAATVRGGASEPT<br>GSPVVSEEPLDLLPTLDLRQEMPPPRVFKSFLSLLFQGLSVLLSLAGDVLVSMYR<br>EVCSIRFLFTAVSLLSLFLSAFWLGLLYLVSPLENEPKEMLTLSEYHERVRSQGQ<br>QLQQLQAELDKLHKEVSTVRAANSERVAKLVFQRLNEDFVRKPDYALSSVGASI<br>DLQKTSHDYADRNTAYFWNRFSFWNYARPPTVILEPHVFPGNCWAFEGDQGQV<br>VIQLPGRVQLSDITLQHPPPSVEHTGGANSAPRDFAVFGLQVYDETEVSLGKFTF<br>DVEKSEIQTFHLQNDPPAAFPKVKIQILSNWGHPRFTCLYRVRAHGVRTSEGAEG<br>SAQGPH |

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| 26 | Human SUCO | MKKHRRALALVSCLFLCSLVWLPSWRVCCKESSSASASSYYSQDDNCALENED<br>VQFQKKDEREGPINAESLGKSGSNLPISPKEHKLKDDSIVDVQNTESKKLSPPVV<br>ETLPTVDLHEESSNAVVDSETVENISSSSTSEITPISKLDEIEKSGTIPIAKPSETEQ<br>SETDCDVGEALDASAPIEQPSFVSPPDSLVGQHIENVSSSHGKGKITKSEFESKV<br>SASEQGGGDPKSALNASDNLKNESSDYTKPGDIDPTSVASPKDPEDIPTFDEWK<br>KKVMEVEKEKSQSMHASSNGGSHATKKVQKNRNNYASVECGAKILAANPEAKS<br>TSAILIENMDLYMLNPCSTKIWFVIELCEPIQVKQLDIANYELFSSTPKDFLVSISDR<br>YPTNKWIKLGTFHGRDERNVQSFPLDEQMYAKYVKMFIKYIKVELLSHFGSEHFC<br>PLSLIRVFGTSMVEEYEEIADSQYHSERQELFDEDYDYPLDYNTGEDKSSKNLLG<br>SATNAILNMVNIAANILGAKTEDLTEGNKSISENATATAAPKMPESTPVSTPVPSP<br>EYVTTEVHTHDMEPSTPDTPKESPIVQLVQEEEEEASPSTVTLLGSGEQEDESSP<br>WFESETQIFCSELTTICCISSFSEYIYKWCSVRVALYRQRSRTALSKGKDYLVLAQ<br>PPLLLPAESVDVSVLQPLSGELENTNIEREAETVVLGDLSSSMHQDDLVNHTVDA<br>VELEPSHSQTLSQSLLLDITPEINPLPKIEVSESVEYEAGHIPSPVIPQESSVEIDNE<br>TEQKSESFSSIEKPSITYETNKVNELMDNIIKEDVNSMQIFTKLSETIVPPINTATVP<br>DNEDGEAKMNIADTAKQTLISVVDSSSLPEVKEEEQSPEDALLRGLQRTATDFYA<br>ELQNSTDLGYANGNLVHGSNQKESVFMRLNNRIKALEVNMSLSGRYLEELSQRY<br>RKQMEEMQKAFNKTIVKLQNTSRIAEEQDQRQTEAIQLLQAQLTNMTQLVSNLSA<br>TVAELKREVSDRQSYLVISLVLCVVLGLMLCMQRCRNTSQFDGDYISKLPKSNQY<br>PSPKRCFSSYDDMNLKRRTSFPLMRSKSLQLTGKEVDPNDLYIVEPLKFSPEKKK<br>KRCKYKIEKIETIKPEEPLHPIANGDIKGRKPFTNQRDFSNMGEVYHSSYKGPPSE<br>GSSETSSQSEESYFCGISACTSLCNGQSQKTKTEKRALKRRRSKVQDQGKLIKT<br>LIQTKSGSLPSLHDIIKGNKEITVGTFGVTAVSGHI |
| 27 | Human SUN3 SUN domain | GASIIEAGTSESYKNNKAKLYWHGIGFLNHEMPPDIILQPDVYPGKCWAFPGSQG<br>HTLIKLATKIIPTAVTMEHISEKVSPSGNISSAPKEFSVYGITKKCEGEEIFLGQFIYN<br>KTGTTVQTFELQHAVSEYLLCVKLNIFSNWGHPKYTCLYRFRVHGTPG |
| 28 | Human SUN5 SUN domain | GASIDFEHTSVTYNHEKAHSYWNWIQLWNYAQPPDVILEPNVTPGNCWAFEGD<br>RGQVTIQLAQKVYLSNLTLQHIPKTISLSGSLDTAPKDFVIYGMEGSPKEEVFLGA<br>FQFQPENIIQMFPLQNQPARAFSAVKVKISSNWGNPGFTCLYRVRVHGSVA |
| 29 | Human SPAG4 SUN domain | GASIDLQKTSHDYADRNTAYFWNRFSFWNYARPPTVILEPHVFPGNCWAFEGD<br>QGQVVIQLPGRVQLSDITLQHPPPSVEHTGGANSAPRDFAVFGLQVYDETEVSL<br>GKFTFDVEKSEIQTFHLQNDPPAAFPKVKIQILSNWGHPRFTCLYRVRAHGVRT |
| 30 | Human SUCO SUN domain | EKSQSMHASSNGGSHATKKVQKNRNNYASVECGAKILAANPEAKSTSAILIENM<br>DLYMLNPCSTKIWFVIELCEPIQVKQLDIANYELFSSTPKDFLVSISDRYPTNKWIK<br>LGTFHGRDERNVQSFPLDEQMYAKYVKMFIKYIKVELLSHFGSEHFCPLSLIRVF<br>GTSM |
| 31 | Human Nesprin-1 | MATSRGASRCPRDIANVMQRLQDEQEIVQKRTFTKWINSHLAKRKPPMVVDDLF<br>EDMKDGVKLLALLEVLSGQKLPCEQGRRMKRIHAVANIGTALKFLEGRKIKLVNIN<br>STDIADGRPSIVLGLMWTIILYFQIEELTSNLPQLQSLSSSASSVDSIVSSETPSPPS<br>KRKVTTKIQGNAKKALLKWVQYTAGKQTGIEVKDFGKSWRSGVAFHSVIHAIRPE<br>LVDLETVKGRSNRENLEDAFTIAETELGIPRLLDPEDVDVDKPDEKSIMTYVAQFL<br>KHYPDIHNASTDGQEDDEILPGFPSFANSVQNFKREDRVIFKEMKVWIEQFERDL<br>TRAQMVESNLQDKYQSFKHFRVQYEMKRKQIEHLIQPLHRDGKLSLDQALVKQS<br>WDRVTSRLFDWHIQLDKSLPAPLGTIGAWLYRAEVALREEITVQQVHEETANTIQ<br>RKLEQHKDLLQNTDAHKRAFHEIYRTRSVNGIPVPPDQLEDMAERFHFVSSTSEL<br>HLMKMEFLELKYRLLSLLVLAESKLKSWIIKYGRRESVEQLLQNYVSFIENSKFFE<br>QYEVTYQILKQTAEMYVKADGSVEEAENVMKFMNETTAQWRNLSVEVRSVRSM<br>LEEVISNWDRYGNTVASLQAWLEDAEKMLNQSENAKKDFFRNLPHWIQQHTAM<br>NDAGNFLIETCDEMVSRDLKQQLLLLNGRWRELFMEVKQYAQADEMDRMKKEY<br>TDCVVTLSAFATEAHKKLSEPLEVSFMNVKLLIQDLEDIEQRVPVMDAQYKIITKTA<br>HLITKESPQEEGKEMFATMSKLKEQLTKVKECYSPLLYESQQLLIPLEELEKQMTS<br>FYDSLGKINEIITVLEREAQSSALFKQKHQELLACQENCKKTLTLIEKGSQSVQKF<br>VTLSNVLKHFDQTRLQRQIADIHVAFQSMVKKTGDWKKHVETNSRLMKKFEESR<br>AELEKVLRIAQEGLEEKGDPEELLRRHTEFFSQLDQRVLNAFLKACDELTDILPEQ<br>EQQGLQEAVRKLHKQWKDLQGEAPYHLLHLKIDVEKNRFLASVEECRTELDRET<br>KLMPQEGSEKIIKEHRVFFSDKGPHHLCEKRLQLIEELCVKLPVRDPVRDTPGTC<br>HVTLKELRAAIDSTYRKLMEDPDKWKDYTSRFSEFSSWISTNETQLKGIKGEAIDT<br>ANHGEVKRAVEEIRNGVTKRGETLSWLKSRLKVLTEVSSENEAQKQGDELAKLS<br>SSFKALVTLLSEVEKMLSNFGDCVQYKEIVKNSLEELISGSKEVQEQAEKILDTEN<br>LFEAQQLLLHHQQKTKRISAKKRDVQQQIAQAQQGEGGLPDRGHEELRKLESTL<br>DGLERSRERQERRIQVTLRKWERFETNKETVVRYLFQTGSSHERFLSFSSLESL<br>SSELEQTKEFSKRTESIAVQAENLVKEASEIPLGPQNKQLLQQQAKSIKEQVKKLE<br>DTLEEDIKTMEMVKTKWDHFGSNFETLSVWITEKEKELNALETSSSAMDMQISQI<br>KVTIQEIESKLSSIVGLEEEAQSFAQFVTTGESARIKAKLTQIRRYGEELREHAQCL<br>EGTILGHLSQQQKFEENLRKIQQSVSEFEDKLAVPIKICSSATETYKVLQEHMDLC<br>QALESLSSAITAFSASARKVVNRDSCVQEAAALQQQYEDILRRAKERQTALENLL<br>AHWQRLEKELSSFLTWLERGEAKASSPEMDISADRVKVEGELQLIQALQNEVVS<br>QASFYSKLLQLKESLFSVASKDDVKMMKLHLEQLDERWRDLPQIINKRINFLQSV<br>VAEHQQFDELLLSFSVVWIKLFLSELQTTSEISIMDHQVALTRHKDHAAEVESKKGE |

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| | | LQSLQGHLAKLGSLGRAEDLHLLQGKAEDCFQLFEEASQVVERRQLALSHLAEF<br>LQSHASLSGILRQLRQTVEATNSMNKNESDLIEKDLNDALQNAKALESAAVSLDGI<br>LSKAQYHLKIGSSEQRTSCRATADQLCGEVERIQNLLGTKQSEADALAVLKKAFQ<br>DQKEELLKSIEDIEERTDKERLKEPTRQALQQRLRVFNQLEDELNSHEHELCWLK<br>DKAKQIAQKDVAFAPEVDREINRLEVTWDDTKRLIHENQGQCCGLIDLMREYQNL<br>KSAVSKVLENASSVIVTRTTIKDQEDLKWAFSKHETAKNKMNYKQKDLDNFTSKG<br>KHLLSELKKIHSSDFSLVKTDMESTVDKWLDVSEKLEENMDRLRVSLSIWDDVLS<br>TRDEIEGWSNNCVPQMAENISNLDNHLRAEELLKEFESEVKNKALRLEELHSKVN<br>DLKELTKNLETPPDLQFIEADLMQKLEHAKEITEVAKGTLKDFTAQSTQVEKFINDI<br>TTWFTKVEESLMNCAQNETCEALKKVKDIQKELQSQQSNISSTQENLNSLCRKY<br>HSAELESLGRAMTGLIKKHEAVSQLCSKTQASLQESLEKHFSESMQEFQEWFLG<br>AKAAAKESSDRTGDSKVLEAKLHDLQNILDSVSDGQSKLDAVTQEGQTLYAHLS<br>KQIVSSIQEQITKANEEFQAFLKQCLKDKQALQDCASELGSFEDQHRKLNLWIHE<br>MEERFNTENLGESKQHIPEKKNEVHKVEMFLGELLAARESLDKLSQRGQLLSEE<br>GHGAGQEGRLCSQLLTSHQNLLRMTKEKLRSCQVALQEHEALEEALQSMWFW<br>VKAIQDRLACAESTLGSKDTLEKRLSQIQDILLMKGEGEVKLNMAIGKGEQALRSS<br>NKEGQRVIQTQLETLKEVWADIMSSSVHAQSTLESVISQWNDYVERKNQLEQW<br>MESVDQKIEHPLQPQPGLKEKFVLLDHLQSILSEAEDHTRALHRLIAKSRELYEKT<br>EDESFKDTAQEELKTQFNDIMTVAKEKMRKVEEIVKDHLMYLDAVHEFTDWLHS<br>AKEELHRWSDMSGDSSATQKKLSKIKELIDSREIGASRLSRVESLAPEVKQNTTA<br>SGCELMHTEMQALRADWKQWEDSVFQTQSCLENLVSQMALSEQEFSGQVAQL<br>EQALEQFSALLKTWAQQLTLLEGKNTDEEIVECWHKGQEILDALQKAEPRTEDLK<br>SQLNELCRFSRDLSTYSGKVSGLIKEYNCLCLQASKGCQNKEQILQQRFRKAFR<br>DFQQWLVNAKITTAKCFDIPQNISEVSTSLQKIQEFLSESENGQHKLNMMLSKGE<br>LLSTLLTKEKAKGIQAKVTAAKEDWKNFHSNLHQKESALENLKIQMKDFEVSAEPI<br>QDWLSKTEKMVHESSNRLYDLPAKRREQQKLQSVLEEIHCYEPQLNRLKEKAQ<br>QLWEGQAASKSFRHRVSQLSSQYLALSNLTKEKVSRLDRIVAEHNQFSLGIKELQ<br>DWMTDAIHMLDSYCHPTSDKSVLDSRTLKLEALLSVKQEKEIQMKMIVTRGESVL<br>QNTSPEGIPTIQQQLQSVKDMWASLLSAGIRCKSQLEGALSKWTSYQDGVRQFS<br>GWMDSMEANLNESERQHAELRDKTTMLGKAKLLNEEVLSYSSLLETIEVKGAGM<br>TEHYVTQLELQDLQERYRAIQERAKEAVTKSEKLVRLHQEYQRDLKAFEVWLGQ<br>EQEKLDQYSVLEGDAHTHETTLRDLQELQVHCAEGQALLNSVLHTREDVIPSGIP<br>QAEDRALESLRQDWQAYQHRLSETRTQFNNVVNKLRLMEQKFQQVDEWLKTA<br>EEKVSPRTRRQSNRATKEIQLHQMKKWHEEVTAYRDEVEEVGARAQEILDESHV<br>NSRMGCQATQLTSRYQALLLQVLEQIKFLEEEIQSLEESESSLSSYSDWYGSTHK<br>NFKNVATKIDKVDTVMMGKKLKTLEVLLKDMEKGHSLLKSAREKGERAVKYLEE<br>GEAERLRKEIHDHMEQLKELTSTVRKEHMTLEKGLHLAKEFSDKCKALTQWIAEY<br>QEILHVPEEPKMELYEKKAQLSKYKSLQQTVLSHEPSVKSVREKGEALLELVQDV<br>TLKDKIDQLQSDYQDLCSIGKEHVFSLEAKVKDHEDYNSELQEVEKWLLQMSGR<br>LVAPDLLETSSLETITQQLAHHKAMMEEIAGFEDRLNNLQMKGDTLIGQCADHLQ<br>AKLKQNVHAHLQGTKDSYSAICSTAQRMYQSLEHELQKHVSRQDTLQQCQAWL<br>SAVQPDLEPSPQPPLSRAEAIKQVKHFRALQEQARTYLDLLCSMCDLSNASVKTT<br>AKDIQQTEQTIEQKLVQAQNLTQGWEEIKHLKSELWIYLQDADQQLQNMKRRHS<br>ELELNIAQNMVSQVKDFVKKLQSKQASVNTIIEKVNKLTKKEESPEHKEINHLNDQ<br>WLDLCRQSNNLCLQREEDLQRTRDYHDCMNVVEVFLEKFTTEWDNLARSDAES<br>TAVHLEALKKLALALQERKYAIEDLKDQKQKMIEHLNLDDKELVKEQTSHLEQRW<br>FQLEDLIKRKIQVSVTNLEELNVVQSRFQELMEWAEEQQPNIAEALKQSPPPDMA<br>QNLLMDHLAICSELEAKQMLLKSLIKDADRVMADLGLNERQVIQKALSDAQSHVN<br>CLSDLVGQRRKYLNKALSEKTQFLMAVFQATSQIQQHERKIMFREHICLLPDDVS<br>KQVKTCKSAQASLKTYQNEVTGLWAQGRELMKEVTEQEKSEVLGKLQELQSVY<br>DSVLQKCSHRLQELEKNLVSRKHFKEDFDKACHWLKQADIVTFPEINLMNESSEL<br>HTQLAKYQNILEQSPEYENLLLTLQRTGQTILPSLNEVDHSYLSEKLNALPRQFNV<br>IVALAKDKFYKVQEAILARKEYASLIELTTQSLSELEAQFLRMSKVPTDLAVEEALS<br>LQDGCRAILDEVAGLGEAVDELNQKKEGFRSTGQPWQPDKMLHLVTLYHRLKR<br>QTEQRVSLLEDTTSAYQEHEKMCQQLERQLKSVKEEQSKVNEETLPAEEKLKMY<br>HSLAGSLQDSGIVLKRVTIHLEDLAPHLDPLAYEKARHQIQSWQGELKLLTSAIGE<br>TVTECESRMVQSIDFQTEMSRSLDWLRRVKAELSGPVYLDLNLQDIQEEIRKIQIH<br>QEEVQSSLRIMNALSHKEKEKFTKAKELISADLEHSLAELSELDGDIQEALRTRQA<br>TLTEIYSQCQRYYQVFQAANDWLEDAQELLQLAGNGLDVESAEENLKSHMEFFS<br>TEDQFHSNLEELHSLVATLDPLIKPTGKEDLEQKVASLELRSQRMSRDSGAQVDL<br>LQRCTAQWHDYQKAREEVIELMNDTEKKLSEFSLLKTSSSHEAEEKLSEHKALVS<br>VVNSFHEKIVALEEKASQLEKTGNDASKATLSRSMTTVWQRWTRLRAVAQDQE<br>KILEDAVDEWTGFNNKVKKATEMIDQLQDKLPGSSAEKASKAELLTLLEYHDTFV<br>LELEQQQSALGMLRQQTLSMLQDGAAPTPGEEPPLMQEITAMQDRCLNMQEKV<br>KTNGKLVKQELKDREMVETQINSVKCWVQETKEYLGNPTIEIDAQLEELQILLTEA<br>TNHRQNIEKMAEEQKEKYLGLYTILPSELSLQLAEVALDLKIRDQIQDKIKEVEQSK<br>ATSQELSRQIQKLAKDLTTILTKLKAKTDNVVQAKTDQKVLGEELDGCNSKLMEL<br>DAAVQKFLEQNGQLGKPLAKKIGKLTELHQQTIRQAENRLSKLNQAASHLEEYNE<br>MLELILKWIEKAKVLAHGTIAWNSASQLREQYILHQTLLEESKEIDSELEAMTEKLQ<br>YLTSVYCTEKMSQQVAELGRETEELRQMIKIRLQNLQDAAKDMKKFEAELKKLQA |

