growth and viability, SUN1 null mice are infertile and deaf. Simultaneous loss of *Sun1* and *Sun2* results in perinatal lethality, indicating a degree of redundancy during embryogenesis [K. Lei *et al.*, *Proc Natl Acad Sci USA* 106: 10207-10212 (2009)].

There is a need to develop alternative methods to ameliorate the negative effects over-accumulation of Sun1 has on cells carrying *Lmna* mutations. The present disclosure aims at providing such a method.

SUMMARY

Surprisingly, the inventors have found that disruption of the LINC complex rather than removal of accumulated Sun1 protein can ameliorate diseases caused by one or more *Lmna* mutations. One way of achieving the disruption is via an expression construct/vector comprising an operably linked transgene, the expression of which generates dominant negative SUN domain protein or mutated endogenous SUN domain protein and/or dominant negative KASH domain protein or mutated endogenous KASH domain protein. The exogenous dominant negative SUN domain and KASH domain proteins act as LINC complex binding competitors, thereby uncoupling the nucleus from its linkage to the cytoskeleton. The mutated SUN domain and KASH domain proteins are endogenous Sun and Nesprin proteins that have been mutated in the SUN or KASH domain, respectively, and cannot form a LINC complex because they cannot bind to their cognate LINC complex partner. These strategies may be used to disrupt the LINC complex to treat, for example, laminopathies. The result was achieved without actively reducing the endogenous SUN1 protein levels. Results shown herein support these claims.

According to a first aspect of the disclosure, there is provided use of an nucleic acid molecule comprising an adeno-associated expression vector (AAV) and a transgene in the manufacture of a medicament for treating a disease caused by a mutation to *LMNA*, wherein the transgene is operably linked to the expression vector, and wherein expression of the transgene in a transfected cell results in disruption of a LInker of Nucleoskeleton and Cytoskeleton (LINC) complex in the transfected cell. Also provided an isolated nucleic acid molecule, wherein the nucleic acid molecule comprises an expression vector and a transgene, whereby the transgene is operably linked to the expression vector, wherein expression of the transgene in a transfected cell results in disruption of a LINC complex in the transfected cell.

In some examples, the expression vector is a cardiac- or cardiomyocyte-specific expression vector.

In some examples, the expression vector comprises a cardiac- or cardiomyocyte-specific promoter selected from the group comprising a cardiac troponin T promoter (cTnT), a α-myosin heavy chain (α-MHC) promoter and a myosin light chain (MLC2v) promoter. Preferably the promoter is cardiac troponin T promoter (cTnT).

In some examples, the cardiomyocyte-specific promoter is chicken cardiac troponin T promoter (cTnT).

In some examples, the expression vector has cardiac tropism/is cardiotropic.

In some examples, the expression vector is a virus expression vector.

In some examples, the virus expression vector is selected from the group comprising Lentivirus, Adenovirus and Adeno-associated virus (AAV). Preferably the virus expression vector is adeno-associated virus (AAV).

In some examples, the AAV vector is selected from the group consisting of AAV9 (serotype 9), AAV1 (serotype 1), AAV6 (serotype 6), AAV8 (serotype 8), AAV2i8 and AAV9.45.

In some examples, the AAV vector is AAV9 (serotype 9).

In some examples, the transgene comprises nucleic acid sequences for expressing a lumenal domain of a SUN domain-containing protein, an N-terminal signal sequence, a signal peptidase cleavage site, and a C-terminal targeting peptide sequence.

In some examples the lumenal domain of the SUN domain-containing protein comprises a coiled coil domain and a SUN domain.

In a preferred example the coiled coil domain is upstream of the SUN domain.

In some examples, the transgene further comprises nucleic acid sequences for expressing an N-terminal signal sequence, a signal peptidase cleavage site, and a C-terminal targeting peptide sequence.

In some examples, the transgene comprises nucleic acid sequences for expressing an N-terminal signal sequence, a signal peptidase cleavage site, and a C-terminal targeting peptide sequence, and either the luminal domain of the SUN domain-containing protein or the SUN domain.

