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(54) DISRUPTING THE LINC COMPLEX FOR TREATING LAMINOPATHY

UNTERBRECHUNG DES LINC-KOMPLEXES ZUR BEHANDLUNG VON LAMINOPATHIE

PERTURBATION DU COMPLEXE LINC POUR LE TRAITEMENT DE LAMINOPATHIES

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(73) Proprietor: **Agency for Science, Technology and Research**
Singapore 138632 (SG)

(72) Inventors:
- **STEWART, Colin Lawson**
  Singapore 138648 (SG)
- **CHAI, Jinfen**
  Singapore 138648 (SG)
- **LEE, Yin Loon**
  Singapore 138648 (SG)
- **BURKE, Brian Edmund**
  Singapore 138648 (SG)

(74) Representative: **Mewburn Ellis LLP**
Aurora Building
Counterslip
Bristol BS1 6BX (GB)

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Description

FIELD OF THE INVENTION

[0001] The present disclosure relates to use of expression vectors and other compounds in methods to disrupt the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, uncoupling the nucleus from its linkage to the cytoskeleton, resulting in amelioration of diseases caused by one or more *Lmna* mutations, so-called laminopathies. More particularly, the present disclosure relates to the expression of dominant negative or mutated SUN domain protein and/or dominant negative or mutated KASH domain protein to disrupt the LINC complex in, for example, cardiomyocytes for suppressing disease progression in dilated cardiomyopathy (DCM).

BACKGROUND

[0002] Dilated Cardiomyopathy (DCM) is the most common disease affecting heart muscle, accounting for approximately 60% of all cardiomyopathies. It is characterized by reduced systolic (contractile) function due to enlargement and thinning of the left ventricular wall or in some cases both ventricles. DCM is associated with sudden heart failure and cardiac death, resulting in high rates of hospital admission, the need for heart transplantation and consequently a high cost burden [J. L. Jefferies and J. A. Towbin, Lancet 375: 752-762 (2010); R. E. Hershberger, et al., Nat Rev Cardiol 10: 531-547 (2013)]. The causes of DCM are varied, but include a variety of extrinsic factors, (viral, autoimmune infiltration, alcohol, and drugs). However 30-40% of all cases have a monogenic basis, with mutations in some 40 genes being linked to DCM. The most frequently mutated gene in DCM is *TTN,* that encodes the giant sarcomeric protein titin, with truncating variants in *TTN* accounting for almost 15-25% of all congenital forms of DCM [D. S. Herman et al., N Engl J Med 366: 619-628 (2012); U. Tayal, S. et al., Genome Med 9: 20 (2017)]. The second most frequently mutated gene is Lamin A *(LMNA)* accounts for as many as 6-8% of congenital DCM patients [U. Tayal, S. et al., Genome Med 9: 20 (2017)].

[0003] *LMNA* induced DCM is characterized by cardiac conduction disease manifested by electrophysiological (ECG) abnormalities, including atrioventricular block, ventricular arrhythmias and fibrillation. The risk of sudden cardiac death is greater in patients with LMNA-cardiomyopathy than patients with other forms of DCM [J. H. Van Berlo et al., Hum Mol Genet 14: 2839-2849 (2005)]. Some 450 different mutations have been identified in the *LMNA* gene, most being missense, resulting in the majority of DCM cases being inherited as autosomal dominants, with this diversity of the mutations complicating genetic approaches to treating *LMNA* induced DCM. To a limited extent *LMNA* induced DCM can be treated by fitting a pacemaker. Ultimately, however, effective treatment at present is accomplished by heart transplantation (R. E. Hershberger and A. Morales, in GeneReviews((R)), M. P. Adam et al., Eds. (Seattle (WA), 1993); G. Captur et al., Heart 104: 468-479 (2018)].

[0004] Mouse lines carrying *Lmna* mutations usually die within a few weeks after birth [T. Sullivan et al., J Cell Biol 147: 913-920 (1999); A. T. Bertrand et al., Hum Mol Genet 21: 1037-1048 (2012); V. Nikolova et al., J Clin Invest 113: 357-369 (2004); A. S. Wang, et al., Differentiation; research in biological diversity, (2015)]. The cause of early death in mice lacking *Lmna* is uncertain due to multiple tissues being affected. Cardiac myopathy is thought to be a major contributing cause, as *Lmna* mutant mice develop DCM with conduction abnormalities and focal myocyte degeneration [V. Nikolova et al., J Clin Invest 113: 357-369 (2004); L. C. Mounkes, et al., Hum Mol Genet 14: 2167-2180 (2005)], although effects on other, as yet undefined, skeletal muscles may contribute to early postnatal death.

[0005] The lamins are nuclear intermediate filament proteins and are the principal constituents of the nuclear lamina, the proteinaceous matrix underlying the inner nuclear membrane (INM). The lamina consists of the A-type lamins, consisting of 2 predominant forms, lamins A and lamin C, derived by alternate splicing of *LMNA,* whereas the two B type lamins *(LMNB* 1 and 2) are each encoded by two genes: *LMNB1* and *LMNB2* [B. Burke and C. L. Stewart, Nat Rev Mol Cell Biol 14: 13-24 (2013)]. The lamina provides structural and mechanical integrity to the nucleus, maintains nuclear shape and position within the cell, as well as being determinants of chromatin organization [T. Sullivan et al., J Cell Biol 147: 913-920 (1999); I. Solovei et al., Cell 152: 584-598 (2013)]. The lamins interact with numerous INM proteins, including Emerin, the Lamina-Associated Polypeptides (LAPs) and the SUN domain proteins [B. van Steensel and A. S. Belmont, Cell 169: 780-791 (2017)], many of which when either mutated or present as a variant are linked to heart disease [H. J. Worman, et al., Cold Spring Harbor perspectives in biology 2: a000760 (2010); C. L. Stewart, et al., Exp Cell Res 313: 2144-2156 (2007)]. Together these proteins comprise an integrated protein network, centered on the lamina, where loss or mutation of the lamins can result in either the mis-localization or a change in expression levels of many lamina associated proteins, (emerin, SUN1, LBR and Lap2α) [T. Sullivan etal., J Cell Biol 147: 913-920 (1999); I. Solovei et al., Cell 152: 584-598 (2013); C. Y. Chen et al., Cell 149: 565-577 (2012); T. V. Cohen et al., Hum Mol Genet 22: 2852-2869 (2013); F. Haque et al., J Biol Chem 285: 3487-3498 (2010)]. Among these proteins, where expression is affected by the loss of *Lmna* or mutation, are SUN1 and Lap2, both of whose levels are increased. In the case of SUN1 the increased level is due to reduced turnover, rather than increased expression, resulting in high levels accumulating in the Golgi which appeared to be cytotoxic at least in the *Lmna*$^{-/-}$ and *Lmna*Δ*9* mouse disease models