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| | | ALEQAQATLTSPEVGRLSLKEQLSHRQHLLSEMESLKPKVQAVQLCQSALRIPED<br>VVASLPLCHAALRLQEEASRLQHTAIQQCNIMQEAVVQYEQYEQEMKHLQQLIE<br>GAHREIEDKPVATSNIQELQAQISRHEELAQKIKGYQEQIASLNSKCKMLTMKAKH<br>ATMLLTVTEVEGLAEGTEDLDGELLPTPSAHPSVVMMTAGRCHTLLSPVTEESG<br>EEGTNSEISSPPACRSPSPVANTDASVNQDIAYYQALSAERLQTDAAKIHPSTSA<br>SQEFYEPGLEPSATAKLGDLQRSWETLKNVISEKQRTLYEALERQQKYQDSLQSI<br>STKMEAIELKLSESPEPGRSPESQMAEHQALMDEILMLQDEINELQSSLAEELVS<br>ESCEADPAEQLALQSTLTVLAERMSTIRMKASGKRQLLEEKLNDQLEEQRQEQA<br>LQRYRCEADELDSWLLSTKATLDTALSPPKEPMDMEAQLMDCQNMLVEIEQKVV<br>ALSELSVHNENLLLEGKAHTKDEAEQLAGKLRRLKGSLLELQRALHDKQLNMQG<br>TAQEKEESDVDLTATQSPGVQEWLAQARTTWTQQRQSSLQQQKELEQELAEQ<br>KSLLRSVASRGEEILIQHSAAETSGDAGEKPDVLSQELGMEGEKSSAEDQMRMK<br>WESLHQEFSTKQKLLQNVLEQEQEQVLYSRPNRLLSGVPLYKGDVPTQDKSAVT<br>SLLDGLNQAFEEVSSQSGGAKRQSIHLEQKLYDGVSATSTWLDDVEERLFVATA<br>LLPEETETCLFNQEILAKDIKEMSEEMDKNKNLFSQAFPENGDNRDVIEDTLGCLL<br>GRLSLLDSVVNQRCHQMKERLQQILNFQNDLKVLFTSLADNKYIILQKLANVFEQ<br>PVAEQIEAIQQAEDGLKEFDAGIIELKRRGDKLQVEQPSMQELSKLQDMYDELMM<br>IIGSRRSGLNQNLTLKSQYERALQDLADLLETGQEKMAGDQKIIVSSKEEIQQLLD<br>KHKEYFQGLESHMILTETLFRKIISFAVQKETQFHTELMAQASAVLKRAHKRGVEL<br>EYILETWSHLDEDQQELSRQLEVVESSIPSVGLVEENEDRLIDRITLYQHLKSSLN<br>EYQPKLYQVLDDGKRLLISISCSDLESQLNQLGECWLSNTNKMSKELHRLETILK<br>HWTRYQSESADLIHWLQSAKDRLEFWTQQSVTVPQELEMVRDHLNAFLEFSKE<br>VDAQSSLKSSVLSTGNQLLRLKKVDTATLRSELSRIDSQWTDLLTNIPAVQEKLH<br>QLQMDKLPSRHAISEVMSWISLMENVIQKDEDNIKNSIGYKAIHEYLQKYKGFKIDI<br>NCKQLTVDFVNQSVLQISSQDVESKRSDKTDFAEQLGAMNKSWQILQGLVTEKI<br>QLLEGLLESWSEYENNVQCLKTWFETQEKRLKQQHRIGDQASVQNALKDCQDL<br>EDLIKAKEKEVEKIEQNGLALIQNKKEDVSSIVMSTLRELGQTWANLDHMVGQLKI<br>LLKSVLDQWSSHKVAFDKINSYLMEARYSLSRFRLLTGSLEAVQVQVDNLQNLQ<br>DDLEKQERSLQKFGSITNQLLKECHPPVTETLTNTLKEVNMRWNNLLEEIAEQLQ<br>SSKALLQLWQRYKDYSKQCASTVQQQEDRTNELLKAATNKDIADDEVATWIQDC<br>NDLLKGLGTVKDSLFFLHELGEQLKQQVDASAASAIQSDQLSLSQHLCALEQALC<br>KQQTSLQAGVLDYETFAKSLEALEAWIVEAEEILQGQDPSHSSDLSTIQERMEEL<br>KGQMLKFSSMAPDLDRLNELGYRLPLNDKEIKRMQNLNRHWSLISSQTTERFSK<br>LQSFLLQHQTFLEKCETWMEFLVQTEQKLAVEISGNYQHLLEQQRAHELFQAEM<br>FSRQQILHSIIIDGQRLLEQGQVDDRDEFNLKLTLLSNQWQGVIRRAQQRRGIIDS<br>QIRQWQRYREMAEKLRKWLVEVSYLPMSGLGSVPIPLQQARTLFDEVQFKEKVF<br>LRQQGSYILTVEAGKQLLLSADSGAEAALQAELAEIQEKWKSASMRLEEQKKKLA<br>FLLKDWEKCEKGIADSLEKLRTFKKKLSQSLPDHHEELHAEQMRCKELENAVGS<br>WTDDLTQLSLLKDTLSAYISADDISILNERVELLQRQWEELCHQLSLRRQQIGERL<br>NEWAVFSEKNKELCEWLTQMESKVSQNGDILIEEMIEKLKKDYQEEIAIAQENKIQ<br>LQQMGERLAKASHESKASEIEYKLGKVNDRWQHLLDLIAARVKKLKETLVAVQQL<br>DKNMSSLRTWLAHIESELAKPIVYDSCNSEEIQRKLNEQQELQRDIEKHSTGVAS<br>VLNLCEVLLHDCDACATDAECDSIQQATRNLDRRWRNICAMSMERRLKIEETWR<br>LWQKFLDDYSRFEDWLKSSERTAAFPSSSGVIYTVAKEELKKFEAFQRQVHECL<br>TQLELINKQYRRLARENRTDSACSLKQMVHEGNQRWDNLQKRVTSILRRLKHFI<br>GQREEFETARDSILVWLTEMDLQLTNIEHFSECDVQAKIKQLKAFQQEISLNHNKI<br>EQIIAQGEQLIEKSEPLDAAIIEEELDELRRYCQEVFGRVERYHKKLIRLPLPDDEH<br>DLSDRELELEDSAALSDLHWHDRSADSLLSPQPSSNLSLSLAQPLRSERSGRDT<br>PASVDSIPLEWDHDYDLSRDLESAMSRALPSEDEEGQDDKDFYLRGAVGLSGD<br>HSALESQIRQLGKALDDSRFQIQQTENIIRSKTPTGPELDTSYKGYMKLLGECSSS<br>IDSVKRLEHKLKEEEESLPGFVNLHSTETQTAGVIDRWELLQAQALSKELRMKQN<br>LQKWQQFNSDLNSIWAWLGDTEEELEQLQRLELSTDIQTIELQIKKLKELQKAVD<br>HRKAIILSINLCSPEFTQADSKESRDLQDRLSQMNGRWDRVCSLLEEWRGLLQD<br>ALMQCQGFHEMSHGLLLMLENIDRRKNEIVPIDSNLDAEILQDHHKQLMQIKHELL<br>ESQLRVASLQDMSCQLLVNAEGTDCLEAKEKVHVIGNRLKLLLKEVSRHIKELEK<br>LLDVSSSQQDLSSWSSADELDTSGSVSPTSGRSTPNRQKTPRGKCSLSQPGPS<br>VSSPHSRSTKGGSDSSLSEPGPGRSGRGFLFRVLRAALPLQLLLLLLIGLACLVP<br>MSEEDYSCALSNNFARSFHPMLRYTNGPPPL |
| 32 | Human Nesprin-2 | MASSPELPTEDEQGSWGIDDLHISLQAEQEDTQKKAFTCWINSQLARHTSPSVIS<br>DLFTDIKKGHVLLDLLEVLSGQQLPRDKGSNTFQCRINIEHALTFLRNRSIKLINIHV<br>TDIIDGNPSIILGLIWTIILHFHIEKLAQTLSCNYNQPSLDDVSVVDSSPASSPPAKK<br>CSKVQARWQMSARKALLLWAQEQCATYESVNVTDFKSSWRNGMAFLAIIHALR<br>PDLIDMKSVKHRSNKDNLREAFRIAEQELKIPRLLEPEDVDVVDPDEKSIMTYVAQ<br>FLQYSKDAPGTGEEAQGKVKDAMGWLTLQKEKLQKLLKDSENDTYFKKYNSLLS<br>FMESFNEEKKSFLDVLSIKRDLDELDKDHLQLREAWDGLDHQINAWKIKLNYALP<br>PPLHQTEAWLQEVEELMDEDLSASQDHSQAVTLIQEKMTLFKSLMDRFEHHSNI<br>LLTFENKDENHLPLVPPNKLEEMKRRINNILEKKFILLLEFHYYKCLVLGLVDEVKS<br>KLDIWNIKYGSRESVELLLEDWHKFIEEKEFLARLDTSFQKCGEIYKNLAGECQNI<br>NKQYMMVKSDVCMYRKNIYNVKSTLQKVLACWATYVENLRLLRACFEETKKEEI<br>KEVPFETLAQWNLEHATLNEAGNFLVEVSNDVVGSSISKELRRLNKRWRKLVSK |