Preferably, the SUN domain protein is SUN1 or SUN2.

In some examples the luminal domain of Sun1 comprises amino acids 458-913 of full-length mouse Sun1 (Uniprot: Q9D666) or its human equivalent comprising the coiled coil

domain and the SUN domain and lacking the transmembrane domain. A schematic of the structure of a dominant negative form of Sun1 is shown in Figure 7.

For SUN domain constructs it is expected that the SUN domain alone (crystal structure solved by the Kutay and Schwartz labs [Sosa *et al., Cell* 149(5):1035-47 (2012)], instead of the entire luminal domain (coiled coil domain and SUN domain) is sufficient to disrupt the SUN-KASH interaction as it is capable of binding to the KASH domain. The human Sun1 SUN domain nucleic acid sequence is set forth in SEQ ID NO: 80. However, the presence of the signal sequence and the KDEL sequence are important for targeting the construct to the perinuclear space.

In some examples, the N-terminal signal sequence is derived from a secretory protein or a Type I transmembrane protein.

Preferably, the secretory protein or Type I transmembrane protein is selected from the group consisting of human serum albumin, proinsulin, transferrin receptor, EGF receptor, pre-pro-opiomelanocortin, pancreatic digestive enzymes (for example, proteases, amylases and lipases), endoplasmic reticulum luminal proteins, for example protein disulphide isomerases, GRP94 and combinations thereof. More preferably, the N-terminal signal sequence is derived from human serum albumin.

In some examples, the N-terminal signal sequence is not preceded at its N-terminus by any other tags.

In some examples, the signal peptidase cleavage site is a signal peptidase cleavage site derived from or is one of the group consisting of human serum albumin, proinsulin, transferrin receptor, EGF receptor, pre-pro-opiomelanocortin, pancreatic digestive enzymes (for example, proteases, amylases and lipases), endoplasmic reticulum lumenal proteins, such as protein disulphide isomerases, GRP94 and combinations thereof. Preferably, the signal peptidase cleavage site is a signal peptidase cleavage site derived from human serum albumin.

In some examples, the C-terminal targeting peptide sequence prevents secretion of a peptide expressed from the transgene according to any example of the disclosure.

In some examples, the C-terminal targeting peptide sequence is a KDEL tetrapeptide Golgi retrieval sequence. Examples of such structures are shown in Figures 11 and 12.

In some examples the transgene comprises a humanized Sun1DN nucleic acid sequence or a humanized Sun2DN nucleic acid sequence. In a preferred example, the

transgene comprises a signal sequence, a humanized Sun1DN nucleic acid sequence and a KDEL sequence as set forth in SEQ ID NO: 4; or the transgene comprises a signal sequence, a humanized Sun2DN nucleic acid sequence and a KDEL sequence as set forth in SEQ ID NO: 5.

In some examples, the transgene further comprises an epitope tag. Preferably the epitope tag is N-terminal, or located anywhere in the nucleic acid molecule except downstream of (after) the C-terminal targeting peptide sequence [for example KDEL], or located anywhere in the nucleic acid molecule except upstream of (before) the N-terminal signal sequence.

In some examples, the epitope tag is selected from the group consisting of cellulose binding domain (CBD), chloramphenicol acetyl transferase (CAT), dihydrofolate reductase (DHFR), one or more FLAG tags, glutathione S-transferase (GST), green fluorescent protein (GFP), haemagglutinin A (HA), histidine (His), Herpes simplex virus (HSV), luciferase, maltose-binding protein (MBP), c-Myc, Protein A, Protein G, streptavidin, T7, thioredoxin, V5, vesicular stomatitis virus glycoprotein (VSV-G), and combinations thereof. Preferably, the epitope tag is haemagglutinin A (HA).

In some examples, the nucleic acid molecule of the disclosure comprises an adeno-associated virus vector (AAV) comprising a chicken cardiac troponin T promoter (cTnT), a transgene according to any example of the disclosure comprising the luminal domain of the SUN domain-containing protein derived from SUN1, an N-terminal signal sequence and a signal peptidase cleavage site which are each derived from human serum albumin, a C-terminal targeting peptide sequence which is a KDEL sequence, and wherein the transgene optionally further comprises haemagglutinin (HA) as an N-terminal epitope tag.