[C. Y. Chen et al., Cell 149: 565-577 (2012); C. Stewart and B. Burke, WO/2013/158046]. However, when SUN1 levels are genetically ablated in mice with *Lmna* mutations, this increases the longevity 3-fold and ameliorates much of the pathology [C. Y. Chen et al., Cell 149: 565-577 (2012); C. Stewart and B. Burke, WO/2013/158046]. The median survival of wild-type or $Sun1^{-/-}$ is > 210 days in a 7 month follow up; $Lmna^{-/-}$ mice had median survival of 41 days; $Lmna^{-/-}$ $Sun1^{+/-}$ mice had a median survival of 54 days; $Lmna^{-/-}$ $Sun1^{-/-}$ mice had a median survival of 104 days ($p < 0.01$ comparing and $Lmna^{-/-}$ $Sun1^{-/-}$). Likewise, whereas all *Lmna*Δ9 mice expired by 30 days after birth, their *Lmna*Δ9$Sun1^{-/-}$ littermates thrived past this date, and most achieved life spans more than twice this duration [C. Y. Chen et al., Cell 149: 565-577 (2012)]. At the cellular level, human fibroblasts harbouring a *LMNA* mutation resulting in Hutchison-Gilford Progeria Syndrome also exhibited increased Sun1 levels. Depleting Sun1 in these cells alleviated nuclear morphology defects, again suggesting that excess Sun1 resulting from LMNA mutation is cytotoxic [C. Y. Chen et al., Cell 149: 565-577 (2012); C. Stewart and B. Burke, WO/2013/158046]

[0006] The SUN (Sad1p, UNC-84) domain proteins share a conserved C-terminal SUN domain and localize to the INM [C. J. Malone, et al., Development 126: 3171-3181 (1999)]. In mammals, SUN1 and SUN2 are the 2 principal SUN proteins that are widely expressed in virtually all tissues. In the perinuclear space, between the INM and outer nuclear membrane (ONM), the C-termini of SUN1 and/or 2 bind to the C-termini (KASH domains) of the different Nesprins/SYNE/KASH proteins that traverse the ONM. Together these 2 families of proteins comprise the LINC complexes that physically couple the interphase nuclei to the cytoskeleton [M. Crisp et al., J Cell Biol 172: 41-53 (2006); E. C. Tapley and D. A. Starr, Curr Opin Cell Biol 25: 57-62 (2013)]. The N-termini of the SUN domain proteins protrude into the nucleoplasm and with SUN1, this region interacts with pre-laminA and nuclear pore complexes. Whether the N- terminus of SUN2 interacts with any nucleoplasmic/NE protein is unclear. In contrast, the bulk of the Nesprins/KASH domain proteins extend into the cytoplasm adjacent to the ONM. There, depending on the particular Nesprin/KASH protein, they interact directly or indirectly with all 3 cytoskeletal protein networks (microtubules, actin microfilaments and intermediate filaments) [H. F. Horn, Current topics in developmental biology 109: 287-321 (2014)]. Together, the SUN and KASH/Nesprin proteins of the LINC complex establish a direct physical connection between the cytoplasmic cytoskeletal networks (and their connections e.g. cell adhesion complexes at the cell membrane) and the interphase nuclear interior or nucleoplasm. The LINC complex is thought to mediate force transmission between the nucleus and cytoskeleton and consequently regulate changes in gene expression/chromatin organization in response to mechanical/physical stimuli [S. G. Alam et al., Scientific reports 6: 38063 (2016)]. Although loss of either SUN1 or SUN2 alone has no overt effect on postnatal 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)].

[0007] Lombardi et al., J Biol Chem (2011) 286(30): 26743-26753, Crisp et al. J Cell Biol (2006) 172(1): 41-53, Zhou et al. Human Molecular Genetics (2017) 26 (12): 2258-2276 and Razafsky and Hodzic, Genesis (2014) 52(4): 359-365 disclose dominant-negative versions of Nesprin and SUN proteins. Chen et al., Cell (2012) 149(3): 565-577 reports that *Lmna-/-* and *Lmna*Δ*9* mouse models of *LMNA* mutation-associated disease are characterised by accumulation of Sun1 protein, and that *Sun1* knockout ameliorates pathology in such models.

[0008] 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 OF INVENTION

[0009] 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.

[0010] The present invention is defined in the claims.

[0011] According to a first aspect of the present disclosure, there is 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.

[0012] In some embodiments, the expression vector is a cardiac- or cardiomyocyte-specific expression vector.

[0013] In some embodiments, 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).

[0014] In some embodiments, the cardiomyocyte-specific promoter is chicken cardiac troponin T promoter (cTnT).

[0015] In some embodiments, the expression vector has cardiac tropism/is cardiotropic.

[0016] In some embodiments, the expression vector is a virus expression vector.

[0017] In some embodiments, 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).

[0018] In some embodiments, 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.

[0019] In some embodiments, the AAV vector is AAV9 (serotype 9).

[0020] In some embodiments, 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.

[0021] In some embodiments the lumenal domain of the SUN domain-containing protein comprises a coiled coil domain and a SUN domain.

[0022] In a preferred embodiment the coiled coil domain is upstream of the SUN domain.

[0023] In some embodiments, 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.

[0024] In some embodiments, 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.

[0025] Preferably, the SUN domain protein is SUN1 or SUN2.

[0026] In some embodiments 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.

[0027] 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.

[0028] In some embodiments, the N-terminal signal sequence is derived from a secretory protein or a Type I transmembrane protein.

[0029] 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.

[0030] In some embodiments, the N-terminal signal sequence is not preceded at its N-terminus by any other tags.

[0031] In some embodiments, 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.

[0032] In some embodiments, the C-terminal targeting peptide sequence prevents secretion of a peptide expressed from the transgene according to any aspect of the present disclosure.

[0033] In some embodiments, the C-terminal targeting peptide sequence is a KDEL tetrapeptide Golgi retrieval sequence. Examples of such structures are shown in Figures 11 and 12.

[0034] In some embodiments the transgene comprises a humanized Sun1DN nucleic acid sequence or a humanized Sun2DN nucleic acid sequence. In a preferred embodiment, 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.

[0035] In some embodiments, 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.

[0036] In some embodiments, 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).

[0037] In some embodiments, the nucleic acid molecule of the present disclosure comprises an adeno-associated virus vector (AAV) comprising a chicken cardiac troponin T promoter (cTnT), a transgene according to any aspect of the present 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.

[0038] According to an embodiment an example of such a vector is shown in Figure 10 and comprises the nucleic acid sequence set forth in SEQ ID NO: 3.

[0039] In some embodiments, the nucleic acid molecule of the present disclosure comprises an adeno-associated virus vector (AAV) comprising a chicken cardiac troponin T promoter (cTnT), a transgene according to any aspect of the present 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.

[0040] According to an embodiment 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.

[0041] Rather than expressing components of a lumenal domain of a SUN domain-containing protein, a KASH domain may be expressed to disrupt a LING complex by competing with endogenous Nesprins (which comprise a KASH domain) for binding to SUN1 and SUN2 domains.

[0042] Accordingly, in some embodiments of the nucleic acid molecule of the present disclosure, the transgene comprises nucleic acid sequences for expressing a KASH domain, and an N-terminal stabiliser polypeptide sequence.

[0043] Preferably, the KASH domain comprises a transmembrane domain and a SUN-interacting peptide.

[0044] 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.

[0045] 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.

[0046] In some embodiments, 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)).

[0047] In preferred embodiments 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.

[0048] In some embodiments 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.

[0049] 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.

[0050] 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.

[0051] In some embodiments, the KASH domain does not comprise any extensions after the last C-terminal amino acid compared to a naturally occurring KASH domain.

[0052] In some embodiments, 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.

[0053] In some embodiments, the N-terminal stabilizer polypeptide sequence forms a discretely folded domain.

[0054] In some embodiments, 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.

[0055] In some embodiments, the N-terminal stabiliser polypeptide sequence is green fluorescent protein (GFP).

[0056] Rather than expressing components of a lumenal domain of a SUN domain-containing protein or a KASH domain to disrupt a LING 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 LING 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 LING complex binding partner.

[0057] 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 LING complex partners.

[0058] Accordingly, in some embodiments of the nucleic acid molecule of the present 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.