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| | | TQLEMNLPLMIKKQDQPTFDNSGNILSKEEKATVEFSTDMSVELPENYNQNIKAG<br>EKHEKENEEFTGQLKVAKDVEKLIGQVEIWEAEAKSVLDQDDVDTSMEESLKHLI<br>AKGSMFDELMARSEDMLQMDIQNISSQESFQHVLTTGLQAKIQEAKEKVQINVVK<br>LIAALKNLTDVSPDLDIRLKMEESQKELESYMMRAQQLLGQRESPGELISKHKEA<br>LIISNTKSLAKYLKAVEELKNNVTEDIKMSLEEKSRDVCAKWESLHHELSLYVQQL<br>KIDIEKGKLSDNILKLEKQINKEKKLIRRGRTKGLIKEHEACFSEEGCLYQLNHHME<br>VLRELCEELPSQKSQQEVKRLLKDYEQKIERLLKCASEIHMTLQPTAGGTSKNEG<br>TITTSENRGGDPHSEAPFAKSDNQPSTEKAMEPTMKFSLASVLRPLQEESIMEKD<br>YSASINSLLERYDTYRDILEHHLQNNKFRITSDFSSEEDRSSSCLQAKLTDLQVIK<br>NETDARWKEFEIISLKLENHVNDIKKPFVIKERDTLKERERELQMTLNTRMESLET<br>ALRLVLPVEKASLLLCGSDLPLHKMAIQGFHLIDADRIYQHLRNIQDSIAKQIEICNR<br>LEEPGNFVLKELHPFDLHAMQNIILKYKTQFEGMNHRVQRSEDTLKALEDFLASL<br>RTAKLSAEPVTDLSASDTQVAQENTLTVKNKEGEIHLMKDKAKHLDKCLKMLDM<br>SFKDAERGDDTSCENLLDAFSIKLSETHGYGVQEEFTEENKLLEACIFKNNELLKN<br>IQDVQSQISKIGLKDPTVPAVKHRKKSLIRLDKVLDEYEEEKRHLQEMANSLPHFK<br>DGREKTVNQQCQNTVVLWENTKALVTECLEQCGRVLELLKQYQNFKSILTTLIQK<br>EESVISLQASYMGKENLKKRIAEIEIVKEEFNEHLEVVDKINQVCKNLQFYLNKMK<br>TFEEPPFEKEANIIVDRWLDINEKTEDYYENLGRALALWDKLFNLKNVIDEWTEKA<br>LQKMELHQLTEEDRERLKEELQVHEQKTSEFSRRVAEIQFLLQSSEIPLELQVME<br>SSILNKMEHVQKCLTGESNCHALSGSTAELREDLDQAKTQIGMTESLLKALSPSD<br>SLEIFTKLEEIQQQILQQKHSMILLENQIGCLTPELSELKKQYESVSDLFNTKKSVL<br>QDHFSKLLNDQCKNFNDWFSNIKVNLKECFESSETKKSVEQKLQKLSDFLTLEG<br>RNSKIKQVDSVLKHVKKHLPKAHVKELISWLVGQEFELEKMESICQARAKELEDS<br>LQQLLRLQDDHRNLRKWLTNQEEKWKGMEEPGEKTELFCQALARKREQFESVA<br>QLNNSLKEYGFTEEEEIIMEATCLMDRYQTLLRQLSEIEEEDKLLPTEDQSFNDLA<br>HDVIHWIKEIKESLMVLNSSEGKMPLEERIQKIKEIILLKPEGDARIETIMKQAESSE<br>APLVQKTLTDISNQWDNTLHLASTYLSHQEKLLLEGEKYLQSKEDLRLMLIELKKK<br>QEAGFALQHGLQEKKAQLKIYKKFLKKAQDLTSLLKELKSQGNYLLECTKNPSFS<br>EEPWLEIKHLHESLLQQLQDSVQNLDGHVREHDSYQVCVTDLNTTLDNFSKEFV<br>SFSDKPVDQIAVEEKLQKLQELENRLSLQDGTLKKILALAKSVKQNTSSVGQKIIK<br>DDIKSLQCKQKDLENRLASAKQEMECCLNSILKSKRSTEKKGKFTLPGREKQATS<br>DVQESTQESAAVEKLEEDWEINKDSAVEMAMSKQLSLNAQESMKNTEDERKVN<br>ELQNQPLELDTMLRNEQLEEIEKLYTQLEAKKAAIKPLEQTECLNKTETGALVLHN<br>IGYSAQHLDNLLQALITLKKNKESQYCVLRDFQEYLAAVESSMKALLTDKESLKVG<br>PLDSVTYLDKIKKFIASIEKEKDSLGNLKIKWENLSNHVTDMDKKLLESQIKQLEHG<br>WEQVEQQIQKKYSQQVVEYDEFTTLMNKVQDTEISLQQQQQHLQLRLKSPEER<br>AGNQSMIALTTDLQATKHGFSVLKGQAELQMKRIWGEKEKKNLEDGINNLKKQW<br>ETLEPLHLEAENQIKKCDIRNKMKETILWAKNLLGELNPSIPLLPDDILSQIRKCKVT<br>HDGILARQQSVESLAEEVKDKVPSLTTYEGSDLNNTLEDLRNQYQMLVLKSTQR<br>SQQLEFKLEERSNFFAIIRKFQLMVQESETLIIPRVETAATEAELKHHHVTLEASQK<br>ELQEIDSGISTHLQELTNIYEELNVFERLFLEDQLKNLKIRTNRIQRFIQNTCNEVEH<br>KIKFCRQFHEKTSALQEEADSIQRNELLLNQEVNKGVKEEIYNLKDRLTAIKCCILQ<br>VLKLKKVFDYIGLNWDFSQLDQLQTQVFEKEKELEEKIKQLDTFEEEHGKYQALL<br>SKMRAIDLQIKKMTEVVLKAPDSSPESRRLNAQILSQRIEKAKCLCDEIIKKLNENK<br>TFDDSFKEKEILQIKLNAEENDKLYKVLQNMVLELSPKELDEKNCQDKLETSLHVL<br>NQIKSQLQQPLLINLEIKHIQNEKDNCEAFQEQVWAEMCSIKAVTAIEKQREENSS<br>EASDVETKLREFEDLQMQLNTSIDLRTNVLNDAYENLTRYKEAVTRAVESITSLEA<br>IIIPYRVDVGNPEESLEMPLRKQEELESTVAHIQDLTEKLGMISSPEAKLQLQYTLQ<br>ELVSKNSAMKEAFKAQETEAERYLENYKCYRKMEEDIYTNLSKMETVLGQSMSS<br>LPLSYREALERLEQSKALVSNLISTKEELMKLRQILRLLRLRCTENDGICLLKIVSAL<br>WEKWLSLLEAAKEWEMWCEELKQEWKFVSEEIEREAIILDNLQEELPEISKTKEA<br>ATTEELSELLDCLCQYGENVEKQQLLLTLLLQRIRSIQNVPESSGAVETVPAFQEI<br>TSMKERCNKLLQKVQKNKELVQTEIQERHSFTKEIIALKNFFQQTTTSFQNMAFQ<br>DHPEKSEQFEELQSILKKGKLTFENIMEKLRIKYSEMYTIVPAEIESQVEECRKALE<br>DIDEKISNEVLKSSPSYAMRRKIEEINNGLHNVEKMLQQKSKNIEKAQEIQKKMW<br>DELDLWHSKLNELDSEVQDIVEQDPGQAQEWMDNLMIPFQQYQQVSQRAECRT<br>SQLNKATVKMEEYSDLLKSTEAWIENTSHLLANPADYDSLRTLSHHASTVQMALE<br>DSEQKHNLLHSIFMDLEDLSIIFETDELTQSIQELSNQVTALQQKIMESLPQIQRMA<br>DDVVAIESEVKSMEKRVSKIKTILLSKEIFDFSPEEHLKHGEVILENIRPMKKTIAEIV<br>SYQVELRLPQTGMKPLPVFQRTNQLLQDIKLLENVTQEQNELLKVVIKQTNEWDE<br>EIENLKQILNNYSAQFSLEHMSPDQADKLPQLQGEIERMEKQILSLNQRKEDLLVD<br>LKATVLNLHQHLKQEQEGVERDRLPAVTSEEGGVAERDASERKLNRRGSMSYL<br>AAVEEEVEESSVKSDNGDEKAEPSPQSWSSLWKHDKDMEEDRASSSSGTIVQE<br>AYGKISTSDNSMAQILTPDSLNTEQGPECSLRPNQTEEGTTPPIEADTLDSSDAQ<br>GGLEPRVEKTRPEPTEVLHACKTQVAELELWLQQANVAVEPETLNADMQQVLE<br>QQLVGCQAMLTEIEHKVAFLLETCKDQGLGDNGATQHEAEALSLKLKTVKCNLE<br>KVQMMLQEKHSEDQHPTILKKSSEPEHQEALQPVNLSELESIVTERPQFSRQKD<br>FQQQQVLELKPMEQKDFIKFIEFNAKKMWPQYCQHDNDTTQESSASNQASSPE<br>NDVPDSILSPQGQNGDKWQYLHHELSSKIKLPLPQLVEPQVSTNMGILPSVTMY<br>NFRYPTTEELKTYTTQLEDLRQEASNLQTQENMTEEAYINLDKKLFELFLTLSQCL |

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| | | SSVEEMLEMPRLYREDGSGQQVHYETLALELKKLYLALSDKKGDLLKAMTWPGE<br>NTNLLLECFDNLQVCLEHTQAAAVCRSKSLKAGLDYNRSYQNEIKRLYHQLIKSK<br>TSLQQSLNEISGQSVAEQLQKADAYTVELENAESRVAKLRDEGERLHLPYALLQE<br>VYKLEDVLDSMWGMLRARYTELSSPFVTESQQDALLQGMVELVKIGKEKLAHGH<br>LKQTKSKVALQAQIENHKVFFQKLVADMLLIQAYSAKILPSLLQNRETFWAEQVTE<br>VKILEEKSRQCGMKLQSLLQKWEEFDENYASLEKDLEILISTLPSVSLVEETEERL<br>VERISFYQQIKRNIGGKHARLYQTLNEGKQLVASVSCPELEGQIAKLEEQWLSLN<br>KKIDHELHRLQALLKHLLSYNRDSDQLTKWLESSQHTLNYWKEQSLNVSQDLDTI<br>RSNINNFFEFSKEVDEKSSLKTAVISIGNQLLHLKETDTATLRASLAQFEQKWTMLI<br>TQLPDIQEKLHQLQMEKLPSRKAITEMISWMNNVEHQTSDEDSVHSPSSASQVK<br>HLLQKHKEFRMEMDYKQWIVDFVNQSLLQLSTCDVESKRYERTEFAEHLGEMN<br>RQWHRVHGMLNRKIQHLEQLLESITESENKIQILNNWLEAQEERLKTLQKPESVIS<br>VQKLLLDCQDIENQLAIKSKALDELKQSYLTLESGAVPLLEDTASRIDELFQKRSS<br>VLTQVNQLKTSMQSVLQEWKIYDQLYDEVNMMTIRFWYCMEHSKPVVLSLETLR<br>CQVENLQSLQDEAESSEGSWEKLQEVIGKLKGLCPSVAEIIEEKCQNTHKRWTQ<br>VNQAIADQLQKAQSLLQLWKAYSNAHGEAAARLKQQEAKFQQLANISMSGNNLA<br>EILPPALQDIKELQHDVQKTKEAFLQNSSVLDRLPQPAESSTHMLLPGPLHSLQR<br>AAYLEKMLLVKANEFEFVLSQFKDFGVRLESLKGLIMHEEENLDRLHQQEKENPD<br>SFLNHVLALTAQSPDIEHLNEVSLKLPLSDVAVKTLQNMNRQWIRATATALERCS<br>ELQGIGLNEKFLYCCEKWIQLLEKIEEALKVDVANSLPELLEQQKTYKMLEAEVSI<br>NQTIADSYVTQSLQLLDTTEIENRPEFITEFSKLTDRWQNAVQGVRQRKGDVDGL<br>VRQWQDFTTSVENLFRFLTDTSHLLSAVKGQERFSLYQTRSLIHELKNKEIHFQR<br>RRTTCALTLEAGEKLLLTTDLKTKESVGRRISQLQDSWKDMEPQLAEMIKQFQST<br>VETWDQCEKKIKELKSRLQVLKAQSEDPLPELHEDLHNEKELIKELEQSLASWTQ<br>NLKELQTMKADLTRHVLVEDVMVLKEQIEHLHRQWEDLCLRVAIRKQEIEDRLNT<br>WVVFNEKNKELCAWLVQMENKVLQTADISIEEMIEKLQKDCMEEINLFSENKLQL<br>KQMGDQLIKASNKSRAAEIDDKLNKINDRWQHLFDVIGSRVKKLKETFAFIQQLDK<br>NMSNLRTWLARIESELSKPVVYDVCDDQEIQKRLAEQQDLQRDIEQHSAGVESV<br>FNICDVLLHDSDACANETECDSIQQTTRSLDRRWRNICAMSMERRMKIEETWRL<br>WQKFLDDYSRFEDWLKSAERTAACPNSSEVLYTSAKEELKRFEAFQRQIHERLT<br>QLELINKQYRRLARENRTDTASRLKQMVHEGNQRWDNLQRRVTAVLRRLRHFT<br>NQREEFEGTRESILVWLTEMDLQLTNVEHFSESDADDKMRQLNGFQQEITLNTN<br>KIDQLIVFGEQLIQKSEPLDAVLIEDELEELHRYCQEVFGRVSRFHRRLTSCTPGL<br>EDEKEASENETDMEDPREIQTDSWRKRGESEEPSSPQSLCHLVAPGHERSGCE<br>TPVSVDSIPLEWDHTGDVGGSSSHEEDEEGPYYSALSGKSISDGHSWHVPDSP<br>SCPEHHYKQMEGDRNVPPVPPASSTPYKPPYGKLLLPPGTDGGKEGPRVLNGN<br>PQQEDGGLAGITEQQSGAFDRWEMIQAQELHNKLKIKQNLQQLNSDISAITTWLK<br>KTEAELEMLKMAKPPSDIQEIELRVKRLQEILKAFDTYKALVVSVNVSSKEFLQTE<br>SPESTELQSRLRQLSLLWEAAQGAVDSWRGGLRQSLMQCQDFHQLSQNLLLW<br>LASAKNRRQKAHVTDPKADPRALLECRRELMQLEKELVERQPQVDMLQEISNSL<br>LIKGHGEDCIEAEEKVHVIEKKLKQLREQVSQDLMALQGTQNPASPLPSFDEVDS<br>GDQPPATSVPAPRAKQFRAVRTTEGEEETESRVPGSTRPQRSFLSRVVRAALPL<br>QLLLLLLLLLACLLPSSEEDYSCTQANNFARSFYPMLRYTNGPPPT |
| 33 | Human Nesprin-3 | MTQQPQDDFDRSVEDAQAWMKAVQDQLQVNDNTQGPRAALEARLWETEKICQ<br>LEPEGRVRVDLVLRMAEALLACCPGDQKPGILARLKDIKAQWEETVTYMTHCHS<br>RIEWVWLHWSEYLLARDEFYRWFQKMMVTLEPHIELQLGLKEKQWQLSHAQVL<br>LHNVDNQAVLLDRLLEEAASLFNRIGDPSVDEDAQKRMKAEYDAVKAKAQKRVD<br>LLEQVAREHEEYQAGVDEFQLWLKAVVEKVNGCLGRNCKLPITQRLSTLQDIAK<br>DFPRGEESLETLEEQSAGVIRNTSPLGAEKITGELEEMRKVLEKLRALWEEEEER<br>LRGLLRSRGAWEQQIKQLEAELSEFRMVLQRLAQEGLQPAAKAGTEDELVAHW<br>RRYSATRAALASEEPRVDRLQAQLKELIVFPHNLKPLSDSVIATIQEYQSLKVKSA<br>RLRNAAAVELWQHFQRPLQDLQLWKALAQRLLEVTASLPDLPSLHTFLPQIEAAL<br>MESSRLKELLTMLQLKKDLLIGIFGQERATALLEQVAGSMRDRDLLHNSLLQRKS<br>KLQSLLAQHKDFGAAFEPLQRKLLDLQVRVQAEKGLQRDLPGKQAQLSRLQGLQ<br>EEGLDLGAQMEAARPLVQENPNHQHKMDQLSSDFQALQRSLEDLVDRCRQSV<br>QEHCTFSHQLLELRQWIVVTTQKLEAHRGEAGPGDAESQEAEFERLVAEFPEKE<br>AQLSLVEAQGWLVMEKSSPEGAAVVQEELRELAESWRALRLLEESLLSLIRNWH<br>LQRMEVDSGKKMVFTNNIPKSGFLINPMDPIPRHRRRANLLQEEEGSHEDFSQLL<br>RNFGQWLQVENSKLVRIIAMRTSTAEDLRTRKSKLQELEARVPEGQHLFENLLRL<br>GPARGTSDELEDLRYQWMLYKSKLKDSGHLLTQSSPGEPTGFQKTRRWRGLG<br>SLFRRACCVALPLQLLLLLFLLLLFLLPIREEDRSCTLANNFARSFTLMLRYNGPPP<br>T |
| 34 | Human Nesprin-4 | MALSLPLGPRLGSEPLNHPPGAPREADIVGCTVCPASGEESTSPEQAQTLGQDS<br>LGPPEHFQGGPRGNEPAAHPPRWSTPSSYEDPAGGKHCEHPISGLEVLEAEQN<br>SLHLCLLGLGRRLQDLEQGLGHWALAQSGMVQLQALQVDLRGAAERVEALLAF<br>GEGLAQRSEPRAWAALEQILRALGAYRDSIFRRLWQLQAQLVSYSLVFEEANTL<br>DQDLEVEGDSDWPGPGGVWGPWAPSSLPTSTELEWDPAGDIGGLGPLGQKTA<br>RTLGVPCELCGQRGPQGRGQGLEEADTSHSRQDMLESGLGHQKRLARHQRHS<br>LLRKPQDKKRQASPHLQDVRLEGNPGAPDPASRQPLTFLLILFLLFLLLVGAMFLL<br>PASGGPCCSHARIPRTPYLVLSYVNGLPPV |