According to an example an example of such a vector is shown in Figure 10 and comprises the nucleic acid sequence set forth in SEQ ID NO: 3.

In some examples, the nucleic acid molecule of the disclosure comprises an adeno-associated virus vector (AAV) comprising a chicken cardiac troponin T promoter (cTnT), a transgene according to any example of the disclosure comprising the luminal domain of the SUN domain-containing protein derived from SUN2, an N-terminal signal sequence and a signal peptidase cleavage site which are each derived from human serum albumin, a C-terminal targeting peptide sequence which is a KDEL sequence, and wherein the transgene optionally further comprises haemagglutinin (HA) as the N-terminal epitope tag.

According to an example an example nucleic acid molecule would comprise the vector structure shown in Figure 10 and the transgene nucleic acid sequence set forth in SEQ ID NO: 5.

Rather than expressing components of a lumenal domain of a SUN domain-containing protein, a KASH domain may be expressed to disrupt a LINC complex by competing with endogenous Nesprins (which comprise a KASH domain) for binding to SUN1 and SUN2 domains.

Accordingly, in some examples of the nucleic acid molecule of the disclosure, the transgene comprises nucleic acid sequences for expressing a KASH domain, and an N-terminal stabiliser polypeptide sequence.

Preferably, the KASH domain comprises a transmembrane domain and a SUN-interacting peptide.

Preferably the transgene comprises nucleic acid sequences for expressing a KASH domain that traverses the outer nuclear membrane, a SUN-interacting KASH peptide that extends into the perinuclear space at the C-terminus, and an N-terminal stabiliser polypeptide sequence in the cytoplasm.

It would be understood that KASH domain constructs with extensions after the last C-terminal amino acid of the naturally occurring KASH domain are not expected to work. i.e. C-terminal tags, or even an additional carboxy-terminal single amino acid, will disrupt KASH interaction with SUN. In addition, a signal sequence on the N-terminus of SUN domain constructs cannot be preceded by any tags.

In some examples, the KASH domain is selected from the group consisting of KASH1 (derived from Nesprin-1 (SYNE1 gene)), KASH2 (derived from Nesprin-2 (SYNE2 gene)), KASH3 (derived from Nesprin-3 (SYNE3 gene)), KASH4 (derived from Nesprin-4 (SYNE4 gene)) and KASH5 (derived from KASH5/CCDC155 (KASH5 gene)).

In preferred examples the KASH 1 domain comprises the human amino acid sequence set forth in SEQ ID NO: 7; the KASH 2 domain comprises the human amino acid sequence set forth in SEQ ID NO: 9; the KASH 3 domain comprises the human amino acid sequence set forth in SEQ ID NO: 11; the KASH 4 domain comprises the human amino acid sequence set forth in SEQ ID NO: 13; and the KASH 5 domain comprises the human amino acid sequence set forth in SEQ ID NO: 15. An alignment of the five KASH amino acid sequences is shown in Figure 14.

In some examples the KASH domain nucleic acid sequence has at least 80%, at least 85%, at least 90%, at least 95% sequence identity or 100% sequence identity to the nucleic acid sequence of the KASH1 domain set forth in SEQ ID NO: 6; the nucleic acid sequence of the KASH2 domain set forth in SEQ ID NO: 8; the nucleic acid sequence of the KASH3 domain set forth in SEQ ID NO: 10; the nucleic acid sequence of the KASH4 domain set forth in SEQ ID NO: 12; or the nucleic acid sequence of the KASH5 domain set forth in SEQ ID NO: 14.

More preferably, for the purpose of clinical use, the KASH domain is the human KASH1 domain of SYNE1 having at least 80%, at least 85%, at least 90%, at least 95% sequence identity or 100% sequence identity to the nucleic acid sequence of the human KASH1 domain set forth in SEQ ID NO: 6.