[0059] Data shown herein (Example 6) suggests that modification of the SUN2 domain or KASH2 domain does not ameliorate Lmna pathology.

[0060] In some embodiments 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.

[0061] In some embodiments, 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.

[0062] In some embodiments, 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.

[0063] In some embodiments, 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.

[0064] In some embodiments, 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.

[0065] In some embodiments, the transgene comprises nucleic acid sequences for expressing a CR!SPR-Cas9 or variant thereof.

[0066] In preferred embodiments, the transgene is a dominant negative construct.

[0067] In some embodiments, the transgene is a humanised transgene.

[0068] In some embodiments, 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.

[0069] In some embodiments, the AAV vector is formulated for delivery into the myocardium of a subject.

[0070] According to a second aspect of the present disclosure there is provided a nucleic acid molecule of any embodiment of the present disclosure for use in treating a disease caused by one or more *Lmna* mutations in a subject.

[0071] In some embodiments of the second aspect, 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.

Table 1

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17811	c.1771T>A	Substitution	p.Cys591Ser	Substitution	Tail	-	Acrogeria, Gottron Type	201200	#
18255	**c.418_438dupCTGCTGAACTCCAAGGAGGCC**	**Duplication**	**p.Leu140 Ala146dup**	**Duplication**	**1B**	-	**Arrhythmogenic cardiomyopathy**	-	-
17439	**c.1039G>A**	**Substitution**	**p.Glu347Lys**	**Substitution**	**2B**	**ARVD7**	**Arrhythmogenic right ventricular cardiomyopathy**	**609160**	%
18492	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**ARVD7**	**Arrhythmogenic right ventricular cardiomyopathy**	**609160**	%
9157	c.1718C>T	Substitution	p.Ser573Leu	Substitution	Tail	-	Arthropathy syndrome, autosomal recessive	-	-
18299	**c.1494G>A**	**Substitution**	**p.Trp498X**	**Substitution**	**Tail**	**AF**	**Atrial fibrillation**	-	-
18301	**c.175C>G**	**Substitution**	**p.Leu59Val**	**Substitution**	**1A**	**APS**	**Atypical progeroid syndrome**	**-**	**-**
8885	**c.169G>C**	**Substitution**	**p.Ala57Pro**	**Substitution**	**1A**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
8886	**c.398G>T**	**Substitution**	**p.Arg133Leu**	**Substitution**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
11462	**c.398G>T**	**Substitution**	**p.Arg133Leu**	**Substitution**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
9232	**c.398G>T**	**Substitution**	**p.Arg133Leu**	**Substitution**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
11509	**c.398G>T**	**Substitution**	**p.Arg133Leu**	**Substitution**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11670	**c.398G>T**	**Substitution**	**p.Arg133Leu**	**Substitution**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
8888	**c.419T>G**	**Substitution**	**p.Leu140Arg**	**Substitution**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
13457	**c.506delT**	**Deletion**	**p.Val169GlyfsX7**	**Frame shift**	**1B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
13350	**c.898G>A**	**Substitution**	**p.Asp300Asn**	**Substitution**	**2B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
17875	**c.898G>A**	**Substitution**	**p.Asp300Asn**	**Substitution**	**2B**	**WRN**	**Atypical Werner syndrome**	**277700**	**#**
11767	**c.1130G>T**	**Substitution**	**p.Arg377Leu**	**Substitution**	**2B**	**AD-SMA**	**Autosomal dominant spinal muscular dystrophy**	**182980**	**#**
11766	**c.1477C>T**	**Substitution**	**p.Gln493X**	**Substitution**	**Tail**	**AD-SMA**	**Autosomal dominant spinal muscular dystrophy**	**182980**	**#**
9205	**c.?**	**Unknown**	**p.Glu33Asp**	**Substitution**	**1A**	**-**	**Axonal neuropathy, muscular dystrophy, cardiac disease**	**-**	**-**
8994	**c.99G>T**	**Substitution**	**p.Glu33Asp**	**Substitution**	**1A**	**-**	**Axonal neuropathy, muscular dystrophy, cardiac disease, leuconychia**	**-**	**-**
12416	**c.1621C>T**	**Substitution**	**p.Arg541Cys**	**Substitution**	**Tail**	**-**	**Cardiac arrhythmia**	**-**	**-**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12399	**c.673C>T**	**Substitution**	**p.Arg225X**	**Substitution**	**L12**	**CCD**	**Cardiac conduction defect**	**115080**	**#**
17808	**c.695G>T**	**Substitution**	**p.Gly232Val**	**Substitution**	**L12**	**CCD**	**Cardiac conduction defect**	**115080**	**#**
13449	**c.799T>C**	**Substitution**	**p.Tyr267His**	**Substitution**	**2B**	**CCD**	**Cardiac conduction defect**	**115080**	**#**
14260	**c.178C>G**	**Substitution**	**p.Arg60Gly**	**Substitution**	**1A**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	**-**	**-**
14256	**c.184C>G**	**Substitution**	**p.Arg62Gly**	**Substitution**	**1A**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	-	-
14018	**c.497G>C**	**Substitution**	**p.Arg166Pro**	**Substitution**	**1B**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	-	-
14258	**c.575A>T**	**Substitution**	**p.Asp192Val**	**Substitution**	**1B**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	-	-
14007	**c.673C>T**	**Substitution**	**p.Arg225X**	**Substitution**	**L12**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	-	-
14015	**c.775T>C**	**Substitution**	**p.Tyr259His**	**Substitution**	**L2**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	**-**	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
14016	**c.775T>C**	**Substitution**	**p.Tyr259His**	**Substitution**	**L2**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	**-**	-
14011	**c.815_818delACAAinsCCAGAC**	**Indel**	**p.Asp272AlafsX208**	**Frame shift**	**2B**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	**-**	-
14012	**c.815_818delACAAinsCCAGAC**	**Indel**	**p.Asp272AlafsX208**	**Frame shift**	**2B**	**-**	**Cardiomyopathy with advanced AV block and arrhythmia**	**-**	-
11652	c.-3_12delGCCATGGAGACCCCG	Deletion	p.Met1_Pro4del	Deletion	Head	CMT2	Charcot-Marie-Tooth disease type 2	118210	#
13374	c.1496_1496delC	Deletion	p.Ala499ValfsX141	Frame shift	Tail	CMT2	Charcot-Marie-Tooth disease type 2	118210	#
17199	c.1910T>C	Substitution	p.Phe637Ser	Substitution	Tail	CMT2	Charcot-Marie-Tooth disease type 2	118210	#
8840	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
11415	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
11416	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8997	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
11840	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
11839	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
11838	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
11837	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
12087	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13414	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13415	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13416	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13417	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13418	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13419	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13420	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13421	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13422	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13423	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13424	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13425	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13426	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13427	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13428	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13429	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13430	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13431	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13432	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13433	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13434	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13435	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13436	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#
13437	c.892C>T	Substitution	p.Arg298Cys	Substitution	2B	CMT2B1	Charcot-Marie-Tooth disease type 2B1	605588	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17886	**c.80C>T**	**Substitution**	**p.Thr27Ile**	**Substitution**	**Head**	**CFTDM**	**Congenital** fiber **type disproportion**	**255310**	**#**
18472	**c.907T>C**	**Substitution**	**p.Ser303Pro**	**Substitution**	**2B**	**CFTDM**	**Congenital fiber type disproportion**	**255310**	**#**
18473	**c.907T>C**	**Substitution**	**p.Ser303Pro**	**Substitution**	**2B**	**CFTDM**	**Congenital fiber type disproportion**	**255310**	**#**
18474	**c.907T>C**	**Substitution**	**p.Ser303Pro**	**Substitution**	**2B**	**CFTDM**	**Congenital fiber type disproportion**	**255310**	**#**
18475	**c.907T>C**	**Substitution**	**p.Ser303Pro**	**Substitution**	**2B**	**CFTDM**	**Congenital fiber type disproportion**	**255310**	**#**
18144	**c.91G>A**	**Substitution**	**p.Glu31Lys**	**Substitution**	**Head**	**CMD**	**Congenital muscular dystrophy**	-	-
18148	**c.91_93delGAG**	**Deletion**	**p.Glu31X**	**Substitution**	**Head**	**CMD**	**Congenital muscular dystrophy**	-	-
17813	**c.93G>C**	**Substitution**	**p.Glu31Asp**	**Substitution**	**Head**	**CMD**	**Congenital muscular dystrophy**	-	-
18146	**c.94_96delAAG**	**Deletion**	**p.Lys32X**	**Substitution**	**1A**	**CMD**	**Congenital muscular dystrophy**	-	-
16402	**c.104T>C**	**Substitution**	**p.Leu35Pro**	**Substitution**	**1A**	**CMD**	**Congenital muscular dystrophy**	-	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
16287	**c.115A>T**	**Substitution**	**p.Asn39Tyr**	**Substitution**	**1A**	**CMD**	**Congenital muscular dystrophy**	-	-
18150	**c.117T>G**	**Substitution**	**p.Asn39Lys**	**Substitution**	**1A**	**CMD**	**Congenital muscular dystrophy**	-	-
18153	**c.143G>C**	**Substitution**	**p.Arg48Pro**	**Substitution**	**1A**	**CMD**	**Congenital muscular dystrophy**	-	-
18156	**c.422T>C**	**Substitution**	**p.Leu141Pro**	**Substitution**	**1B**	**CMD**	**Congenital muscular dystrophy**	-	-
13462	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**CMD**	**Congenital muscular dystrophy**	-	-
16404	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**CMD**	**Congenital muscular dystrophy**	-	-
17758	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**CMD**	**Congenital muscular dystrophy**	-	-
18158	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**CMD**	**Congenital muscular dystrophy**	-	-
11816	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**CMD**	**Congenital muscular dystrophy**	-	-
18161	**c.1117A>G**	**Substitution**	**p.Ile373Val**	**Substitution**	**2B**	**CMD**	**Congenital muscular dystrophy**	-	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
18166	c.1118T>A	Substitution	p.Ile373Asn	Substitution	2B	CMD	Congenital muscular dystrophy	-	-
13460	c.1139T>C	Substitution	p.Leu380Ser	Substitution	2B	CMD	Congenital muscular dystrophy	-	-
18169	c.1147G>A	Substitution	p.Glu383Lys	Substitution	2B	CMD	Congenital muscular dystrophy	-	-
18171	c.1147G>A	Substitution	p.Glu383Lys	Substitution	2B	CMD	Congenital muscular dystrophy	-	-
18163	c.1151A>G	Substitution	p.Glu384Gly	Substitution	2B	CMD	Congenital muscular dystrophy	-	-
11817	c.1162C>T	Substitution	p.Arg388Cys	Substitution	Tail	CMD	Congenital muscular dystrophy	-	-
17473	c.1330_1338dupGAGGTGGAT	Duplication	p.Glu444_Asp446dup	Duplication	Tail	CMD	Congenital muscular dystrophy	-	-
11818	c.1368_1370delCAA	Deletion	p.Asn456del	Deletion	Tail	CMD	Congenital muscular dystrophy	-	-