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| 35 | Human KASH5 | MDLPEGPVGGPTAEMYLRERPEEARLGMPVSLEEQILNSTFEACDPQRTGTVAV AQVLAYLEAVTGQGPQDARLQTLANSLDPNGEGPKATVDLDTFLVVMRDWIAAC QLHGGLELEEETAFQGALTSRQLPSGCPEAEEPANLESFGGEDPRPELQATADL LSSLEDLELSNRRLVGENAKLQRSMETAEEGSARLGEEILALRKQLHSTQQALQF AKAMDEELEDLKTLARSLEEQNRSLLAQARQAEKEQQHLVAEMETLQEENGKLL AERDGVKKRSQELAMEKDTLKRQLFECEHLICQRDTILSERTRDVESLAQTLEEY RVTTQELRLEISRLEEQLSQTYEGPDELPEGAQLRRVGWTELLPPSLGLEIEAIRQ KQEVATADLSNPLCGVWQWEEVIHETSEETEFPSEAPAGGQRNFQGEPAHPEE GRKEPSMWLTRREEEEDAESQVTADLPVPLGAPRPGDIPENPPERPARRELQQ ALVPVMKKLVPVRRRAWGQLCLPPQRLRVTRHPLIPAPVLGLLLLLLLSVLLLGPS PPPTWPHLQLCYLQPPPV |
| 36 | Human LRMP | MESTPFSGVANQIHTLCERPTYGEVKDGALDVKRQHKCPGPTSGPSPGTNLSG CIRMNDDPSMEENGVERVCPESLLQSREYSSLPLPRHTSSTDGTITSSDPGLEIL NMASCDLDRNSLCKKEEDTRSASPTIEAQGTSPAHDNIAFQDSTSKDKTILNLEA KEEPETIEEHKKEHASGDSVVSPLPVTTVKSVNLRQSENTSANEKEVEAEFLRLS LGFKCDWFTLEKRVKLEERSRDLAEENLKKEITNCLKLLESLTPLCEDDNQAQEII KKLEKSIKFLSQCAARVASRAEMLGAINQESRVSKAVEVMIQHVENLKRMYAKEH AELEELKQVLLQNERSFNPLEDDDDCQIKKRSASLNSKPSSLRRVTIASLPRNIGN AGMVAGMENNDRFSRRSSSWRILGSKQSEHRPSLPRFISTYSWADAEEEKCEL KTKDDSEPSGEETVERTRKPSLSEKKNNPSKWDVSSVYDTIASWATNLKSSIRK ANKALWLSIAFIVLFAALMSFLTGQLFQKSVDAAPTQQEDSWTSLEHILWPFTRLR HNGPPPV |
| 37 | Human Nesprin-1 KASH domain | RGFLFRVLRAALPLQLLLLLLIGLACLVPMSEEDYSCALSNNFARSFHPMLRYTNG PPPL |
| 38 | Human Nesprin-2 KASH domain | RSFLSRVVRAALPLQLLLLLLLLLACLLPSSEEDYSCTQANNFARSFYPMLRYTNG PPPT |
| 39 | Human Nesprin-3 KASH domain | GSLFRRACCVALPLQLLLLLFLLLLFLLPIREEDRSCTLANNFARSFTLMLRYNGPP PT |
| 40 | Human Nesprin-4 KASH domain | DPASRQPLTFLLILFLLFLLLVGAMFLLPASGGPCCSHARIPRTPYLVLSYVNGLPP V |
| 41 | Human KASH5 KASH domain | LIPAPVLGLLLLLLLSVLLLGPSPPPTWPHLQLCYLQPPPV |
| 42 | Human LRMP KASH domain | QLFQKSVDAAPTQQEDSWTSLEHILWPFTRLRHNGPPPV |
| 43 | SUN1(360-812); DNSUN1 | DDPQDVFKPTTSRLKQPLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLR ELTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMS HLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSW RHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKG DLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVN SALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSP RVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPK DFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSN WGHPEYTCLYRFRVHGEPVK |
| 44 | SUN1(616-812) | GITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLF GIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKT LSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDT AFQIVELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 45 | SUN2(258-717); DNSUN2 | DEGWEARDSSPHFQAEQRVMSRVHSLERRLEALAAEFSSNWQKEAMRLERLEL RQGAPGQGGGGGLSHEDTLALLEGLVSRREAALKEDFRRETAARIQEELSALRA EHQQDSEDLFKKIVRASQESEARIQQLKSEWQSMTQESFQESSVKELRRLEDQL AGLQQELAALALKQSSVAEEVGLLPQQIQAVRDDVESQFPAWISQFLARGGGGR VGLLQREEMQAQLRELESKILTHVAEMQGKSAREAAASLSLTLQKEGVIGVTEEQ VHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLSLFGIPLWY HSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPKALSPNS TISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMATYQVVEL RILTNWGHPEYTCIYRFRVHGEPAH |
| 46 | SUN2(522-717) | GVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLSLF GIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPK ALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMAT YQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 47 | DNSUN1 w/SP and KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGPALDDPQDVFKPTTSRLKQPLQGDSEAF PWHWMSGVEQQVASLSGQCHHHGENLRELTTLLQKLQARVDQMEGGAAGPS ASVRDAVGQPPRETDFMAFHQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQK TISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREM VKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTK QLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSI LSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVV RLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYD QDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |

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| 48 | DNSUN2 w/SP and KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGPALDEGWEARDSSPHFQAEQRVMSRVH SLERRLEALAAEFSSNWQKEAMRLERLELRQGAPGQGGGGGLSHEDTLALLEG LVSRREAALKEDFRRETAARIQEELSALRAEHQQDSEDLFKKIVRASQESEARIQ QLKSEWQSMTQESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGLLP QQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTHVA EMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALE SGGASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGP QQGFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLG KFTYDQDGEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPAHK DEL |
| 49 | SUN2(324-717) (Zhou *et al.*, 2012) | SHEDTLALLEGLVSRREAALKEDFRRETAARIQEELSALRAEHQQDSEDLFKKIV RASQESEARIQQLKSEWQSMTQESFQESSVKELRRLEDQLAGLQQELAALALKQ SSVAEEVGLLPQQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQL RELESKILTHVAEMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYS EDRIGLADYALESGGASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDV HPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGF DEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCI YRFRVHGEPAH |
| 50 | SUN2(335-717) (Sosa *et al.*, 2012) | LVSRREAALKEDFRRETAARIQEELSALRAEHQQDSEDLFKKIVRASQESEARIQ QLKSEWQSMTQESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGLLP QQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTHVA EMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALE SGGASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGP QQGFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLG KFTYDQDGEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 51 | SUN2(398-717) (Zhou *et al.*, 2012) | MTQESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGLLPQQIQAVRD DVESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTHVAEMQGKSA REAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVIS TRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVR LSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQD GEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 52 | SUN2(433-717) (Zhou *et al.*, 2012) | SSVAEEVGLLPQQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQL RELESKILTHVAEMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYS EDRIGLADYALESGGASVISTRCSETYETKTALLSLFGIPLWYHSQSPRVILQPDV HPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGF DEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMATYQVVELRILTNWGHPEYTCI YRFRVHGEPAH |
| 53 | SUN2(467-717) (Wang *et al.*, 2012) | RGGGGRVGLLQREEMQAQLRELESKILTHVAEMQGKSAREAAASLSLTLQKEG VIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLS LFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHV PKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTM ATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 54 | SUN2(507-717) (Sosa *et al.*, 2012) | AAASLSLTLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTR CSETYETKTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLS ARIRPTAVTLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGE PIQTFHFQAPTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 55 | SUN2(514-717) (Sosa *et al.*, 2012) | TLQKEGVIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYET KTALLSLFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAV TLEHVPKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQ APTMATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 56 | SUN2(520-717) (Zhou *et al.*, 2012) | VIGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLS LFGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHV PKALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTM ATYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 57 | SUN2(521-717) (Sosa *et al.*, 2012) | IGVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLSL FGIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVP KALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMA TYQVVELRILTNWGHPEYTCIYRFRVHGEPAH |
| 58 | Sun1(458-913); DnSun1 (Crisp *et al.*, 2006) | DDSKGMHRPGPLPPSPPPKVDHKASQWPQESDMGQKVASLSAQCHNHDERLA ELTVLLQKLQIRVDQVDDGREGLSLWVKNVVGQHLQEMGTIEPPDAKTDFMTFH HDHEVRLSNLEDVLRKLTEKSEAIQKELEETKLKAGSRDEEQPLLDRVQHLELEL NLLKSQLSDWQHLKTSCEQAGARIQETVQLMFSEDQQGGSLEWLLEKLSSRFV SKDELQVLLHDLELKLLQNITHHITVTGQAPTSEAIVSAVNQAGISGITEAQAHIIVN NALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALLSLFGVPLWYFSQSP RVVIQPDIYPGNCWAFKGSQGYLVVRLSMKIYPTTFTMEHIPKTLSPTGNISSAPK DFAVYGLETEYQEEGQPLGRFTYDQEGDSLQMFHTLERPDQAFQIVELRVLSN WGHPEYTCLYRFRVHGEPIQ |
| 59 | Sun1(668-913) (Xu *et al.*, 2018) | SRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPTSEAIVSAVNQAGISGITEAQA HIIVNNALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALLSLFGVPLWYF |

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| | | SQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMKIYPTTFTMEHIPKTLSPTGNIS SAPKDFAVYGLETEYQEEGQPLGRFTYDQEGDSLQMFHTLERPDQAFQIVELRV LSNWGHPEYTCLYRFRVHGEPIQ |
| 60 | Sun2(410-731) (Nie *et al.*, 2016) | LLLLLGAGVSLWGQGNFFSLLPVLNWTAMQPTQRVDDSKGMHRPGPLPPSPPP KVDHKASQWPQESDMGQKVASLSAQCHNHDERLAELTVLLQKLQIRVDQVDDG REGLSLWVKNVVGQHLQEMGTIEPPDAKTDFMTFHHDHEVRLSNLEDVLRKLTE KSEAIQKELEETKLKAGSRDEEQPLLDRVQHLELELNLLKSQLSDWQHLKTSCEQ AGARIQETVQLMFSEDQQGGSLEWLLEKLSSRFVSKDELQVLLHDLELKLLQNIT HHITVTGQAPTSEAIVSAVNQAGISGITEAQAHIIVNNALKLYSQDKTGMVDFALES GGGSILSTRCSETYETKTALLSLFGVPLWYFSQSPRVVIQPDIYPGNCWAFKGSQ GYLVVRLSMKIYPTTFTMEHIPKTLSPTGNISSAPKDFAVYGLETEYQEEGQPLGR FTYDQEGDSLQMFHTLERPDQAFQIVELRVLSNWGHPEYTCLYRFRVHGEPIQ |
| 61 | Sun2(458-731) (Jahed *et al.*, 2016) | PSPPPKVDHKASQWPQESDMGQKVASLSAQCHNHDERLAELTVLLQKLQIRVD QVDDGREGLSLWVKNVVGQHLQEMGTIEPPDAKTDFMTFHHDHEVRLSNLEDV LRKLTEKSEAIQKELEETKLKAGSRDEEQPLLDRVQHLELELNLLKSQLSDWQHL KTSCEQAGARIQETVQLMFSEDQQGGSLEWLLEKLSSRFVSKDELQVLLHDLEL KLLQNITHHITVTGQAPTSEAIVSAVNQAGISGITEAQAHIIVNNALKLYSQDKTGM VDFALESGGGSILSTRCSETYETKTALLSLFGVPLWYFSQSPRVVIQPDIYPGNC WAFKGSQGYLVVRLSMKIYPTTFTMEHIPKTLSPTGNISSAPKDFAVYGLETEYQ EEGQPLGRFTYDQEGDSLQMFHTLERPDQAFQIVELRVLSNWGHPEYTCLYRF RVHGEPIQ |
| 62 | Sun2(482-731) (Nie *et al.*, 2016) | ASLSAQCHNHDERLAELTVLLQKLQIRVDQVDDGREGLSLWVKNVVGQHLQEM GTIEPPDAKTDFMTFHHDHEVRLSNLEDVLRKLTEKSEAIQKELEETKLKAGSRD EEQPLLDRVQHLELELNLLKSQLSDWQHLKTSCEQAGARIQETVQLMFSEDQQG GSLEWLLEKLSSRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPTSEAIVSAVN QAGISGITEAQAHIIVNNALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTA LLSLFGVPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMKIYPTTFTME HIPKTLSPTGNISSAPKDFAVYGLETEYQEEGQPLGRFTYDQEGDSLQMFHTLER PDQAFQIVELRVLSNWGHPEYTCLYRFRVHGEPIQ |
| 63 | SUN1(404-812) | HHHGENLRELTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFH QEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQ LKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRF SSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITE AQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPL WYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPT GNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQI VELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 64 | SUN1(455-812) | HQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELD QLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQR FSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGIT EAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIP LWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSP TGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAF QIVELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 65 | SUN1(495-812) | GEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLF SEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTS EAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRC SETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSM MIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGE SLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 66 | SUN1(562-812) | QRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGAS GITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLF GIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKT LSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDT AFQIVELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 67 | SUN1(601-812) | TSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILST RCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLS MMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDG ESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVK |
| 68 | SUN1(636-812) | DKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIY PGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLE NEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTC LYRFRVHGEPVK |
| 69 | SUN1(404-812) w/KDEL | HHHGENLRELTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFH QEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQ LKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRF SSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITE AQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPL WYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPT |

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| | | GNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQI VELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 70 | SUN1(455-812) w/KDEL | HQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELD QLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQR FSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGIT EAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIP LWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSP TGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAF QIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 71 | SUN1(495-812) w/KDEL | GEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLF SEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTS EAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRC SETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSM MIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGE SLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 72 | SUN1(562-812) w/KDEL | QRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGAS GITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLF GIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKT LSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDT AFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 73 | SUN1(601-812) w/KDEL | TSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILST RCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLS MMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDG ESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 74 | SUN1(616-812) w/KDEL | GITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLF GIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKT LSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDT AFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 75 | SUN2(522-717) w/KDEL | GVTEEQVHHIVKQALQRYSEDRIGLADYALESGGASVISTRCSETYETKTALLSLF GIPLWYHSQSPRVILQPDVHPGNCWAFQGPQGFAVVRLSARIRPTAVTLEHVPK ALSPNSTISSAPKDFAIFGFDEDLQQEGTLLGKFTYDQDGEPIQTFHFQAPTMAT YQVVELRILTNWGHPEYTCIYRFRVHGEPAHKDEL |
| 76 | Signal Peptide | MKWVTFISLLFLFSSAYSRGVFRRD |
| 77 | ER retention motif | KDEL |
| 78 | HA tag | YPYDVPDYA |
| 79 | ALFA Tag | SRLEEELRRRLTE |
| 80 | SUN1(360-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGD DPQDVFKPTTSRLKQPLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLRE LTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMSHL EDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRH VKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDL QTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSA LKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRV VIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDF AVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWG HPEYTCLYRFRVHGEPVKKDEL |
| 81 | SUN1(404-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGH HHGENLRELTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQE HEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLK SELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSS QFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQ ARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLW YFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTG NISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIV ELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 82 | SUN1(455-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGH QEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQ LKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRF SSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITE AQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPL WYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPT GNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQI VELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 83 | SUN1(495-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGG EQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFS EDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSE AVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCS ETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMI |

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|---|---|---|
| | | HPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESL QMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 84 | SUN1(562-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGQ RFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGI TEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGI PLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLS PTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAF QIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 85 | SUN1(601-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGTS EAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRC SETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSM MIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGE SLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 86 | SUN1(616-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGGI TEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGI PLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLS PTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAF QIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 87 | SUN1(636-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGD KTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYP GNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLEN EYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCL YRFRVHGEPVKKDEL |
| 88 | Human SUN1 CC1, CC2 α1, CC2 α2, CC2 α3 | GEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLF SEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTS EAVVSAVSEAGASGITEAQARAIVNSALKL |
| 89 | Human SUN1 CC2 α1, CC2 α2, CC2 α3 | KGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAI VNSALKL |
| 90 | Human SUN1 CC2 α2, CC2 α3 | TSEAVVSAVSEAGASGITEAQARAIVNSALKL |
| 91 | Human SUN2 CC1, CC2 α1, CC2 α2, CC2 α3 | QESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGLLPQQIQAVRDDV ESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTHVAEMQGKSARE AAASLSLTLQKEGVIGVTEEQVHHIVKQALQR |
| 92 | Human SUN2 CC2 α1, CC2 α2, CC2 α3 | REEMQAQLRELESKILTHVAEMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIV KQALQR |
| 93 | Human SUN2 CC2 α2, CC2 α3 | AAASLSLTLQKEGVIGVTEEQVHHIVKQALQR |
| 94 | SUN1(483-812) | LEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERV DVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVT HHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFAL ESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKG SQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLL GQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEP VK |
| 95 | SUN1(483-812) w/KDEL | LEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERV DVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVT HHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFAL ESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKG SQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLL GQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEP VKKDEL |
| 96 | SUN1(483-812) w/SP, HA tag, ALFA tag, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGSYPYDVPDYAPSRLEEELRRRLTEPTGLE QTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDV QVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHH VSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALES GGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQ GYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLG QFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPV KKDEL |
| 97 | Human SUN1 partial SMART coil-coiled 2, CC1, CC2 α1, CC2 α2, CC2 α3 | LEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERV DVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVT HHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKL |
| 98 | Human SUN2 partial SMART coil-coiled 2, CC1, CC2 | IQQLKSEWQSMTQESFQESSVKELRRLEDQLAGLQQELAALALKQSSVAEEVGL LPQQIQAVRDDVESQFPAWISQFLARGGGGRVGLLQREEMQAQLRELESKILTH VAEMQGKSAREAAASLSLTLQKEGVIGVTEEQVHHIVKQALQR |

| | | |
|---|---|---|
| | α1, CC2 α2, CC2 α3 | |
| 99 | SUN1(360-812) w/KDEL | DDPQDVFKPTTSRLKQPLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLR ELTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMS HLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSW RHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKG DLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVN SALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSP RVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPK DFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSN WGHPEYTCLYRFRVHGEPVKKDEL |
| 100 | Minimal human serum albumin signal peptide | MKWVTFISLLFLFSSAYS |
| 101 | SUN1(360-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSDDPQDVFKPTTSRLKQPLQGDSEAFPWHWMSGVEQ QVASLSGQCHHHGENLRELTTLLQKLQARVDQMEGGAAGPSASVRDAVGQPP RETDFMAFHQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAVGEQLLPT VEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLFSEDQQG GSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAV SEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRCSETYETK TALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFT LEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQAL KRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 102 | SUN1(404-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSHHHGENLRELTTLLQKLQARVDQMEGGAAGPSASVR DAVGQPPRETDFMAFHQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTISAV GEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVKLLF SEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQLPTS EAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILSTRC SETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSM MIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGE SLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 103 | SUN1(455-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSHQEHEVRMSHLEDILGKLREKSEAIQKELEQTKQKTIS AVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDAVQERVDVQVREMVK LLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQL PTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSILS TRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRL SMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQD GESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 104 | SUN1(495-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCETVDA VQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRDLQLQ ILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTG MVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGN CWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEY QEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYR FRVHGEPVKKDEL |
| 105 | SUN1(483-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSLEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWR HVKTGCETVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGD LQTMLRDLQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNS ALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPR VVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKD FAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNW GHPEYTCLYRFRVHGEPVKKDEL |
| 106 | SUN1(562-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSQRFSSQFVSKGDLQTMLRDLQLQILRNVTHHVSVTKQ LPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSIL STRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVR LSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQ DGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 107 | SUN1(601-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQDKT GMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPG NCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENE YQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLY RFRVHGEPVKKDEL |
| 108 | SUN1(616-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSGITEAQARAIVNSALKLYSQDKTGMVDFALESGGGSIL STRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVR LSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQ DGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 109 | SUN1(636-812) w/SP, KDEL | MKWVTFISLLFLFSSAYSDKTGMVDFALESGGGSILSTRCSETYETKTALMSLFGI PLWYFSQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLS |

| | | |
|---|---|---|
| | | PTGNISSAPKDFAVYGLENEYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAF QIVELRIFSNWGHPEYTCLYRFRVHGEPVKKDEL |
| 110 | shRNA sequence targeting *Dsp* | AACTGTGTGAGGCTAAGGCTG |
| 111 | shRNA targeting LacZ | AAATCGCTGATTTGTGTAGTC |
| 112 | SUN1(360-812) w/SP, Linker, ALFA tag, Linker, KDEL | MKWVTFISLLFLFSSAYSRGVFRRDGPSGGSRLEEELRRRLTETGGDDPQDVFK PTTSRLKQPLQGDSEAFPWHWMSGVEQQVASLSGQCHHHGENLRELTTLLQKL QARVDQMEGGAAGPSASVRDAVGQPPRETDFMAFHQEHEVRMSHLEDILGKL REKSEAIQKELEQTKQKTISAVGEQLLPTVEHLQLELDQLKSELSSWRHVKTGCE TVDAVQERVDVQVREMVKLLFSEDQQGGSLEQLLQRFSSQFVSKGDLQTMLRD LQLQILRNVTHHVSVTKQLPTSEAVVSAVSEAGASGITEAQARAIVNSALKLYSQD KTGMVDFALESGGGSILSTRCSETYETKTALMSLFGIPLWYFSQSPRVVIQPDIYP GNCWAFKGSQGYLVVRLSMMIHPAAFTLEHIPKTLSPTGNISSAPKDFAVYGLEN EYQEEGQLLGQFTYDQDGESLQMFQALKRPDDTAFQIVELRIFSNWGHPEYTCL YRFRVHGEPVKKDEL |

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The present disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organisational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present disclosure will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including 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.

It must be noted that, as used in the specification and the appended claims, the singular forms 'a,' 'an,' and 'the' include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from 'about' one particular value, and/or to 'about' another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent 'about,' it will be understood that the particular value forms another embodiment.

Methods described herein may preferably be performed *in vitro*. The term '*in vitro*' is intended to encompass procedures performed with cells in culture whereas the term '*in vivo*' is intended to encompass procedures with/on intact multi-cellular organisms.

**Brief Description of the Figures**

Embodiments and experiments illustrating the principles of the present disclosure will now be discussed with reference to the accompanying figures.

**Figures 1A to 1D.** Functional rescue of LMNA N195K iPSC-CMs by DNSUN1. (**1A**) Schematic of the experimental set-up for functional assays conducted using the CardioExcyte system. (**1B**) Illustrative snapshots of EFP in control and LMNA N195K iPSC-CMs, and LMNA N195K iPSC-CMs transduced with DNSUN1. (**1C**) Diagrams showing the phenotypes observed in LMNA N195K iPSC-CMs during EFP measurements, namely the increased coefficient of variation, indicating arrhythmia, as well as an increase in amplitude and increased beat rate. DNSUN1 induction results in the rescue or mitigation of these phenotypes. (**1D**) Diagram showing a decrease in amplitude observed in LMNA N195K iPSC-CMs during contractility measurements, and the introduction of DNSUN1 results in rescue of this phenotype. Each dot corresponds to a well.

**Figures 2A and 2B.** DNSUN1 Reduces Microtubule Networks in Cardiomyocytes. (**2A**) Immunostaining using the specified anti-tubulin antibodies and the nuclear stain DAPI in non-transduced CMs and CMs transduced with DNSUN1. The top panels include magnified views of insets in Tyr-alpha-Tub. Treated cells exhibit a weaker and less uniformly distributed perinuclear microtubule signal. (**2B**) Quantification of microtubule intensity normalized to DAPI, with each dot representing a distinct imaged field. Statistical significance was determined by Mann-Whitney U-test.

**Figure 3.** Diagram showing the experimental set-up of *in vivo* Proximity Labelling (BioID).

**Figure 4.** Diagram showing the Desmin Proximity Network in healthy and DSP-linked diseased cardiomyocytes. The proximity score, represented as a grey gradient, reflects the closeness of proteins belonging to distinct functional units (squared boxes) to the Desmin bait. The bait (DES) is distinctly outlined with a continuous black border. Among the acquired interactions, the most striking discovery lies in the association between Desmin and the MTOC (Microtubule Organizing Center) in the disease state. Proteins notably impacted by the disease state are marked with black dashed lines. IF, Intermediate Filament. AJ, Adherens Junction.

**Figures 5A, 5B and 5C.** Schematic Representation of Microtubule and MTOC in an Adult Cardiomyocyte. (**5A**) Cardiomyocytes transition from mitotic (i) to postmitotic (ii) shortly after birth, changing the localisation of microtubule nucleation factors, such as microtubule organising centres (MTOCs) and, thus, the polarity of the network. Mature cardiomyocytes are comprised of three microtubule populations*:* interfibrillar, cortical, and perinuclear. (**5B**) Magnification of the interaction sites between microtubules and Desmin, Z-Disc. Each myofibril in the cardiomyocyte contains many individual units called the sarcomere. The sarcomere is composed primarily of actin and myosin. α-Actinin serves to anchor actin at the Z-disc. Desmin*,* cross-linked by plectin*,* is also present at the Z-disc and can reinforce microtubules at this location. (**5C**) Magnification of the interaction sites between microtubules, Desmin and nuclear envelope. Rectangular boxes highlight the strengthened interaction between Desmin and the subdistal appendage of the MTOC in the disease state. Furthermore, the schematic drawing depicts the site of action for DNSUN1 (SUN1-2) disassembling the connection between the nuclear envelope and the cytoskeleton. Figures 5A and 5B were adapted from Uchida, K., Scarborough, E. A., & Prosser, B. L. (2021).

**Figures 6A and 6B.** Increase in total and detyrosinated microtubule levels in shDSP-diseased hearts. (**6A**) Western blot comparing total and Detyrosinated Tubulin levels in healthy control and shDSP-diseased hearts, with Beta-actin serving as the loading control. (**6B**) Quantification of the fold change of total and Detyrosinated Tubulin, normalised to the control (shLacZ), Bar height indicated mean, error bar Standard Deviation. Statistical analysis conducted using a 2-way ANOVA. * $P \leq 0.05$; ** $P \leq 0.01$

**Figures 7A and 7B.** Cardiac function in mice after *Dsp* knockdown (KD) and upon rescue by DNSUN1. (**7A**) Diagram showing the design and timeline of *in vivo* rescue experiment. Ctrl shLacZ – control AAV expressing shRNA targeting LacZ; Ctrl cTnT-EGFP – control AAV expressing EGFP from a cardiac troponin promoter; shDSP – AAV expressing shRNA targeting Dsp, cTnT-DNSUN1 – AAV expressing dominant negative SUN1 from a cardiac troponin promoter. (**7B**) Mice with *Dsp* KD have reduced ejection fraction (EF) and fractional shortening (FS) but mice in Rescue group have similar EF and FS as Control groups. (****P < 0.0001; ***P < 0.001; one-way ANOVA, mean ± SD). From Day 84, Group 4 consists of 3 mice as 1 mouse was deceased due to overexposure to anaesthesia while preparing for echocardiogram. From Day 133, Group 2 consists of 4 mice as 1 mouse was deceased due to accidental hypoxia and suffocation in parallel experiments involving an electrocardiogram tunnel that was too small for the mouse's size.

**Figures 8A and 8B.** Cardiac function in the second batch of 20 mice after *Dsp* knockdown (KD) and upon rescue by DNSUN1. (**8A**) Diagram showing the design and timeline of the *in vivo* rescue experiment. Ctrl shLacZ – control AAV expressing shRNA targeting LacZ; Ctrl cTnT-EGFP – control AAV expressing EGFP from a cardiac troponin promoter; shDSP – AAV expressing shRNA targeting Dsp, cTnT-DNSUN1 – AAV expressing dominant negative SUN1 from a cardiac troponin promoter. (**8B**) Mice with *Dsp* KD have reduced EF% and FS%. Data were analyzed form the total number of animals (N) per group of treatment as indicated in (A). (****P < 0.0001; one-way ANOVA, mean ± SD).

**Figures 9A and 9B.** Cardiac function in mice after *TTN* knockdown (KD) and upon rescue by DNSUN1. Mice with *TTN* KD have reduced ejection fraction (EF) (**9A**) and fractional shortening (FS) (**9B**) compared to the control group. Mice treated with DNSUN1 have statistically significant higher EF and FS compared to the disease group. (****P < 0.0001; ***P < 0.001; one-way ANOVA, mean ± SD). Ctrl – control AAV expressing shRNA targeting LacZ; EGFP – AAV expressing EGFP ; shTTN – AAV expressing shRNA targeting Titin, DNSUN1 – AAV expressing dominant negative SUN1.

**Figures 10A, 10B and 10C.** DNSun1 treatment reduces tubulin levels and improves cardiac function in an LMNA-DCM Mouse Model. (**10A**) Western blot comparing tubulin levels in healthy control (WT), LMNA-DCM diseased hearts, and LMNA-DCM mice treated with DNSUN1, with GAPDH serving as the loading control. (**10B**) Quantification of the fold change in tubulin levels, normalized to the control (WT). Bar height indicates the mean, and error bars represent the standard deviation. Statistical analysis was conducted using ordinary one-way ANOVA. **P ≤ 0.01; ****P ≤ 0.0001. (**10C**) Negative correlation between LVEF (EF %) and normalized tubulin expression in control (WT), diseased (LMNA-DCM), and treated mice (LMNA-DCM + DNSUN1).

**Figures 11A and 11B.** Increase in microtubule levels in shTTN-diseased hearts. (**11A**) Western blot comparing tubulin levels in healthy control and shTTN diseased hearts, with Histone H2B serving as the loading control. Western Blot was performed using a standard protocol available on the Bio-Rad website with antibodies specific to α-tubulin and histone H2B. (**11B**) Quantification of the fold change of tubulin, normalised to the control (shLacZ), Bar height indicates, mean, error bar, Standard Deviation. Statistical analysis conducted using unpaired t-test. * $P \leq 0.05$.

**Examples**

**Example 1: Uncoupling LINC complex using DNSUN1 reversed pathological microtubule organisation in human iPSC-derived cardiomyocytes**

DNSUN1 competes with endogenous SUN proteins for binding to KASH proteins in the perinuclear space of cardiomyocytes, thereby uncoupling the nuclear interior from the cytoskeleton (Crisp, M. et al. J. Cell Biol. 172, 41–53 (2006)).

Based on the above findings, and the fact that various heart conditions (*e.g.* cardiomyopathies, irrespective of their genetic or non-genetic origin as explained above), share a common characteristic: a substantial and maladaptive rise in microtubule density within cardiomyocytes, the inventors investigated whether DNSUN1 could potentially serve as an effective treatment for multiple conditions by reversing the formation of pathological microtubule networks.

The inventors' initially tested their hypothesis using an in vitro model of LMNA cardiomyopathies. This decision was based on substantial evidence indicating the effectiveness of DNSUN1 in treating LMNA cardiomyopathy in mouse models (Chai, R.J., Werner, H., Li, P.Y. et al. Disrupting the LINC complex by AAV mediated gene transduction prevents progression of Lamin induced cardiomyopathy. Nat Commun 12, 4722 (2021). https://doi.org/10.1038/s41467-021-24849-4, WO 2019/143300 A1 and WO 2021/010898 A1).

The in vitro model refers specifically to *LMNA* N195K cardiomyopathy. Asparagine (N)-to-lysine (K) substitution at amino acid 195 in the variant of the A-type lamins causes DCM (dilated cardiomyopathies) in humans (Fatkin, et al. (1999) N. Engl. J. Med., 341, 1715–1724).

LMNA-N195K mutated patients' induced Pluripotent Stem Cell-derived cardiomyocytes (iPSC-CMs) exhibit both electrophysiological abnormalities and nuclear morphological irregularities. Consequently, they represent a suitable model for rescue experiments. (Shemer Y et al. (2021) Int J Mol Sci. 23;22(15):7874).

1.1 LINC complex uncoupling by DNSUN1 reverses electrophysiological irregularities of LMNA N195K iPSC-CMs

The CardioExcyte system was employed for electrophysiological studies of LMNA N195K iPSC-CMs.

The CardioExcyte system enables the simultaneous measurement of both impedance and extracellular field potential (EFP) from a single well. An electrode positioned at the well's bottom gauges impedance (resistance) introduced by the monolayer on top of the electrode. The configuration of the cells forming the monolayer influences impedance, with flat cells (e.g., relaxed CMs) leading to high impedance, and round cells (e.g., contracted CMs) resulting in low impedance. By capturing impedance changes with high temporal resolution, it becomes possible to assess cell contractility. Furthermore, fluctuations in membrane potential in beating CMs influence ion concentrations trapped between the monolayer and the detecting electrode. Therefore, measuring ion concentration (i.e., EFP) with high temporal resolution serves as a proxy for changes in CM's membrane potential.

EFP analysis of LMNA N195K iPSC-CMs revealed an arrhythmic phenotype along with as an increase in beat rate and amplitude when compared to normal isogenic CMs (Control) (Figures 1B and 1C). Contractility measurements indicated a decrease in amplitude when compared to normal isogenic CMs (Figure 1D). Notably, treatment of LMNA N195K iPSC-CMs with AAV6-DNSUN1 results in the rescue or mitigation of all these disease phenotypes (Figures 1B, 1C and 1D), namely reversal of the arrhythmic phenotype as evidenced by a decrease in the coefficient of variation, decrease in the amplitude of the membrane potential and in the beat rate (Figure 1C), and reversal of impaired contractility as evidenced by an increase in the amplitude of impedance.