It would be understood that due to the redundancy in the genetic code, a nucleic acid sequence may have less than 100% identity and still encode the same amino acid sequence.

In some examples, the KASH domain does not comprise any extensions after the last C-terminal amino acid compared to a naturally occurring KASH domain.

In some examples, the N-terminal stabiliser polypeptide sequence is selected from the group consisting of green fluorescent protein (GFP), cellulose binding domain (CBD), chloramphenicol acetyl transferase (CAT), dihydrofolate reductase (DHFR), glutathione S-transferase (GST), luciferase, maltose-binding protein (MBP), Protein A, Protein G, streptavidin, thioredoxin, DHFR, including multiples and combinations thereof.

In some examples, the N-terminal stabilizer polypeptide sequence forms a discretely folded domain.

In some examples, the vector is the adeno-associated virus vector (AAV) comprising a cardiac troponin T promoter (cTnT), and the transgene comprises nucleic acid sequences for expressing a KASH domain, and an N-terminal stabiliser polypeptide sequence, wherein the KASH domain is selected from the group comprising KASH1, KASH2, KASH3, KASH4 and KASH5.

In some examples, the N-terminal stabiliser polypeptide sequence is green fluorescent protein (GFP).

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 examples of the nucleic acid molecule of the disclosure, the transgene comprises nucleic acid sequences for expressing a CRISPR-Cas or other synthetic nuclease system to modify nucleic acid that encodes the SUN domain or KASH domain of endogenous Sun or Nesprin protein, respectively.

Data shown herein (Example 6) suggests that modification of the SUN2 domain or KASH2 domain does not ameliorate Lmna pathology.

In some examples the CRISPR-Cas 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 examples, the transgene comprises nucleic acid sequences for expressing a CRISPR-Cas with a gRNA nucleic acid sequence comprising 5'-GCACAATAGCCTCGGATGTCG-3' (SEQ ID NO: 66) to modify the SUN domain of mouse Sun1.

In some examples, the transgene comprises nucleic acid sequences for expressing a CRISPR-Cas with a gRNA nucleic acid sequence targeting the human SUN1 domain set forth in SEQ ID NO: 80. Preferably, the gRNA nucleic acid sequence targets the end of exon 20 comprising a nucleic acid sequence set forth in SEQ ID NO: 81. More preferably, the gRNA nucleic acid sequence targets a SUN1 nucleic acid sequence selected from the group comprising SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63; SEQ ID NO: 64 and SEQ ID NO: 65 set forth in Table 3.

In some examples, the transgene comprises nucleic acid sequences for expressing a CRISPR-Cas with a gRNA nucleic acid sequence comprising 5'-

CCGTTGGTATATCTGAGCAT-3' (SEQ ID NO: 34) to modify the KASH domain of mouse Syne-1.

In some examples, the transgene comprises nucleic acid sequences for expressing a CRISPR-Cas with a gRNA nucleic acid sequence targeting the human KASH domain set forth in SEQ ID NO: 6. Preferably, the gRNA nucleic acid sequence comprises a nucleic sequence selected from the group comprising SEQ ID NO: 44; SEQ ID NO: 45; SEQ ID NO: 46; SEQ ID NO: 47; SEQ ID NO: 48; SEQ ID NO: 49; SEQ ID NO: 50; SEQ ID NO: 51; SEQ ID NO: 52; SEQ ID NO: 53 and SEQ ID NO: 54 set forth in Table 3.

In some examples, the transgene comprises nucleic acid sequences for expressing a CRISPR-Cas9 or variant thereof.

In preferred examples, the transgene is a dominant negative construct.

In some examples, the transgene is a humanised transgene.

In some examples, expression of the transgene results in the disruption of the protein-protein interaction between SUN and KASH domains of the LINC complex. Preferably, the disruption of the protein-protein interaction between SUN and KASH of the LINC complex occurs between the protein interactions selected from the group consisting of Sun1+Nesprin-1, Sun2+Nesprin-1, Sun1+Nesprin-2, Sun1+Nesprin-3, Sun2+Nesprin-2, and Sun2+Nesprin-3. More preferably, the disruption of the protein-protein interaction between SUN and KASH of the LINC complex occurs between the protein interactions of Sun1 and Nesprin-1.