18238	c.1489-14_1489-7delTTTCTCCT	Deletion	p.?	Unknown	Unknown	CMD	Congenital muscular dystrophy	-	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
16951	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	T2D	DIABETES MELLITUS, NONINSULIN-DEPENDENT; NIDDM	125853	#
13171	**c.?**	**Unknown**	**p.Lys260Asn**	**Substitution**	**L2**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
8832	**c.16C>T**	**Substitution**	**p.Gln6X**	**Substitution**	**Head**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
9397	**c.28_29insA**	**Insertion**	**p.Thr10AsnfsX31**	**Frame shift**	**Head**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
11627	**c.31delC**	**Deletion**	**p.Arg11AlafsX85**	**Frame shift**	**Head**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
14254	**c.73C>G**	**Substitution**	**p.Arg25Gly**	**Substitution**	**Head**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
13155	**c.82C>T**	**Substitution**	**p.Arg28Trp**	**Substitution**	**Head**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
13563	**c.155T>C**	**Substitution**	**p.Leu52Pro**	**Substitution**	**1A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
13379	**c.176T>G**	**Substitution**	**p.Leu59Arg**	**Substitution**	**1A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13378	**c.176T>G**	**Substitution**	**p.Leu59Arg**	**Substitution**	**1A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8748	**c.178C>G**	**Substitution**	**p.Arg60Gly**	**Substitution**	**1A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13192	**c.203_208delAGGTGG**	**Deletion**	**p.Glu68_Val69del**	**Deletion**	**1A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17742	**c.232G>A**	**Substitution**	**p.Lys78Glu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9163	**c.244G>A**	**Substitution**	**p.Glu82Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16139	**c.244G>A**	**Substitution**	**p.Glu82Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8749	**c.254T>G**	**Substitution**	**p.Leu85Arg**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8865	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11618	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13080	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13157	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14030	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16966	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17777	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13613	**c.274C>T**	**Substitution**	**p.Leu92Phe**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8844	**c.289A>G**	**Substitution**	**p.Lys97Glu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11620	**c.289A>G**	**Substitution**	**p.Lys97Glu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13159	**c.289A>G**	**Substitution**	**p.Lys97Glu**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13082	**c.302G>C**	**Substitution**	**p.Arg101Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16954	**c.302G>C**	**Substitution**	**p.Arg101Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8843	c.331G>T	Substitution	p.Glu111X	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
11619	c.331G>T	Substitution	p.Glu111X	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13199	c.331G>T	Substitution	p.Glu111X	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
16935	c.348_349insG	Insertion	p.Lys117GlufsX10	Frame shift	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13089	c.357-1G>T	Substitution	p.?	Unknown	Unknown	CMD1A	Dilated cardiomyopathy 1A	115200	#
9148	c.394G>C	Substitution	p.Ala132Pro	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
17029	c.394G>C	Substitution	p.Ala132Pro	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
17028	c.394G>C	Substitution	p.Ala132Pro	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
9297	c.425_426insGGCACTGGAGGCTCTGCTGAA	Insertion	p.Leu141_Asn142insLysAspLeuAspAlaLeuLeu	Insertion	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
9010	c.427T>C	Substitution	p.Ser143Pro	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11473	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11474	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11475	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11476	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11477	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9150	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16162	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16163	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16164	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16165	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17038	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17037	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17036	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17024	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17022	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17021	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17020	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17019	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17018	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17023	**c.427T>C**	**Substitution**	**p.Ser143Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17661	**c.448A>T**	**Substitution**	**p.Thr150Ser**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8879	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13161	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13162	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13163	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13328	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13617	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13091	**c.497G>C**	**Substitution**	**p.Arg166Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16968	**c.497G>C**	**Substitution**	**p.Arg166Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16969	**c.497G>C**	**Substitution**	**p.Arg166Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13165	**c.548T>C**	**Substitution**	**p.Leu183Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
18267	**c.563T>G**	**Substitution**	**p.Leu188Arg**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16160	**c.565C>T**	**Substitution**	**p.Arg189Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8845	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9011	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11507	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9149	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11621	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11782	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11790	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13167	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16167	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17034	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17033	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17032	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17031	**c.568C>T**	**Substitution**	**p.Arg190Trp**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13093	**c.569G>A**	**Substitution**	**p.Arg190Gln**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13330	**c.569G>A**	**Substitution**	**p.Arg190Gln**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16976	**c.569G>A**	**Substitution**	**p.Arg190Gln**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9165	**c.575A>G**	**Substitution**	**p.Asp192Gly**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
14200	c.575A>G	Substitution	p.Asp192Gly	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
8750	c.585C>G	Substitution	p.Asn195Lys	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
12393	c.585C>A	Substitution	p.Asn195Lys	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
11668	c.607G>A	Substitution	p.Glu203Lys	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
16978	c.607G>A	Substitution	p.Glu203Lys	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
8751	c.608A>G	Substitution	p.Glu203Gly	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13332	c.608A>T	Substitution	p.Glu203Val	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13095	c.629T>G	Substitution	p.Ile210Ser	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
16971	c.629T>G	Substitution	p.Ile210Ser	Substitution	1B	CMD1A	Dilated cardiomyopathy 1A	115200	#
8901	c.640-10A>G	Substitution	p.?	Unknown	Unknown	CMD1A	Dilated cardiomyopathy 1A	115200	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8864	**c.644T>C**	**Substitution**	**p.Leu215Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16980	**c.644T>C**	**Substitution**	**p.Leu215Pro**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13169	**c.656A>C**	**Substitution**	**p.Lys219Thr**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13335	**c.656A>C**	**Substitution**	**p.Lys219Thr**	**Substitution**	**1B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11669	**c.673C>T**	**Substitution**	**p.Arg225X**	**Substitution**	**L12**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17607	**c.673C>T**	**Substitution**	**p.Arg225X**	**Substitution**	**L12**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17609	**c.673C>T**	**Substitution**	**p.Arg225X**	**Substitution**	**L12**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13097	**c.700C>T**	**Substitution**	**p.Gln234X**	**Substitution**	**L12**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13201	**c.736C>T**	**Substitution**	**p.Gln246X**	**Substitution**	**2A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17786	**c.736C>T**	**Substitution**	**p.Gln246X**	**Substitution**	**2A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17779	**c.767T>G**	**Substitution**	**p.Val256Gly**	**Substitution**	**2A**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11809	**c.800A>G**	**Substitution**	**p.Tyr267Cys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13173	**c.800A>G**	**Substitution**	**p.Tyr267Cys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11810	**c.855delG**	**Deletion**	**p.Ala287LeufsX191**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11613	**c.908_909delCT**	**Deletion**	**p.Ser303CysfsX26**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
12384	**c.908_909delCT**	**Deletion**	**p.Ser303CysfsX26**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13206	**c.936+1G>T**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16805	**c.937-11C>G**	**Substitution**	**p.Leu313GlyfsX31**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8846	**c.949G>A**	**Substitution**	**p.Glu317Lys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11617	**c.949G>A**	**Substitution**	**p.Glu317Lys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13175	**c.949G>A**	**Substitution**	**p.Glu317Lys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13176	**c.949G>A**	**Substitution**	**p.Glu317Lys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13621	**c.949G>A**	**Substitution**	**p.Glu317Lys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13100	**c.952G>A**	**Substitution**	**p.Ala318Thr**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16956	**c.952G>A**	**Substitution**	**p.Ala318Thr**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11700	**c.959delT**	**Deletion**	**p.Leu320fsX160**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9409	**c.959delT**	**Deletion**	**p.Leu320fsX160**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9391	**c.959delT**	**Deletion**	**p.Leu320fsX160**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14032	**c.959delT**	**Deletion**	**p.Leu320fsX160**	**Frame shift**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
12389	**c.961C>T**	**Substitution**	**p.Arg321X**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