The observed rescue by DNSUN1 of electrophysiological irregularities in LMNA N195K iPSC-CMs renders this in vitro system a suitable tool for deciphering the mechanism of action of DNSUN1.

1.2 DNSUN1 is capable of reversing pathological microtubule organisation in iPSC-CM

To examine the impact of DNSUN1 on the microtubule network in iPSC-CM, immunostaining was performed using antibodies targeting tyrosinated and detyrosinated microtubules. In untreated LMNA N195K iPSC-CMs, a distinct microtubule network was visible around the nuclear periphery. Conversely, cardiomyocytes treated with DNSUN1 displayed a more evenly distributed pattern through the cytoplasm and reduced microtubule density in the cytoplasm compared to the untreated LMNA N195K iPSC-CMs (Figure 2). These findings suggest that DNSUN1 is capable of reversing pathological microtubule organisation found in disease states.

Based on the findings described in this Example, the inventors propose a novel mechanism by which inhibition of the LINC complex (*e.g.* by DNSUN1) rescues cardiomyopathies, such as *LMNA*-related cardiomyopathy: the mechanism is through mitigating the pathological effect of dysfunctional microtubules. Two potentially additive protective effects can be anticipated:

1. Protection of the nucleus against excessive forces stemming from disorganised microtubule structures, consequently lowering the risk of DNA damage.
2. Protection against impaired contractile properties resulting from alterations in microtubule load-bearing and force transmission with the sarcomeres. The uncoupling of the nuclear envelope

from the cytoskeleton mediated by disrupting the LINC complex by DNSUN1 could diminish the anchoring sites of the perinuclear microtubule population, leading to a reduction in their stability.

The present data show that inhibition of the LINC complex (*e.g.* by DNSUN1) is expected to serve as an effective treatment for multiple conditions associated with aberrant microtubule density or aberrant microtubule organisation within cardiomyocytes.

1.3 Materials and Methods

*Production of AAV6*

AAV6 was produced by the triple transfection method and purified with methods adapted from Arden et al., J Biol Methods. 2016;3(2):e38 or Strobel et al., Hum Gene Ther Methods. 2015;26(4):147-57.

*iPSC-CM experiments*

Human iPSC line derived from the PGP1 donor (Personal Genome Project) were engineered to carry a homozygous point mutation in LMNA (N195K) using the CRISPR/Cas9 system. The iPSC line was then directed to differentiate into beating cardiomyocytes (CMs) and cultured following a published protocol (Lian, X., et al. PNAS. (2012) 109(27), E1848-57).

For the functional assay (Figure 1A), cells were plated in each CardioExcyte well (Nanion technologies). One day post-seeding, LMNA-N195K iPSC-CMs and controls (WT) were treated with AAV6-DNSUN1 (encoding DNSUN1, SEQ ID NO:112) or AAV6-GFP. Seeded cells were stimulated with Endothelin1. EFP and contractility were then assessed according to the manufacturer's protocol. Statistical analysis was conducted using one-way ANOVA using GraphPad Prism. The experiment was repeated 3 times. Each dot in Figures 1C and 1D represents a well.

For the immunofluorescence experiment, the CMs were plated in 96-well plastic Phenoplates coated with Matrigel One-day post-seeding treated cells were transduced with AAV6 carrying DNSUN1. Seven days post-transduction, the cells were fixed with 4% paraformaldehyde and subjected to immunostaining using a standard protocol with antibodies specific to microtubules. Images were captured from treated and non-treated wells, three from each, using the Perkin Elmer Opera Phenix (High-Content) microscopy system. Mean fluorescence intensity of microtubules at the region of interest was analysed using IMARIS 8.2 software (Bitplane) and normalized to mean nuclear intensity. For statistical analysis, Mann-Whitney U-test was performed using Prism software.

**Example 2: *In vivo* Proximity Labelling (BioID) identifies aberrant microtubule organisation in DSP-linked cardiomyopathy.**

2.1 Identifying changes in the Desmin proximity network between healthy heart and DSP-linked diseased heart

To identify potential cytoskeletal rearrangements associated with Desmoplakin cardiomyopathy, an in vivo comparison of the Desmin interactome was conducted between healthy and shDSP-induced diseased hearts (Figure 3).

The method used to identify the proteins interacting with Desmin was BioID. BioID is described in Roux K. et al., Curr Protoc Protein Sci. 2018, 91: 19.23.1-19.23.5, which is hereby incorporated by reference in its entirety. The method screens for physiologically relevant protein interactions that occur in living cells. This technique harnesses a promiscuous biotin ligase to biotinylate proteins based on proximity. The ligase is fused to a protein of interest and expressed in cells, where it biotinylates proximal endogenous proteins. Because it is a rare protein modification in nature, biotinylation of these endogenous proteins by BioID fusion proteins enables their selective isolation and identification with standard biotin-affinity capture.

Desmin is a component of intermediate filaments and serves as the crucial protein bridging the intercalated disc (via direct binding to desmoplakin) to the nuclear envelope (through interaction with the LINC complex via Plectin).

The inventors identified 18 proteins exhibiting differential proximity with Desmin in the DSP-linked pathology samples over the healthy heart tissue samples. Among these, 9 show increased proximity, while 9 exhibit reduced proximity. Among the proteins displaying decreased proximity, DSP represents a quality control, since the overall levels of this protein have been reduced by shRNA to induce the disease state.

The most relevant changes observed in the diseased state are the increased association of Desmin with the Microtubule Organizing Center (MTOC) (Figure 4). Specifically, an increased proximity of Desmin with Ccdc61, Cc2d2a, Nhsl2, Plekha6, and Dvl3 was present in the disease state.

The MTOC functions as one of the key microtubule nucleation factors, playing a significant role in shaping the polarity of the microtubule network. Adult cardiomyocytes lose the typical centrosomal MTOC and instead rely on non-centrosomal MTOCs situated around the nuclear envelope for microtubule nucleation (Figure 5A). In this way, the entire cardiomyocyte nuclear envelope is coopted as a MTOC. Within adult cardiomyocytes, three distinct microtubule populations exist: interfibrillar, cortical, and perinuclear (Figure 5A, ii). Desmin is proposed to function as a mechanical anchor for microtubules, confining their distribution to specific spatial regions (Uchida K., et al. (2022)).

The enhanced association observed between the intermediate filament (Desmin) and the MTOC signifies a pathological link, resulting in dysfunctional microtubule organisation in DSP-linked cardiomyopathy.

2.2 Materials and Methods

The methods for shRNA vector design (shLacZ and shDSP) and production of AAV9 are described in Example 4.2 herein.

*In vivo proximity labelling study (BioID)*

Desmin (Uniprot Accession number P17661) was tagged with BirA, as per Roux et al. Curr Protoc Protein Sci. 2018, 91: 19.23.1-19.23.5, and an Alfa tag, and AAV9 vectors were manufactured. C57BL6 mice were injected with AAV9 viruses through retro-orbital administration. The selection of animals into different experimental groups was randomized. The mouse hearts were harvested post-injection following approved anaesthesia and euthanasia protocols.

*Streptavidin affinity pulldown*

Streptavidin affinity pulldown was performed with a protocol adapted from Roux K. et al., Curr Protoc Protein Sci. 2018, 91: 19.23.1-19.23.5, which is incorporated herein by reference in its entirety.

*Proteomic Mass Spectrometry-based quantitative analysis*

The proteins from the pulldown were then sent to a mass spec facility for analysis. For analysis, tandem mass tag (TMT) raw intensity values underwent Log2-fold-change (L2FC) calculation and enrichment analysis by calculating the L2FC of proteins from shDSP samples (disease state) relative to shLacZ control samples (healthy).

**Example 3: Abnormal Microtubule Levels and Composition in shDSP-Diseased Hearts**

3.1 *In vivo* studies identify an increase in total and detyrosinated microtubule levels in shDSP-Diseased Hearts

To further corroborate the association between DSP-linked cardiomyopathy and aberrant microtubule density and organization, an analysis was conducted comparing the levels of total and detyrosinated microtubules in the hearts of healthy and shDSP-diseased mice.

Previous studies have shown that Desmin preferentially associates with detyrosinated microtubules and an increase in the detyrosinated microtubule population has been linked to impaired function in animal models of heart disease (Robison P. et al. Science 352: aaf0659 (2016)).

The levels of total tubulin (β-tubulin) and detyrosinated tubulin (dTyr-tubulin) between healthy control heart samples (shLacZ) and hearts affected by DSP-linked disease (shDSP) were analysed by Western blot and quantified (Figure 6). In the shDSP diseased hearts, a 2-fold increase in total microtubules and a 2.5-fold rise in the detyrosinated microtubule population was observed (Figure 6B). These results corroborate the proteomic proximity data of Example 2, showing an elevation in the microtubule population (dTyr) that interacts with Desmin in the disease state.

3.2 Materials and Methods

*Western blot*

Western Blot was performed using a standard protocol available on the Bio-Rad website with antibodies specific to Desmoplakin, Beta Actin, total tubulin and detyrosinated α-tubulin.

**Example 4: Uncoupling LINC complex using DNSUN1 attenuated DSP-linked cardiomyopathy**

The present data in Examples 2 and 3 herein demonstrate that Desmoplakin cardiomyopathy is part of a larger group of cardiomyopathies that share a common downstream pathway, characterized by a maladaptive increase in microtubule density.

Inhibition of the LINC complex (*e.g.* by DNSUN1) is expected to serve as an effective treatment for multiple conditions associated with aberrant microtubule density or aberrant microtubule organisation within cardiomyocytes.

In vivo rescue of cardiac function in a mouse model of Desmoplakin cardiomyopathy was achieved using DNSUN1 as a therapeutic treatment.

4.1. Evaluating the consequence of loss of DSP on heart function of mice, and the effect of LINC complex inhibition on pathology associated with DSP deficiency

To evaluate the consequence of loss of DSP to the heart function of mice, and the feasibility of rescuing pathology associated with loss of DSP through LINC complex inhibition, the inventors depleted Dsp using shRNA delivered by adeno-associated virus serotype 9 (AAV9), and assessed heart function via echocardiography following administration of AAV encoding DNSUN1, or a control vector encoding eGFP (Figure 7A).

Echocardiography measurement revealed that the cardiac function of shDsp-transduced mice started to decline six weeks after viral transduction (Figure 7B). At six weeks, ejection fraction (EF) and fractional shortening (FS) of shDsp-transduced mice (EF = 48.4% ± 5.27%, P = 0.0029 compared to control Group 1; FS = 24.3% ± 3.11%, P = 0.0016 compared to control Group 1) was reduced compared to that of mice transduced with control LacZ shRNA (EF = 56.5% ± 1.35%, FS = 29.6% ± 0.85%). Analysis of ejection fraction and fractional shortening over time showed a marked progression towards heart failure in DSP knockdown mice compared to the control group (Figure 7B). In the control shLacZ/cTnT-EGFP mice (Group 1), ejection fraction (EF) and fractional shortening (FS) were in the healthy range of 58.3% ± 2.21% and 30.7% ± 1.32%, respectively. At week 19, the ejection fraction (EF) and fractional shortening (FS) of shDsp/cTnT-EGFP-transduced mice (Group 3) further diminished compared to Control Group 1 ($P < 0.0001$) to 32.36% ± 10.3%, P = 0.0001 and 15.6% ± 5.55%, respectively. These results indicate that Dsp expression is critical for heart contractile function, and inhibition of Dsp expression causes dilated cardiomyopathy (DCM) in mice.

SUN domain proteins play a crucial role in anchoring KASH domain proteins to the outer nuclear membrane (ONM) and facilitate the connection between the nucleus and the cytoskeleton. In this study, the inventors disrupted the LINC complex by introducing a dominant-negative SUN1 construct (DNSUN1)

into cardiomyocytes (CMs) using AAV9 as a delivery vehicle. DNSUN1 competes with endogenous SUN proteins for binding to KASH proteins in the perinuclear space of CMs, thereby uncoupling the LINC complex (Crisp, M. et al. Coupling of the nucleus and cytoplasm: role of the LINC complex. J. Cell Biol. 172, 41–53 (2006)).

Compared to the mice injected with shDsp/cTnT-eGFP (Group 3, DSP KD), hearts of mice injected with shDsp/AAV9-DNSUN1 (Group 4, Rescue) demonstrated healthy contractile function (Figure 7B). This was evident from the normal ejection fraction (EF) and fractional shortening (FS) of 58.5% ± 1.17%, P = 0.007 and 30.8% ± 0.29%, P = 0.004 compared to DSP KD (Group 3), respectively, six weeks after viral transduction. Notably, the rescue of heart function by DNSUN1 was observed throughout the 19 weeks of the time-course study. After 19 weeks, EF and FS of mice injected with shDSP/cTnT-DNSUN1 were maintained in the healthy range of 56% ± 2.58%, P = 0.0012 and 29.5% ± 1.68%, P = 0.0008, respectively, when compared to DSP KD (Group 3). These data suggest that LINC complex inhibition can ameliorate cardiomyopathy caused by desmosome/desmoplakin deficiency.

Moreover, disrupting the LINC complex with AAV9-DNSUN1 was well tolerated in mice. Mice injected with AAV9-DNSUN1 + shLacZ showed normal cardiac performance throughout the entire time-course of the study (Figure 7B). After 19 weeks, the EF and FS of these mice were still maintained in the normal range of 58.1% ± 5.012% and 30.7% ± 3.26%, respectively.

In summary, the inventors demonstrated the in vivo efficacy and safety of a DNSUN1 construct for treating cardiomyopathy caused by desmosome/desmoplakin deficiency.

Next, to assess the reproducibility of the data from the first in vivo rescue study, the cardiac function via echocardiography of an additional second batch of 20 mice was evaluated with a similar experimental design as the first study, except that EF and FS was measured from day 103 post-AAV injection (Figure 8A).

Consistent with the results of the first study, in comparison to Dsp KD mice injected with shDsp/cTnT-eGFP, the hearts of Dsp KD mice treated with AAV9-DNSUN1 exhibited healthy contractile function (Figure 8B). This was evident from the normal ejection fraction (EF) and fractional shortening (FS) values, which were measured as 58.6% ± 1.29% (P < 0.0001) and 29.7% ± 0.78% (P <0.0001), respectively, 15 weeks after AAV9-cTnT-DNSUN1 and AAV9-cTnT-shLacZ viral transduction. Importantly, the improvement in heart function mediated by DNSUN1 was observed consistently over the course of the 19-week study. At the end of the 19-week period, the EF and FS values of Group 4 Rescue mice injected with AAV9-DNSUN1 remained within the healthy range at 57.1 ± 2.27 (P <0.0001) and 29.9 ± 1.42 (P < 0.0001) when compared to Group 3 DSP KD mice, respectively. These findings confirm that LINC complex inhibition (e.g. achieved using DNSUN1) ameliorates cardiomyopathy caused by desmosome/desmoplakin deficiency (e.g. induced via depletion of Dsp).

Furthermore, the disruption of the LINC complex using AAV9-DNSUN1 was also well tolerated in the second group of mice. Mice injected with AAV9-DNSUN1 + shLacZ exhibited normal cardiac performance

throughout the entire duration of the study (Figure 8B). Even after 19 weeks, the EF and FS values of these mice were maintained within the normal range at 59.8% ± 0.69% and 31.8% ± 0.39%, respectively.

In summary, this study successfully demonstrated the effectiveness and safety of the DNSUN1 construct in vivo, suggesting the potential of LINC complex inhibition as a treatment for diseases characterised by desmosome/desmoplakin deficiency/insufficiency or dysfunction, e.g. cardiomyopathies associated with mutation to genes encoding desmosome proteins.

This study also successfully demonstrated the effectiveness and safety of the DNSUN1 construct in vivo in the treatment of desmosome-related cardiomyopathy characterized by a downstream pathway with a maladaptive increase in microtubule density.