In some examples, the AAV vector is formulated for delivery into the myocardium of a subject.

Also provided is a nucleic acid molecule of any example of the disclosure for use in treating a disease caused by one or more *Lmna* mutations in a subject.

In some examples, the disease is selected from the group consisting of restrictive dermopathy, familial partial lipodystrophy (for example, Dunnigan type), mandibuloacral dysplasia with type A lipodystrophy, metabolic syndrome, Charcot-Marie-Tooth disease type 2, Charcot-Marie-Tooth disease type 2B1 and diseases presented in normal font in Table 1.

In some examples, the nucleic acid molecule according to any example of the disclosure is for use in treating cardiovascular disease in a subject.

In some examples, the disease or the cardiovascular disease is characterised by the presence of at least one *Lmna* mutation.

Preferably, the cardiovascular disease is selected from the group consisting of laminopathy, cardiomyopathy, such as dilated cardiomyopathy (DCM), dilated cardiomyopathy 1A, dilated cardiomyopathy with conduction system defects, cardiomyopathy with advanced AV block and arrhythmia, lone atrial fibrillation; muscular dystrophy (often associated with cardiomyopathy), such as cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal dominant), cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal recessive), cardiomyopathy associated with Limb-girdle muscular dystrophy type 1B, cardiomyopathy associated with congenital muscular dystrophy; premature aging syndromes (thought to be primarily vascular, but may have cardiac involvement) such as cardiomyopathy associated with Atypical Werner syndrome, cardiomyopathy associated with Hutchinson-Gilford progeria syndrome and the like, as well as diseases presented in bold font in Table 1.

Also provided is an adeno-associated virus vector (AAV) comprising a cardiac troponin T promoter (cTnT), and the transgene according to any example of the disclosure.

Also provided is a pharmaceutical composition comprising the nucleic acid molecule according to any example of the disclosure for treating a disease.

In some examples the disease is a laminopathy.

In some examples the pharmaceutical composition comprising the nucleic acid molecule according to the disclosure is for use in treating a cardiovascular disease in a subject.

Also provided is a method of treating a disease in a subject, the method comprising administration of a pharmaceutically effective amount of the nucleic acid molecule according to any example of the disclosure, or the pharmaceutical composition of the disclosure.

In some examples of the method of treating a disease in a subject, the disease is characterised by the presence of at least one *Lmna* mutation.

In some examples of the method of treating a disease in a subject, the method comprises:

(i) testing a sample obtained from a subject suspected of having a disease for the presence or absence of at least one *Lmna* mutation;

wherein the presence of at least one *Lmna* mutation indicates that the subject is to be administered the pharmaceutical composition of the disclosure or the nucleic acid molecule of the disclosure.

In some examples of the method of treating a disease in a subject, the *Lmna* mutation(s) affect(s) lamin A isoform, or lamin C isoform of the *Lmna* gene, or both lamin A/C isoforms.

In some examples of the method of treating a disease in a subject, the disease is selected from the group consisting of restrictive dermopathy, familial partial lipodystrophy (for example, Dunnigan type), mandibuloacral dysplasia with type A lipodystrophy, metabolic syndrome, Charcot-Marie-Tooth disease type 2, Charcot-Marie-Tooth disease type 2B1 and diseases presented in normal font in Table 1.

In some examples of the method of treating a disease in a subject, the disease is a cardiovascular disease, wherein the cardiovascular disease is selected from the group consisting of laminopathy, cardiomyopathy, such as dilated cardiomyopathy (DCM), dilated cardiomyopathy 1A, dilated cardiomyopathy with conduction system defects, cardiomyopathy with advanced AV block and arrhythmia, lone atrial fibrillation; muscular dystrophy (often associated with cardiomyopathy), such as cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal dominant), cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal recessive), cardiomyopathy associated with Limb-girdle muscular dystrophy type 1B, cardiomyopathy associated with congenital muscular dystrophy; premature aging syndromes (thought to be primarily vascular, but may have cardiac involvement) such as cardiomyopathy associated with Atypical Werner syndrome, cardiomyopathy associated with Hutchinson-Gilford progeria syndrome; and diseases presented in bold font in Table 1.