14077	**c.961C>T**	**Substitution**	**p.Arg321X**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14093	**c.961C>T**	**Substitution**	**p.Arg321X**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17737	**c.961C>T**	**Substitution**	**p.Arg321X**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17738	**c.961C>T**	**Substitution**	**p.Arg321X**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9155	**c.976T>A**	**Substitution**	**p.Ser326Thr**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11760	**c.[992G>A; =]+[=; 1039G>A]**	**Substitution**	**p.[Arg331Glu; =]+[=; Glu347Lys]**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11811	**c.992G>C**	**Substitution**	**p.Arg331Pro**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14075	**c.992G>A**	**Substitution**	**p.Arg331Gln**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17477	**c.1003C>T**	**Substitution**	**p.Arg335Trp**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
18486	**c.1039G>A**	**Substitution**	**p.Glu347Lys**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9012	c.1046G>T	Substitution	p.Arg349Leu	Substitution	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13561	c.1048G>C	Substitution	p.Ala350Pro	Substitution	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
14204	c.1057C>A	Substitution	p.Gln353Lys	Substitution	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
14072	c.1063C>T	Substitution	p.Gln355X	Substitution	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
17475	c.1070A>C	Substitution	p.Asp357Ala	Substitution	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13116	c.1072G>T	Substitution	p.Glu358X	Substitution	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
9152	c.1085_1085delT	Deletion	p.Leu363TrpfsX117	Frame shift	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
17026	c.1085_1085delT	Deletion	p.Leu362TrpfsX117	Frame shift	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
16157	c.1102_1130dupGCCCTGGACATGGAGATCCACGCCTACCG	Duplication	p.Lys378ProfsX112	Frame shift	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13102	c.1114delG	Deletion	p.Glu372ArgfsX107	Frame shift	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8866	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8869	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8880	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9160	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9162	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
12330	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13178	**c.1130G>T**	**Substitution**	**p.Arg377Leu**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13623	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14034	**c.1130G>A**	**Substitution**	**p.Arg377His**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17664	**c.1150G>T**	**Substitution**	**p.Glu384X**	**Substitution**	**2B**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17482	c.1157+1G>T	Substitution	p.Arg386SerfsX21	Frame shift	2B	CMD1A	Dilated cardiomyopathy 1A	115200	#
13104	c.1163G>A	Substitution	p.Arg388His	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
16958	c.1163G>A	Substitution	p.Arg388His	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
13106	c.1195C>T	Substitution	p.Arg399Cys	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
16960	c.1195C>T	Substitution	p.Arg399Cys	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
17782	c.1197_1240delTGGCCGTGCTTCCTCTCACTCATCCCAGACACAGGGTGGGGGCA	Deletion	p.Gly400ArgfsX11	Frame shift	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
17789	c.1292C>G	Substitution	p.Ser431X	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
14082	c.1294C>T	Substitution	p.Gln432X	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#
14084	c.1294C>T	Substitution	p.Gln432X	Substitution	Tail	CMD1A	Dilated cardiomyopathy 1A	115200	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
14085	**c.1294C>T**	**Substitution**	**p.Gln432X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14086	**c.1294C>T**	**Substitution**	**p.Gln432X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13108	**c.1307_1308insGCAC**	**Insertion**	**p.Ser437HisfsX1**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16962	**c.1307_1308insGCAC**	**Insertion**	**p.Ser437HisfsX1**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14088	**c.1318G>A**	**Substitution**	**p.Val440Met**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14090	**c.1318G>A**	**Substitution**	**p.Val440Met**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14091	**c.1318G>A**	**Substitution**	**p.Val440Met**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11812	**c.1370delA**	**Deletion**	**p.Lys457SerfsX21**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
12397	**c.1380+1G>A**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9413	**c.1397_1397delA**	**Deletion**	**p.Asn466IlefsX14**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9161	**c.1397_1397delA**	**Deletion**	**p.Asn466IlefsX14**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13110	**c.1412G>A**	**Substitution**	**p.Arg471His**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16973	**c.1412G>A**	**Substitution**	**p.Arg471His**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13112	**c.1424_1425insAGA**	**Insertion**	**p.Gly474_Asp475insGlu**	**Insertion**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11508	**c.1443C>G**	**Substitution**	**p.Tyr481X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17791	**c.1443C>G**	**Substitution**	**p.Tyr481X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17480	**c.1489-1G>T**	**Substitution**	**p.Ile497_Glu536del**	**Deletion**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13180	**c.1492T>A**	**Substitution**	**p.Trp498Arg**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9153	**c.1493_1493delG**	**Deletion**	**p.Ala499LeufsX47**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
12395	**c.1512_1513insAG**	**Insertion**	**p.Thr505ArgfsX44**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17784	**c.1526_1527insC**	**Insertion**	**p.Thr510TyrfsX42**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17797	**c.1526_1527insA**	**Insertion**	**p.Thr510TyrfsX42**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17486	**c.1549C>T**	**Substitution**	**p.Gln517X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17484	**c.1560G>A**	**Substitution**	**p.Trp520X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13625	**c.1567G>A**	**Substitution**	**p.Gly523Arg**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11624	**c.1579_1580insCTGC**	**Insertion**	**p.Arg527ProfsX26**	**Frame shift**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17558	**c.1583C>T**	**Substitution**	**p.Thr528Met**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9307	**c.1621C>T**	**Substitution**	**p.Arg541Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9283	**c.1621C>A**	**Substitution**	**p.Arg541Ser**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9151	**c.1621C>A**	**Substitution**	**p.Arg541Ser**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13592	**c.1621C>T**	**Substitution**	**p.Arg541Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14202	**c.1621C>A**	**Substitution**	**p.Arg541Ser**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
16472	**c.1621C>G**	**Substitution**	**p.Arg541Gly**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17774	**c.1621C>T**	**Substitution**	**p.Arg541Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
17775	**c.1621C>T**	**Substitution**	**p.Arg541Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13559	**c.1622G>A**	**Substitution**	**p.Arg541His**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9392	**c.1718C>T**	**Substitution**	**p.Ser573Leu**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13182	**c.1718C>T**	**Substitution**	**p.Ser573Leu**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13183	**c.1718C>T**	**Substitution**	**p.Ser573Leu**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14038	**c.1718C>T**	**Substitution**	**p.Ser573Leu**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17667	**c.1879C>T**	**Substitution**	**p.Arg624Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13565	**c.1904G>A**	**Substitution**	**p.Gly635Asp**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
8833	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
9018	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11500	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11501	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
11502	**c.1930C>A**	**Substitution**	**p.Arg644His**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13185	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
13337	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**
14080	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13114	**c.1960C>T**	**Substitution**	**p.Arg654X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
16964	**c.1960C>T**	**Substitution**	**p.Arg654X**	**Substitution**	**Tail**	**CMD1A**	**Dilated cardiomyopathy 1A**	**115200**	#
11788	**c.?**	**Unknown**	**p.Tyr481X**	**Substitution**	**Tail**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	**-**	-
17886	**c.80C>T**	**Substitution**	**p.Thr27Ile**	**Substitution**	**Head**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	**-**	-
13486	**c.106C>T**	**Substitution**	**p.Gln36X**	**Substitution**	**1A**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	**-**	-
11792	**c.158A>T**	**Substitution**	**p.Glu53Val**	**Substitution**	**1A**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
18301	**c.175C>G**	**Substitution**	**p.Leu59Val**	**Substitution**	**1A**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
11791	**c.481G>A**	**Substitution**	**p.Glu161Lys**	**Substitution**	**1B**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13204	c.514-1G>A	Substitution	p.?	Unknown	Unknown	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
11793	c.556G>A	Substitution	p.Glu186Lys	Substitution	1B	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
11789	c.568C>T	Substitution	p.Arg190Trp	Substitution	1B	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
11787	c.575A>G	Substitution	p.Asp192Gly	Substitution	1B	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
18470	c.683A>T	Substitution	p.Glu228Val	Substitution	L12	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
17868	c.871G>A	Substitution	p.Glu291Lys	Substitution	2B	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
17870	c.949G>A	Substitution	p.Glu317Lys	Substitution	2B	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-
13078	c.1069G>C	Substitution	p.Asp357His	Substitution	2B	DCM-CD	Dilated cardiomyopathy with conduction system defects	-	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13208	**c.1157+1G>A**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
18490	**c.1412G>A**	**Substitution**	**p.Arg471His**	**Substitution**	**Tail**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
11814	**c.1526_1527insC**	**Insertion**	**p.Thr510TyrfsX42**	**Frame shift**	**Tail**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
9008	**c.1621C>T**	**Substitution**	**p.Arg541Cys**	**Substitution**	**Tail**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
17578	**c.1711C>A**	**Substitution**	**p.=**	**Silent**	**Not**	**DCM-CD affected**	**Dilated cardiomyopathy with conduction system defects**	-	-
17880	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**DCM-CD**	**Dilated cardiomyopathy with conduction system defects**	-	-
18305	c.1774G>A	Substitution	p.Gly592Arg	Substitution	Tail	DAPJ	Distal acroosteolysis, poikiloderma and joint stiffness	-	-
13581	c.1 930C>T	Substitution	p.Arg644Cys	Substitution	Tail	-	Distal motor neuropathy	-	-