4.2. Materials and Methods

*Design of cTNT-DNSUN1 and cTNT-eGFP constructs*

The amino acid sequence of the DNSUN1 polypeptide employed in the experimental examples (SEQ ID NO:112) comprises a human serum albumin signal peptide (SEQ ID NO:76), an Alfa epitope tag (SEQ ID NO:79) flanked by linker amino acids, and the lumenal domain of human SUN1 consisting of the C-terminal 453 amino acids of human SUN1, and the KDEL endoplasmic reticulum retention signal (SEQ ID NO:99). The DNSUN1 sequence was obtained by gene synthesis, restriction digested, and ligated into an Adeno-associated virus (AAV) transfer plasmid containing the cTnT cardiac promoter and a rabbit beta-globin polyA tail. An AAV transfer plasmid instead comprising the coding sequence for GFP was also prepared, for the preparation of AAV vectoring GFP, for use as an experimental control.

*shRNA vector design*

shRNA targeting *Dsp* was designed to target 21 base-pair gene-specific regions. The sequence of mouse *Dsp* shRNA is AACTGTGTGAGGCTAAGGCTG (SEQ ID NO:110). The sequence against LacZ, AAATCGCTGATTTGTGTAGTC (SEQ ID NO:111), was used as the control shRNA. The shRNA cassette based on a miR-155 backbone was inserted at the 3' end of the transgene cassette of an AAV-*cTnT-EGFP* vector (Wakimoto *et al.*, Curr Protoc Mol Biol. 2016 Jul 1;115:23.16.1-23.16.9.)

*Production of AAV9*

Adeno-associated viruses were produced by PackGene Biotech Inc using transient triple transfection of HEK293T cells with an adenoviral helper plasmid, a rep/cap plasmid encoding AAV2 Rep and AAV9 cap, and transgene-specific plasmids. AAV9 vectors were purified by iodixanol ultracentrifugation. Viral titre was determined using alkaline gel electrophoresis with a plasmid DNA standard (Kohlbrenner and Weber, Methods Mol. Biol. (2017) 1521 :91-107), gel imaging on a ChemiDoc imaging system (Bio-Rad Laboratories, Hercules, CA, USA) and image quantification with Image Lab software (Bio-Rad Laboratories).

*In vivo* rescue study

Mice were kept in ventilated animal barrier facilities. These facilities maintained a 12-hour cycle of light and dark, with the temperature regulated at 21 ± 1 °C and humidity maintained between 55% and 70%. The animals had unrestricted access to food and water.

C57BL6 mice were injected with AAV9 viruses through retro-orbital administration. The injection was carried out at a volume of 5 µL/g, using an insulin syringe. The selection of animals into different experimental groups was randomized. The AAV9 injections were performed while the animals were under anaesthesia using a mixture of 1.5% Isoflurane and oxygen. Prior to administration, the AAV9 working solution was freshly prepared. Depending on the viral genome concentration, the corresponding AAV9 stock solutions were diluted with PBS containing 0.001% Pluronic F-68.

*Echocardiography*

Echocardiography, performed with the Prospect T1 ultrasound systems from S-Sharp Inc., was used to assess cardiac function. Prior to the ultrasound examination, mice were shaved one day in advance. The animals were then anesthetized using a mixture of 1.5% isoflurane and oxygen. B-mode and M-mode readings were obtained while the mice's heart rates were ranging from 450 bpm to 350 bpm. The cardiac package of the Prospect T1 software version 3.1.34.2884 (S-Sharp Inc.) was utilized to calculate FS (fractional shortening), and EF (ejection fraction) from the parasternal long axis. For the diastolic and systolic state, measurements of the left ventricular interior diameter, interventricular septum, and left ventricular posterior wall were obtained from the parasternal short-axis.

*Statistical analysis*

Statistical analysis was conducted using Graphpad Prism 9.5.0. The results are presented as the mean value accompanied by the standard deviation (±SD). Data were analyzed using One-way ANOVA. To assess the significance of cardiac data among multiple groups, Tukey's post hoc test was employed. ns, non-significant, $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

**Example 5: Uncoupling LINC complex using DNSUN1 attenuated TTN-linked cardiomyopathy**

The present data in Examples 1, 2, 3, 4 and 12 show that inhibition of the LINC complex (*e.g.* by DNSUN1) is expected to serve as an effective treatment for multiple cardiomyopathies associated with aberrant microtubule density or aberrant microtubule organisation within cardiomyocytes.

The authors opted to evaluate the therapeutic potential of DNSUN1 in TTN-cardiomyopathy. Titin cardiomyopathy is a genetic cardiomyopathy conceptually distinct from Desmoplakin cardiomyopathy since the causative mutation affects a sarcomeric protein instead of a desmosomal protein.

Titin acts as a colossal molecular spring, regulating sarcomere extension during cardiomyocyte relaxation. As the cardiomyocyte elongates tensile force is exerted on the nucleus through microtubules and LINC complexes (Zhong Z, Wilson KL, Dahl KN. Methods Cell Biol. 2010;98:97-119; ElSaygh, Jude

MD*; Zaher, Anas MD*; Peterson, Stephen J. MD*,†; Parikh, Manish A. MD*,†; Frishman, William H. MD‡. Cardiology in Review ():10.1097)

The authors suggest that reduced/malfunctional Titin in TTN-Cardiomyopathy leads to exaggerated sarcomere extension during cardiomyocyte relaxation, thereby intensifying tensile forces exerted on the nucleus by microtubule, consequently causing nuclear abnormalities and damage.

Cardiac specimens from subjects with TTN Truncating Mutations exhibit myocyte nuclei with abnormal morphological characteristics (Daniel S. Herman, Lien Lam, Matthew R.G. Taylor, et al., N Engl J Med 2012; 366:619-628)

This Example demonstrates that *in vivo* rescue of cardiac function in a mouse model of Titin cardiomyopathy is achieved using DNSUN1 as a therapeutic treatment, and that Titin cardiomyopathy is associated with microtubule dysfunction.

5.1. *In vivo* studies identify an increase in total microtubule levels in shTTN-diseased hearts

To further corroborate the association between TTN-linked cardiomyopathy and aberrant microtubule density, an analysis was conducted to compare the levels of total microtubules in the hearts of healthy and shTTN-diseased mice.

Previous studies have demonstrated that, in response to myocardial stress, the microtubule network typically remodels through densification. It has been shown that microtubule network density, as an isolated variable, is inversely related to contractile function both in vivo and in vitro (Caporizzo MA, Prosser BL. Nat Rev Cardiol. 2022).

In the current example, levels of total tubulin (β-tubulin) were analysed by Western blot and quantified, comparing healthy control heart samples (shLacZ) with hearts affected by TTN-linked disease (shTTN) (Figure 11A). A two-fold increase in the total microtubule population was observed in the shTTN-diseased hearts (Figure 11B), further corroborating that aberrant microtubule density is associated with TTN-linked disease.

The present findings suggest a therapeutic effect of inhibition of the LINC complex (*e.g.* by DNSUN1) on Titin cardiomyopathies, as DNSUN1 is demonstrated to rescue contractile dysfunction by targeting cardiac microtubules.

5.2. Evaluating the consequence of loss of TTN on heart function of mice, and the effect of LINC complex inhibition on pathology associated with TTN deficiency

To evaluate the consequence of loss of TTN to the heart function of mice, and the feasibility of rescuing pathology associated with loss of TTN through LINC complex inhibition, the inventors depleted Titin using shRNA delivered by adeno-associated virus serotype 9 (AAV9) and assessed heart function via

echocardiography following administration of AAV encoding DNSUN1, or a control vector encoding eGFP.

Echocardiography measurement revealed that the cardiac function of shTTN-transduced mice declines nine weeks after viral transduction (Figures 9A and 9B). At nine weeks, ejection fraction (EF) and fractional shortening (FS) of shTTN-transduced mice (EF = 39% ± 4.3%, P = <0.0001 compared to control Group 1; FS = 19% ± 2.4%, <0.0001 compared to control Group 1) was reduced compared to that of mice transduced with control LacZ shRNA (EF = 53% ± 1.6%, FS = 27% ± 1%). These results indicate that TTN expression is critical for heart contractile function, and inhibition of TTN expression causes dilated cardiomyopathy (DCM) in mice.

In comparison to the mice injected with shTTN (Group 2, TTN KD), the hearts of mice injected with shTTN/AAV9-DNSUN1 (Group 3, Rescue) exhibited improved contractile function (see Figures 9A and 9B). This was evident from the ejection fraction (EF) and fractional shortening (FS) of 46% ± 2.6%, P = 0.006 and 22% ± 1.6%, P = 0.0007 compared to TTN KD (Group 2), respectively, nine weeks after viral transduction.

These data suggest that LINC complex inhibition can ameliorate cardiomyopathy caused by Titin deficiency.

In summary, the inventors demonstrated the in vivo efficacy of a DNSUN1 construct for treating cardiomyopathies associated with aberrant microtubule density or organization in three conceptually distinct cardiomyopathies; namely, LMNA cardiomyopathies, in which the causative mutation is in a lamin protein; Desmoplakin cardiomyopathy, in which the causative mutation affects a desmosomal protein; and Titin cardiomyopathy, in which the causative mutation affects a sarcomeric protein.

5.3. Materials and Methods

*Design of DNSUN1 construct*

The amino acid sequence of the DNSUN1 polypeptide employed in the experimental examples is SEQ ID NO:104.

*shRNA vector design*

shRNA targeting *TTN* was designed to target 21 base-pair gene-specific regions (Liao et al., Sci Rep. 2019 Nov 8;9(1):16330).

The methods for vector design (shLacZ and eGFP) and production of AAV9 are described in Example 4.2 herein.

The methods for the *in vivo* rescue study, Echocardiography and statistical analysis are described in Example 4.2 herein.

**Example 6: LINC complex disruption by expression of dominant-negative LINC complex proteins alleviates pathophysiology in DSP and TTN cardiomyopathies**

Mice having DSP or TTN cardiomyopathy are administered nucleic acid encoding dominant-negative Sun2, or dominant-negative KASH1, KASH2, KASH3, KASH4 or KASH-5. The mouse models of DSP or TTN cardiomyopathy are detailed in Example 4 and Example 5 above.

Mice having DSP or TTN cardiomyopathy treated with a LINC complex inhibitor have higher cardiac ejection fraction and fractional shortening as compared to untreated mice or mice treated with vehicle only.

**Example 7: RNAi-mediated knockdown of LINC complex component expression alleviates pathophysiology in DSP and TTN cardiomyopathies**

Mice having DSP or TTN cardiomyopathy are administered with nucleic acid encoding shRNA or siRNA targeting LINC complex components. The mouse models of DSP or TTN cardiomyopathy are detailed in Example 4 and Example 5 above.

The shRNA/siRNA target a SUN domain-containing protein or a KASH domain-containing protein, as defined herein.

Mice having DSP or TTN cardiomyopathy treated with a LINC complex inhibitor have higher cardiac ejection fraction and fractional shortening as compared to untreated mice or mice treated with vehicle only.

**Example 8: Gene editing of LINC complex components alleviates pathophysiology in DSP and TTN cardiomyopathies**

Mice constituting mouse models of DSP and TTN cardiomyopathy are administered with nucleic acid encoding target nucleic acid editing systems (including CRISPR/Cas9 gene editing systems) for disrupting LINC complex components. The mouse models of DSP or TTN cardiomyopathy are detailed in Example 4 and Example 5 above.

The gene editing systems 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.

Mice having DSP or TTN cardiomyopathy treated with a LINC complex inhibitor have higher cardiac ejection fraction and fractional shortening as compared to untreated mice or mice treated with vehicle only.

**Example 9: LINC complex disruption by expression of dominant-negative LINC complex proteins alleviates pathophysiology in cardiac diseases characterized by microtubule dysfunction**

The inventors further explore the effect of LINC complex disruption in disease models that are characterised by microtubule dysfunction.

Animals constituting animal models of diabetic cardiomyopathy, dystrophic cardiomyopathy, heart failure with preserved ejection fraction (HFpEF), heart failure with reduced ejection fraction (HFrEF) or myocardial infarction, representing cardiac diseases associated with microtubule dysfunction, are administered a nucleic acid encoding dominant-negative Sun1, Sun2, or dominant-negative KASH1, KASH2, KASH3, KASH4 or KASH5.

The animal models of diabetic cardiomyopathy include the streptozotocin (STZ) rodent model of diabetes (Shiels H et al. Stable microtubules contribute to cardiac dysfunction in the streptozotocin-induced model of type 1 diabetes in the rat. Mol. Cell. Biochem 294, 173–180 (2007)).

The animal models of dystrophic cardiomyopathy include the mdx mouse model (Prosser BL, Ward CW & Lederer WJ X-ROS Signaling: Rapid Mechano-Chemo Transduction in Heart. Science 333, 1440–1445 (2011); Prins KW, Asp ML, Zhang H, Wang W, & Metzger JM Microtubule-Mediated Misregulation of Junctophilin-2 Underlies T-Tubule Disruptions and Calcium Mishandling in mdx Mice. JACC: Basic to Transl. Sci 1, 122–130 (2016))

The animal models of heart failure with preserved ejection fraction include ZSF1 obese rats (O-ZSF1, Schulz L, Werner S, Böttner J, Adams V, Lurz P, Besler C, Thiele H, Büttner P. Tubulin expression and modification in heart failure with preserved ejection fraction (HFpEF). Sci Rep. 2022 Sep 21;12(1):15734), mice treated with high-fat diet (HFD) in combination with moderate transverse aortic constriction (mTAC) or mice treated with high-fat diet (HFD) in combination with l-arginine methyl ester (L-NAME) (Ranjbarvaziri S, Zeng A, Wu I, Greer-Short A, Farshidfar F, Budan A, Xu E, Shenwai R, Kozubov M, Li C, Van Pell M, Grafton F, MacKay CE, Song X, Priest JR, Argast G, Mandegar MA, Hoey T, Yang J. Targeting HDAC6 to treat heart failure with preserved ejection fraction in mice. Nat Commun. 2024 Feb 26;15(1):1352).

The animal models of heart failure with reduced ejection fraction include transverse aortic constriction (TAC) mouse models (Li L, Zhang Q, Zhang X, Zhang J, Wang X, Ren J, Jia J, Zhang D, Jiang X, Zhang J, Mei H, Chen B, Hu J, Huang Y. Microtubule associated protein 4 phosphorylation leads to pathological cardiac remodeling in mice. EBioMedicine. 2018 Nov;37:221-235).

The animal models of myocardial infarction include coronary artery ligation in a mouse (Li L, Zhang Q, Zhang X, Zhang J, Wang X, Ren J, Jia J, Zhang D, Jiang X, Zhang J, Mei H, Chen B, Hu J, Huang Y. Microtubule associated protein 4 phosphorylation leads to pathological cardiac remodeling in mice. EBioMedicine. 2018 Nov;37:221-235).

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

Streptozotocin (STZ) rodent models of diabetes treated with a LINC complex inhibitor have improved diastolic or systolic cardiac function, or less cardiac death or cardiac fibrosis, compared to untreated STZ rodents, or rodents treated with vehicle only.

Mice having the mdx mutation, subjected to isoproterenol stress (Prins KW, Asp ML, Zhang H, Wang W, & Metzger JM Microtubule-Mediated Misregulation of Junctophilin-2 Underlies T-Tubule Disruptions and Calcium Mishandling in mdx Mice. JACC: Basic to Transl. Sci 1, 122–130 (2016)) and treated with a LINC complex inhibitor have improved systolic function or an extended lifespan as compared to untreated mice, or mice treated with vehicle only.

Animals having heart failure with preserved ejection fraction, such as O-ZSF1 rats, HFD + mTAC mice or HFD + L-NAME mice, treated with a LINC complex inhibitor have reduced cardiac hypertrophy or diastolic dysfunction as compared to untreated disease animals, or disease animals treated with vehicle only.

Animals having heart failure with reduced ejection fraction, such as TAC mice, treated with a LINC complex inhibitor have improved systolic or diastolic function or left ventricular morphology or cardiac fibrosis as compared to untreated disease animals, or disease animals treated with vehicle only.