In some examples of the method of treating a disease in a subject, the subject is a non-human mammal, such as a mouse, or a human.

In some examples of the method, the mouse is an N195K mouse (Lmna N195K/N195K), or a *Lmna* conditional knockout (Lmnaflox/flox).

Also provided is use of the pharmaceutical composition according to the disclosure or the nucleic acid molecule according to the disclosure in the manufacture of a medicament for treating a disease caused by one or more *Lmna* mutations.

In some examples the disease is *Lmna* mutation-related cardiovascular disease.

In some examples the disease is selected from the group consisting of restrictive dermopathy, familial partial lipodystrophy (for example, Dunnigan type), mandibuloacral dysplasia with type A lipodystrophy, metabolic syndrome, Charcot-Marie-Tooth disease type 2, Charcot-Marie-Tooth disease type 2B1 and diseases presented in normal font in Table 1.

In some examples the cardiovascular disease is selected from the group consisting of laminopathy, cardiomyopathy, such as dilated cardiomyopathy (DCM), dilated cardiomyopathy 1A, dilated cardiomyopathy with conduction system defects, cardiomyopathy with advanced AV block and arrhythmia, lone atrial fibrillation; muscular dystrophy (often associated with cardiomyopathy), such as cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal dominant), cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal recessive), cardiomyopathy associated with Limb-girdle muscular dystrophy type 1B, cardiomyopathy associated with congenital muscular dystrophy; premature aging syndromes (thought to be primarily vascular, but may have cardiac involvement) such as cardiomyopathy associated with Atypical Werner syndrome, cardiomyopathy associated with Hutchinson-Gilford progeria syndrome and the like, as well as diseases presented in bold font in Table 1.

Furtherprovided is a method for screening for drug candidates capable of inhibiting or disrupting the LINC complex in a cell.

Accordingly, in some examples there is provided a method for screening for drug candidates capable of inhibiting the interaction of the proteins of a LINC complex in a cell, which comprises:

(a) combining the proteins of said LINC complex in the presence of a drug to form a first complex;

(b) combining the proteins in the absence of said drug to form a second complex;

(c) measuring the amount of said first complex and said second complex; and

(d) comparing the amount of said first complex with the amount of said second complex,

wherein if the amount of said first complex is less than the amount of said second complex, then the drug is a drug candidate for inhibiting the interaction of the proteins of said LINC complex in a cell.

In some examples the drug candidate disrupts the protein-protein interaction between SUN and KASH of the LINC complex. Preferably the drug candidate disrupts the interaction between Sun1 and Nesprin-1 proteins.

In some examples said screening is an in vitro screening.

In some examples said complex is measured by an ELISA method.

In some examples recombinant SUN domain is immobilized on a solid surface and recombinant KASH domain is labelled with an enzyme that can generate a colorimetric or chemiluminescent readout. Compounds that fail to inhibit the SUN-KASH interaction will result in a well in the plate where the recombinant SUN would bind to the enzyme-linked KASH domain. Following wash steps and incubation with colorimetric or chemiluminescent enzyme substrates, the presence of the SUN-KASH interaction can be detected in standard plate readers. If the compound can inhibit SUN-KASH interaction, then following the wash step, the KASH domain would be removed, and there would be reduced or no enzymatic reaction in the well.

In some examples if the amount of said first complex is less than the amount of said second complex, then said drug is a drug candidate for inhibiting the interaction of said proteins.

In some examples said complex is measured by a fluorescence anisotropy method.

In some examples the fluorescence anisotropy method employs recombinant SUN and KASH domains. In some examples the KASH domain is fluorescently labelled with a fluorescein moiety and fluorescence anisotropy of the KASH domain interacting with SUN domain may be measured using standard equipment such as a plate reader incorporating a fluorescence spectrometer function.