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17813	c.93G>C	Substitution	p.Glu31Asp	Substitution	Head	-	Dropped head syndrome	-	-
9020	c.94_96delAAG	Deletion	p.Lys32del	Deletion	1A	-	Dropped head syndrome	-	-
13476	c.116A>G	Substitution	p.Asn39Ser	Substitution	1A	-	Dropped head syndrome	-	-
13466	c.149G>C	Substitution	p.Arg50Pro	Substitution	1A	-	Dropped head syndrome	-	-
13464	c.745C>T	Substitution	p.Arg249Trp	Substitution	2A	-	Dropped head syndrome	-	-
13470	c.905T>C	Substitution	p.Leu302Pro	Substitution	2B	-	Dropped head syndrome	-	-
13468	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	-	Dropped head syndrome	-	-
13482	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	-	Dropped head syndrome	-	-
13484	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	-	Dropped head syndrome	-	-
13474	c.1358G>C	Substitution	p.Arg453Pro	Substitution	Tail	-	Dropped head syndrome	-	-
13472	c.1364G>C	Substitution	p.Arg455Pro	Substitution	Tail	-	Dropped head syndrome	-	-
13478	c.1366A>G	Substitution	p.Asn456Asp	Substitution	Tail	-	Dropped head syndrome	-	-
13480	c.1381-2A>G	Substitution	p.?	Unknown	Unknown	-	Dropped head syndrome	-	-