Animals having myocardial infarction, such as through coronary artery ligation, treated with a LINC complex inhibitor have improved systolic or diastolic function or cardiac fibrosis as compared to untreated disease animals, or disease animals treated with vehicle only.

**Example 10: RNAi-mediated knockdown of LINC complex component expression alleviates pathophysiology in cardiomyopathies and other heart diseases characterized by microtubule dysfunction**

The inventors investigate the effect of inhibitory nucleic acids targeting LINC complex components using the animal models of diabetic cardiomyopathy, dystrophic cardiomyopathy, heart failure with preserved ejection fraction (HFpEF), heart failure with reduced ejection fraction (HFrEF) or myocardial infarction, which are described in Example 9. The animals are administered nucleic acids encoding shRNA or siRNA targeting LINC complex components.

The shRNA/siRNA target a SUN domain-containing protein or a KASH domain-containing protein, as defined herein.

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

Streptozotocin (STZ) rodent models of diabetes treated with a LINC complex inhibitor have improved diastolic or systolic cardiac function, or less cardiac death or cardiac fibrosis, compared to untreated STZ rodents, or rodents treated with vehicle only.

Mice having the mdx mutation subjected to isoproterenol stress (Prins KW, Asp ML, Zhang H, Wang W, & Metzger JM Microtubule-Mediated Misregulation of Junctophilin-2 Underlies T-Tubule Disruptions and Calcium Mishandling in mdx Mice. JACC: Basic to Transl. Sci 1, 122–130 (2016)) and treated with a LINC

complex inhibitor have improved systolic function or an extended lifespan as compared to untreated mice, or mice treated with vehicle only.

Animals having heart failure with preserved ejection fraction, such as O-ZSF1 rats, HFD + mTAC mice or HFD + L-NAME mice, treated with a LINC complex inhibitor have reduced cardiac hypertrophy or diastolic dysfunction as compared to untreated disease animals, or disease animals treated with vehicle only.

Animals having heart failure with reduced ejection fraction, such as TAC mice, treated with a LINC complex inhibitor have improved systolic or diastolic function or left ventricular morphology or cardiac fibrosis as compared to untreated disease animals, or disease animals treated with vehicle only.

Animals having myocardial infarction, such as through coronary artery ligation, treated with a LINC complex inhibitor have improved systolic or diastolic function or cardiac fibrosis as compared to untreated disease animals, or disease animals treated with vehicle only.

**Example 11: Gene editing of LINC complex components alleviates pathophysiology in cardiomyopathies and other heart diseases characterized by microtubule dysfunction**

The inventors investigate the effect of gene editing systems for disrupting LINC complex components using the animal models of diabetic cardiomyopathy, dystrophic cardiomyopathy, heart failure with preserved ejection fraction (HFpEF), heart failure with reduced ejection fraction (HFrEF) or myocardial infarction, which are described in Example 9. The animals are administered nucleic acids encoding gene editing systems (including CRISPR/Cas9 systems) for disrupting LINC complex components.

The gene editing systems 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 nucleic acids are delivered using adeno-associated virus, adenovirus or lentivirus vectors, or nanoparticles.

Streptozotocin (STZ) rodent models of diabetes treated with a LINC complex inhibitor have improved diastolic or systolic cardiac function, or less cardiac death or cardiac fibrosis, compared to untreated STZ rodents, or rodents treated with vehicle only.

Mice having the mdx mutation subjected to isoproterenol stress (Prins KW, Asp ML, Zhang H, Wang W, & Metzger JM Microtubule-Mediated Misregulation of Junctophilin-2 Underlies T-Tubule Disruptions and Calcium Mishandling in mdx Mice. JACC: Basic to Transl. Sci 1, 122–130 (2016)) treated with a LINC complex inhibitor have improved systolic function or an extended lifespan as compared to untreated mice, or mice treated with vehicle only.

Animals having heart failure with preserved ejection fraction, such as O-ZSF1 rats, HFD + mTAC mice or HFD + L-NAME mice, treated with a LINC complex inhibitor have reduced cardiac hypertrophy or diastolic dysfunction as compared to untreated disease animals, or disease animals treated with vehicle only.

Animals having heart failure with reduced ejection fraction, such as TAC mice, treated with a LINC complex inhibitor have improved systolic or diastolic function or left ventricular morphology or cardiac fibrosis as compared to untreated disease animals, or disease animals treated with vehicle only.

Animals having myocardial infarction, such as through coronary artery ligation, treated with a LINC complex inhibitor have improved systolic or diastolic function or cardiac fibrosis as compared to untreated disease animals, or disease animals treated with vehicle only.

**Example 12: Uncoupling LINC complex using DNSUN1 reversed pathological microtubule organisation in LMNA cardiomyopathies *in vivo***

Following the results of Example 1, the inventors next investigated the ability of DNSUN1 to rescue the deleterious effects of aberrant microtubule density in a LMNA mutation-associated dilated cardiomyopathy *in vivo*, in a mouse model.

8-12 weeks old LmnaFF:mcm mice harbouring cardiac-specific tamoxifen-inducible Cre recombinase were injected with tamoxifen (TMX) to induce deletion of the Lmna gene. Two days after TMX treatment, the mice received a single dose ($5 \times 10^{13}$ vg/kg) of AAV9 encoding DNSUN1 (LMNA + DNSUN1). Control conditions included wild-type mice (WT) and LmnaFF mice administered with TMX (LMNA DCM).

Cardiac function was evaluated by echocardiography, and aberrant microtubule density was analysed by Western blot (Figure 10A). A 5.5-fold increase in the total microtubule population was observed in the LMNA-DCM diseased hearts, whereas DNSUN1 treatment reduced this increase to 2.9-fold (Figures 10A and 10B).

The inventors then examined whether this modification correlated with functional deficits in mouse cardiac function. The data showed that microtubule density inversely correlated with left ventricular ejection fraction (EF%), a primary indicator of cardiac contractility (Figure 10C). Myocardium from mice with LMNA-DCM demonstrated considerably depressed EF and substantially increased tubulin levels. DNSUN1-treated mice showed a variable but consistent decrease in tubulin expression, positively correlating with improved ejection fraction (Figure 10C).

Together, these data demonstrate that a modified cytoskeletal network impedes contractile function in cardiomyocytes from diseased hearts and that inhibition of the LINC complex (*e.g.* by DNSUN1) could serve as an effective treatment for multiple conditions by reversing the formation of pathological microtubule networks. This represents a new, comprehensive strategy for improving cardiac function in both genetic and non-genetic cardiomyopathies.

12.1 Materials and Methods

*Production of AAV9*

Adeno-associated viruses were produced by PackGene Biotech Inc using transient triple transfection of HEK293T cells with an adenoviral helper plasmid, a rep/cap plasmid encoding AAV2 Rep and AAV9 cap, and transgene-specific plasmids. AAV9 vectors were purified by iodixanol ultracentrifugation. Viral titre was determined using alkaline gel electrophoresis with a plasmid DNA standard (Kohlbrenner and Weber, Methods Mol. Biol. (2017) 1521 :91-107), gel imaging on a ChemiDoc imaging system (Bio-Rad Laboratories, Hercules, CA, USA) and image quantification with Image Lab software (Bio-Rad Laboratories) or by digital PCR.

*Lmna deletion dilated cardiomyopathy (DCM) mouse model*

Mouse strains were maintained on a 12 h light/dark cycle in ventilated animal barrier facilities with the temperature set to 21 ± 1°C, humidity at 55–70% and with food and water provide ad libitum, in accordance with all ethical regulations.

To generate the Lmna deletion-associated mouse model of dilated cardiomyopathy, mice of the conditional “knockout” or “floxed” LmnaFlx/Flx mouse strain described in Wang et al., Differentiation. (2015) 89(1-2):11-21 were crossed with mice harbouring a tamoxifen-inducible Cre recombinase under control of a cardiomyocyte-specific alpha-myosin heavy chain promoter (αMHC-MerCreMer, abbreviated to mcm) purchased from the Jackson Laboratory (JAX stock number 005657; see also Chai et al., Nat Commun (2021) 12(1):4722). To induce dilated cardiomyopathy in these LmnaFF:mcm mice, 40 mg/kg tamoxifen was injected with intraperitoneally, resulting in deletion of Lmna specifically in cardiomyocytes. Tamoxifen (Merck) solution (16 g/L) was prepared in corn oil (Sigma).

*Echocardiography*

Cardiac function was evaluated by ultrasound recordings using Prospect-T1 (S-Sharp). Mice were shaved 1 day before ultrasound examination. Animals were anaesthetized with 1.5% isoflurane mixed with oxygen. Readings of B-mode and M-mode were taken at heart rates between 450 bpm and 350 bpm. Fractional Shortening (FS) and Ejection Fraction (EF) were calculated from the parasternal long axis using the equipment’s software analysis tool.

*Western blot*

Western Blot was performed using a standard protocol available on the Bio-Rad website with antibodies specific to α-tubulin, DNsun1 and GAPDH.

**References**

A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. The entirety of each of these references is incorporated herein.

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**Claims:**

1. A LINC complex inhibitor for use in a method of treating or preventing cardiomyopathy, wherein the cardiomyopathy is not cardiomyopathy associated with a laminopathy.

2. Use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing cardiomyopathy, wherein the cardiomyopathy is not cardiomyopathy associated with a laminopathy.

3. A method of treating or preventing cardiomyopathy, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject, wherein the cardiomyopathy is not cardiomyopathy associated with a laminopathy.

4. The LINC complex inhibitor for use according to claim 1, the use according to claim 2, or the method according to claim 3, wherein the cardiomyopathy is selected from dilated cardiomyopathy, arrhythmogenic cardiomyopathy, hypertrophic cardiomyopathy and restrictive cardiomyopathy.

5. The LINC complex inhibitor for use according to claim 1 or claim 4, the use according to claim 2 or claim 4, or the method according to claim 3 or claim 4, wherein the cardiomyopathy is associated with mutation to a gene encoding a desmosome protein.

6. The LINC complex inhibitor for use according to any one of claims 1, 4 or 5, the use according to any one of claims 2, 4 or 5, or the method according to any one of claims 3 to 5, wherein the cardiomyopathy is associated with mutation to a gene selected from: *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*.

7. The LINC complex inhibitor for use according to any one of claims 1 and 4 to 6, the use according to any one of claims 2 and 4 to 6, or the method according to any one of claims 3 to 6, wherein the cardiomyopathy is characterised by microtubule dysfunction.

8. A LINC complex inhibitor for use in a method of treating or preventing a disease or condition characterised by desmosome deficiency or dysfunction.

9. Use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing disease or condition characterised by desmosome deficiency or dysfunction.

10. A method of treating or preventing disease or condition characterised by desmosome deficiency or dysfunction, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject.

11. The LINC complex inhibitor for use according to claim 8, the use according to claim 9, or the method according to claim 10, wherein the disease or condition characterised by desmosome deficiency or dysfunction is associated with mutation to a gene encoding a desmosome protein.

12. The LINC complex inhibitor for use according to claim 8 or claim 11, the use according to claim 9 or claim 11, or the method according to claim 10 or claim 11, wherein the disease or condition characterised by desmosome deficiency or dysfunction is associated with mutation to a gene selected from: *DSP*, *DES*, *DSG2*, *DSC2*, *PKP2*, and *JUP*.

13. The LINC complex inhibitor for use according to any one of claims 8, 11 or 12, the use according to any one of claims 9, 11 or 12, or the method according to any one of claims 10 to 12, wherein the disease or condition characterised by desmosome deficiency or dysfunction is characterised by cardiomyopathy.

14. The LINC complex inhibitor for use according to any one of claims 8 and 11 to 13, the use according to any one of claims 9 and 11 to 13, or the method according to any one of claims 10 to 13, wherein the disease or condition characterised by desmosome deficiency or dysfunction is characterised by microtubule dysfunction.

15. A LINC complex inhibitor for use in a method of treating or preventing a disease or condition characterised by microtubule dysfunction.

16. Use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing a disease or condition characterised by microtubule dysfunction.

17. A method of treating or preventing a disease or condition characterised by microtubule dysfunction, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject.

18. The LINC complex inhibitor for use according to claim 15, the use according claim 16, or the method according to 17, wherein the disease or condition characterised by microtubule dysfunction is or is characterised by cardiomyopathy.

19. The LINC complex inhibitor for use according to claim 1 or claim 18, the use according to claim 2 or claim 18, or the method according to claim 3 or claim 18, wherein the cardiomyopathy is selected from dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic cardiomyopathy, diabetic cardiomyopathy and ischaemic cardiomyopathy.

20. The LINC complex inhibitor for use according to any one of claims 1, 4, 5, 7, 8, 11, 12, 14, 15, 18 or 19, the use according to any one of claims 2, 4, 5, 7, 9, 11, 12, 14, 16, 18 or 19, or the method according to any one of claims 3 to 5, 7, 10 to 12, 14 or 17 to 19, wherein the treating or preventing comprises administering a nucleic acid comprising or encoding a LINC complex inhibitor to a subject.

21. The LINC complex inhibitor for use according to any one of claims 1, 4, 5, 7, 8, 11, 12, 14, 15, 18, 19 or 20, the use according to any one of claims 2, 4, 5, 7, 9, 11, 12, 14, 16, 18, 19 or 20, or the method according to any one of claims 3 to 5, 7, 10 to 12, 14, or 17 to 20, wherein the treating or preventing comprises administering a nucleic acid encoding a LINC complex inhibiting polypeptide to a subject.

22. The LINC complex inhibitor for use according to claim 21, the use according to claim 21, or the method according to claim 21, wherein the LINC complex inhibiting polypeptide comprises: (i) an inhibitory region comprising an amino acid sequence corresponding to the α3 helix of the CC2 region and the SUN domain of a SUN domain-containing protein, and (ii) an endoplasmic reticulum retention motif.

23. The LINC complex inhibitor for use according to claim 21 or claim 22, the use according to claim 21 or claim 22, or the method according to claim 21 or claim 22, wherein the LINC complex inhibiting polypeptide comprises, or consists essentially of an amino acid sequence having at least 80% amino acid sequence identity to the amino acid sequence of SEQ ID NO:99, 72, 95, 71, 69, 70 or 73.

24. The LINC complex inhibitor for use according to any one of claims 21 to 23, the use according to any one of claims 21 to 23, or the method according to any one of claims 21 to 23, wherein the nucleic acid encoding a LINC complex inhibiting polypeptide to a subject is comprised in an adeno-associated virus (AAV) vector.

25. A LINC complex inhibitor for use in a method of treating or preventing a disease or condition characterised by sarcomere deficiency or dysfunction.

26. Use of a LINC complex inhibitor in the manufacture of a medicament for use in treating or preventing disease or condition characterised by sarcomere deficiency or dysfunction.

27. A method of treating or preventing disease or condition characterised by sarcomere deficiency or dysfunction, comprising administering a therapeutically- or prophylactically-effective amount of a LINC complex inhibitor to a subject.

28. The LINC complex inhibitor for use according to claim 25, the use according to claim 26, or the method according to claim 27, wherein the disease or condition characterised by sarcomere deficiency or dysfunction is associated with mutation to a gene encoding a sarcomere protein.

29. The LINC complex inhibitor for use according to claim 25 or claim 28, the use according to claim 26 or claim 28, or the method according to claim 27 or claim 28, wherein the disease or condition characterised by sarcomere deficiency or dysfunction is associated with mutation to a gene selected from: *TTN, MYH7, TNNT2, TNNI3, TNNC1, TPM1, MYBPC3, MYL2, MYL3, CSRP3, RBM20, ACTN2, TCAP and ACTC1*.

30. The LINC complex inhibitor for use according to any one of claims 25, 28 or 29, the use according to any one of claims 26, 28 or 29, or the method according to any one of claims 27 to 29, wherein the disease or condition characterised by sarcomere deficiency or dysfunction is characterised by cardiomyopathy.

31. The LINC complex inhibitor for use according to any one of claims 25 and 28 to 30, the use according to any one of claims 26 and 28 to 30, or the method according to any one of claims 27 to 30, wherein the

disease or condition characterised by sarcomere deficiency or dysfunction is characterised by microtubule dysfunction.