In some examples if the amount of said first complex is less than the amount of said second complex there will be a difference in the fluorescence anisotropy of the fluorescent KASH and said drug is a drug candidate for inhibiting the interaction of said proteins.

CLAIMS:

1. Use of an nucleic acid molecule comprising an adeno-associated expression vector (AAV) and a transgene in the manufacture of a medicament for treating a disease caused by a mutation to *LMNA*, wherein the transgene is operably linked to the expression vector, and wherein expression of the transgene in a transfected cell results in disruption of a LInker of Nucleoskeleton and Cytoskeleton (LINC) complex in the transfected cell.

2. The use according to claim 1, wherein expression of the transgene results in the disruption of the protein-protein interaction between SUN and KASH of the LINC complex.

3. The use according to claim 1 or claim 2, wherein transgene comprises nucleic acid sequences for expressing the SUN domain of a SUN domain-containing protein, an N-terminal signal sequence, a signal peptidase cleavage site, and a C-terminal targeting peptide sequence preventing secretion of a peptide expressed from the transgene.

4. The use according to claim 3, wherein the transgene comprises nucleic acid sequences for expressing the lumenal domain of the SUN domain-containing protein, wherein the lumenal domain comprises a coiled coil domain and a SUN domain.

5. The use according to claim 3 or claim 4, wherein the SUN domain-containing protein is SUN1 or SUN2.

6. The use according to any one of claims 3 to 5, wherein the C-terminal targeting peptide sequence is a KDEL sequence.

7. The use according to claim 1 or claim 2, wherein the transgene comprises nucleic acid sequences for expressing a KASH domain, and an N-terminal stabiliser polypeptide sequence.

8. The use according to claim 7, wherein the KASH domain comprises a transmembrane domain and a SUN-interacting peptide.

9. The use according to claim 7 or claim 8, wherein the KASH domain is selected from the group consisting of KASH1, KASH2, KASH3, KASH4, and KASH5.

10. The use according to any one of claims 1 to 9, wherein the AAV is a cardiac- or cardiomyocyte-specific expression vector.

11. The use according to any one of claims 1 to 10, wherein in the AAV vector is selected from the group consisting of: AAV9, AAV1, AAV6, AAV8, AAV2i8, and AAV9.45.

12. The use according to any one of claims 1 to 11, wherein the AAV comprises a cardiac- or cardiomyocyte-specific promoter.

13. The use according to any one of claims 1 to 12, wherein the AAV comprises a cardiac- or cardiomyocyte-specific promoter selected from the group comprising a cardiac

troponin T promoter (cTnT), a α-myosin heavy chain (α-MHC) promoter and a myosin light chain (MLC2v) promoter.

14. The use according to any one of claims 1 to 13, wherein the disease is selected from the group consisting of: a cardiovascular disease, restrictive dermopathy, familial partial lipodystrophy, mandibuloacral dysplasia with type A lipodystrophy, metabolic syndrome, Charcot-Marie-Tooth disease type 2, Charcot-Marie-Tooth disease type 2B1 and diseases presented in normal font in Table 1.

15. The use according to claim 14, wherein the cardiovascular disease is selected from the group consisting of: laminopathy, cardiomyopathy, dilated cardiomyopathy (DCM), dilated cardiomyopathy 1A, dilated cardiomyopathy with conduction system defects, cardiomyopathy with advanced AV block and arrhythmia, lone atrial fibrillation; muscular dystrophy, cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal dominant), cardiomyopathy associated with Emery-Dreifuss muscular dystrophy (autosomal recessive), cardiomyopathy associated with Limb-girdle muscular dystrophy type 1B, cardiomyopathy associated with congenital muscular dystrophy; premature aging syndromes, cardiomyopathy associated with Atypical Werner syndrome, cardiomyopathy associated with Hutchinson-Gilford progeria syndrome, and diseases presented in bold font in Table 1.