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11652	**c.-3_12delGCCATGGAGACCCCG**	**Deletion**	**p.Met1_Pro4del**	**Deletion**	**Head**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8743	**c.16C>T**	**Substitution**	**p.Gln6X**	**Substitution**	**Head**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8796	**c.16C>T**	**Substitution**	**p.Gln6X**	**Substitution**	**Head**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11746	**c.31delC**	**Deletion**	**p.Arg11AlafsX85**	**Frame shift**	**Head**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9262	**c.73C>G**	**Substitution**	**p.Arg25Gly**	**Substitution**	**Head**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9376	**c.74G>C**	**Substitution**	**p.Arg25Pro**	**Substitution**	**Head**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9177	**c.94_96delAAG**	**Deletion**	**p.Lys32del**	**Deletion**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8998	**c.94_96delAAG**	**Deletion**	**p.Lys32del**	**Deletion**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9014	**c.94_96delAAG**	**Deletion**	**p.Lys32del**	**Deletion**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13594	**c.98A>G**	**Substitution**	**p.Glu33Gly**	**Substitution**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11747	**c.99G>C**	**Substitution**	**p.Glu33Asp**	**Substitution**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8999	**c.103C>G**	**Substitution**	**p.Leu35Val**	**Substitution**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11748	c.116A>G	Substitution	p.Asn39Ser	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16329	c.116A>G	Substitution	p.Asn39Ser	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
18174	c.116A>G	Substitution	p.Asn39Ser	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9377	c.127G>A	Substitution	p.Ala43Thr	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8797	c.134A>G	Substitution	p.Tyr45Cys	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16331	c.134A>G	Substitution	p.Tyr45Cys	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13605	c.136A>G	Substitution	p.Ile46Val	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13545	c.139G>C	Substitution	p.Asp47His	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9378	c.148C>A	Substitution	p.Arg50Ser	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8798	c.149G>C	Substitution	p.Arg50Pro	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8799	c.188T>G	Substitution	p.Ile63Ser	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9175	c.188T>A	Substitution	p.Ile63Asn	Substitution	1A	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11633	**c.188T>A**	**Substitution**	**p.Ile63Asn**	**Substitution**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13192	**c.203_208delAGGTGG**	**Deletion**	**p.Glu68_Val69del**	**Deletion**	**1A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
12477	**c.265C>T**	**Substitution**	**p.Arg89Cys**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13596	**c.265C>T**	**Substitution**	**p.Arg89Cys**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
17193	**c.266G>T**	**Substitution**	**p.Arg89Leu**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9410	**c.334_336delGAG**	**Deletion**	**p.Glu112del**	**Deletion**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9000	c.334_336delGAG	Deletion	p.Glu112del	Deletion	1B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16291	c.357C>T	Substitution	p.=	Silent	Not affected	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
17182	c.367_369delAAG	Deletion	p.Lys123del	Deletion	1B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9379	c.398G>C	Substitution	p.Arg133Pro	Substitution	1B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11632	c.419T>C	Substitution	p.Leu140Pro	Substitution	1B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9015	c.428C>T	Substitution	p.Ser143Phe	Substitution	1B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12609	**c.428C>T**	**Substitution**	**p.Ser143Phe**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8795	**c.448A>C**	**Substitution**	**p.Thr150Pro**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16334	**c.448A>C**	**Substitution**	**p.Thr150Pro**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13453	**c.485T>C**	**Substitution**	**p.Leu162Pro**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16396	**c.566_567delGGinsCC**	**Indel**	**p.Arg189Pro**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16299	**c.568_570dupCGG**	**Duplication**	**p.Arg190dup**	**Duplication**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9380	**c.588_596delGCTGCAGAC**	**Deletion**	**p.Arg196_Thr199delinsSer**	**Indel**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16301	**c.618C>G**	**Substitution**	**p.Phe206Leu**	**Substitution**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
12382	**c.625delA**	**Deletion**	**p.Asn209ThrfsX271**	**Frame shift**	**1B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8800	**c.665A>C**	**Substitution**	**p.His222Pro**	**Substitution**	**L12**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13547	**c.694G>C**	**Substitution**	**p.Gly232Arg**	**Substitution**	**L12**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8801	**c.695G>A**	**Substitution**	**p.Gly232Glu**	**Substitution**	**L12**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12485	**c.695G>A**	**Substitution**	**p.Gly232Glu**	**Substitution**	**L12**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9001	**c.743T>C**	**Substitution**	**p.Leu248Pro**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16336	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16337	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16338	**c.745C>T**	**Substitution**	**p.Arg249Trp**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16342	**c.[745C>T; 1930C>T]**	**Substitution**	**p.[Arg249Trp; Arg644Cys]**	**Substitution**	**2A, Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8783	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11380	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11381	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8802	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9002	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9023	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11631	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11749	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11750	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13552	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16344	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16345	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
18176	**c.746G>A**	**Substitution**	**p.Arg249Gln**	**Substitution**	**2A**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11751	**c.775T>G**	**Substitution**	**p.Tyr259Asp**	**Substitution**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9640	**c.781_783delAAG**	**Deletion**	**p.Lys261del**	**Deletion**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9411	**c.781_783delAAG**	**Deletion**	**p.Lys261del**	**Deletion**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9381	**c.781_783delAAG**	**Deletion**	**p.Lys261del**	**Deletion**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13584	**c.781_783delAAGinsGTGGAGCAGTATAAGAAA**	**Indel**	**p.Lys261delinsValGluGlnTyrLysLys**	**Indel**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
16149	**c.788T>C**	**Substitution**	**p.Leu263Pro**	**Substitution**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
17403	**c.788T>C**	**Substitution**	**p.Leu263Pro**	**Substitution**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
17751	**c.788T>C**	**Substitution**	**p.Leu263Pro**	**Substitution**	**L2**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
17405	**c.799T>C**	**Substitution**	**p.Tyr267His**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9003	**c.800A>G**	**Substitution**	**p.Tyr267Cys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11809	**c.800A>G**	**Substitution**	**p.Tyr267Cys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13173	c.800A>G	Substitution	p.Tyr267Cys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16303	c.802T>C	Substitution	p.Ser268Pro	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16305	c.810G>A	Substitution	p.=	Silent	Not affected	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16307	c.810G>A	Substitution	p.=	Silent	Not affected	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16309	c.810+1G>A	Substitution	p.?	Unknown	Unknown	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16347	c.812T>C	Substitution	p.Leu271Pro	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
18186	**c.832G>C**	**Substitution**	**p.Ala278Pro**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8803	**c.881A>C**	**Substitution**	**p.Gln294Pro**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16349	**c.881A>C**	**Substitution**	**p.Gln294Pro**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16311	**c.883T>C**	**Substitution**	**p.Ser295Pro**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8863	**c.907T>C**	**Substitution**	**p.Ser303Pro**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16351	**c.907T>C**	**Substitution**	**p.Ser303Pro**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8786	**c.1007G>A**	**Substitution**	**p.Arg336Gln**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16314	**c.1064_1066delAGC**	**Deletion**	**p.Gln355del**	**Deletion**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8804	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11388	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11816	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9167	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11478	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11479	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11480	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11752	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
12479	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
12483	**c.1072G>A**	**Substitution**	**p.Glu358Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13598	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13603	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16353	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16354	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16355	c.1072G>A	Substitution	p.Glu358Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16316	c.1081G>A	Substitution	p.Glu361Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8806	c.1112T>A	Substitution	p.Met371Lys	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
18184	c.1124C>G	Substitution	p.Ala375Gly	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11634	c.1130G>T	Substitution	p.Arg377Leu	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11693	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
12405	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
18223	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12474	**c.1142A>C**	**Substitution**	**p.Glu381Ala**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11629	**c.1157G>A**	**Substitution**	**p.Arg386Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13653	**c.1157G>T**	**Substitution**	**p.Arg386Met**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16357	**c.1157G>A**	**Substitution**	**p.Arg386Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16358	**c.1157G>A**	**Substitution**	**p.Arg386Lys**	**Substitution**	**2B**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16319	**c.1158-2A>G**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11817	c.1162C>T	Substitution	p.Arg388Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9281	c.1187A>G	Substitution	p.Gln396Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8862	c.1201C>T	Substitution	p.Arg401Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9176	c.1201C>T	Substitution	p.Arg401Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9302	c.1201C>T	Substitution	p.Arg401Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9004	c.1337A>T	Substitution	p.Asp446Val	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
16321	**c.1346G>A**	**Substitution**	**p.Gly449Asp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
18192	**c.1346G>T**	**Substitution**	**p.Gly449Val**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8744	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8787	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11382	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11383	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11384	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8807	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11389	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11390	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8836	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9005	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9304	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11614	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11753	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
12481	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13590	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13607	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13609	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13611	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16363	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16364	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16365	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16366	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
16367	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16368	**c.1357C>T**	**Substitution**	**p.Arg453Trp**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16323	**c.1361T>C**	**Substitution**	**p.Leu454Pro**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
9513	**c.1367A>T**	**Substitution**	**p.Asn456Ile**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
8810	**c.1368C>A**	**Substitution**	**p.Asn456Lys**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
11818	**c.1368_1370delCAA**	**Deletion**	**p.Asn456del**	**Deletion**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17446	c.1368_1370delCAA	Deletion	p.Asn456del	Deletion	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
17447	c.1368_1370delCAA	Deletion	p.Asn456del	Deletion	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16370	c.1381-2A>G	Substitution	p.?	Unknown	Unknown	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16372	c.[1381-1G>T; 1381G>T]	Substitution	p.[?; Asp461 Tyr]	Unknown, Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16325	c.1399T>C	Substitution	p.Trp467Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8791	c.1406T>C	Substitution	p.Ile469Thr	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
18194	**c.1466T>C**	**Substitution**	**p.Leu489Pro**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16327	**c.1488+1G>A**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
13180	**c.1492T>A**	**Substitution**	**p.Trp498Arg**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
16375	**c.1526dupC**	**Duplication**	**p.Thr510TyrfsX42**	**Frame shift**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
18188	**c.1540T>A**	**Substitution**	**p.Trp514Arg**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**
18190	**c.1540T>A**	**Substitution**	**p.Trp514Arg**	**Substitution**	**Tail**	**EDMD2**	**Emery-Dreifuss muscular dystrophy, autosomal dominant**	**181350**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12434	c.1558T>G	Substitution	p.Trp520Gly	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
18179	c.1558T>C	Substitution	p.Trp520Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8811	c.1559G>C	Substitution	p.Trp520Ser	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8745	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11344	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8792	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8812	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11391	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8838	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8859	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11423	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11630	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11754	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16377	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
18182	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8793	c.1583C>A	Substitution	p.Thr528Lys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8814	c.1583C>A	Substitution	p.Thr528Lys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
11392	c.1583C>A	Substitution	p.Thr528Lys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9006	c.1583C>G	Substitution	p.Thr528Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16379	c.1583C>A	Substitution	p.Thr528Lys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16381	c.1583C>G	Substitution	p.Thr528Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16382	c.1583C>G	Substitution	p.Thr528Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16407	c.1583C>G	Substitution	p.Thr528Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
18196	c.1583C>G	Substitution	p.Thr528Arg	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
18297	c.1588C>T	Substitution	p.Leu530Phe	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
8747	c.1589T>C	Substitution	p.Leu530Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16384	c.1621C>A	Substitution	p.Arg541Ser	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9007	c.1622G>A	Substitution	p.Arg541His	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16387	c.1622G>C	Substitution	p.Arg541 Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13588	c.1633C>T	Substitution	p.Arg545Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
16389	c.1804G>A	Substitution	p.Arg541 Pro	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9383	c.1871G>A	Substitution	p.Arg624His	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9154	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13570	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13571	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
13572	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13573	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
16392	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	EDMD2	Emery-Dreifuss muscular dystrophy, autosomal dominant	181350	#
9523	c.664C>T	Substitution	p.His222Tyr	Substitution	L12	EDMD3	Emery-Dreifuss muscular dystrophy, autosomal recessive	604929	#
17407	c.674G>A	Substitution	p.Arg225Gln	Substitution	L12	EDMD3	Emery-Dreifuss muscular dystrophy, autosomal recessive	604929	#
17573	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	EDMD3	Emery-Dreifuss muscular dystrophy, autosomal recessive	604929	#
13319	c.1580G>C	Substitution	p.Arg527Pro	Substitution	Tail	EDMD3	Emery-Dreifuss muscular dystrophy, autosomal recessive	604929	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
18345	c.?	Unknown	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8847	c.82C>T	Substitution	p.Arg28Trp	Substitution	Head	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
18291	c.139G>A	Substitution	p.Asp47Asn	Substitution	1A	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8861	c.178C>G	Substitution	p.Arg60Gly	Substitution	1A	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
14260	c.178C>G	Substitution	p.Arg60Gly	Substitution	1A	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8848	c.184C>G	Substitution	p.Arg62Gly	Substitution	1A	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
14256	c.184C>G	Substitution	p.Arg62Gly	Substitution	1A	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12100	c.398G>T	Substitution	p.Arg133Leu	Substitution	1B	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
14258	c.575A>T	Substitution	p.Asp192Val	Substitution	1B	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17624	c.667G>A	Substitution	p.Glu223Lys	Substitution	L12	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11775	c.688G>A	Substitution	p.Asp230Asn	Substitution	L12	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11776	c.1195C>T	Substitution	p.Arg399Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17066	c.1232G>A	Substitution	p.Gly411Asp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12096	c.1315C>T	Substitution	p.Arg439Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17208	c.1315C>T	Substitution	p.Arg439Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11542	c.[1318G>A; =]+[=; 1445G>A]	Substitution	p.[Val440Met; =]+[=; Arg482Gln]	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8781	c.1394G>A	Substitution	p.Gly465Asp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12601	c.1411C>G	Substitution	p.Arg471Gly	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8754	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11358	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11359	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11360	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11361	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11362	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11363	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11364	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11365	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8773	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11374	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11375	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11376	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11377	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11378	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11379	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8816	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11393	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11394	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11395	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11396	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11397	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8834	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
9213	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
9156	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11543	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11544	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11662	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11692	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11699	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12101	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12102	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12103	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12104	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12105	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
14066	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16433	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16434	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16682	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17866	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17195	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17464	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17465	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17466	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17467	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17733	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17735	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17744	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17936	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
18484	c.1444C>T	Substitution	p.Arg482Trp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8753	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11354	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11355	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11356	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11357	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8763	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8764	c.1445G>T	Substitution	p.Arg482Leu	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8768	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11370	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11371	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11372	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11373	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8822	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8868	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
9305	c.1445G>T	Substitution	p.Arg482Leu	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11663	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11667	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12098	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12387	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Unknown	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12418	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Unknown	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12423	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
13150	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13367	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16285	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16436	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16437	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16438	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16439	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16440	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
18138	c.1445G>A	Substitution	p.Arg482Gln	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8765	c.1458G>C	Substitution	p.Lys486Asn	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11366	c.1458G>C	Substitution	p.Lys486Asn	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8767	c.1458G>T	Substitution	p.Lys486Asn	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
9319	c.1488+5G>C	Substitution	p.Ile497_Met664delinsValThrGlyArgAlaLeuGlyThrLeuGlyArgProTrpValAlaMetGlyAlaLeuGly X	Indel	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17455	c.1683G>C	Substitution	p.=	Silent	Not affected	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11777	c.1718C>T	Substitution	p.Ser573Leu	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
8780	c.1745G>A	Substitution	p.Arg582His	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16429	c.1745G>A	Substitution	p.Arg582His	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16430	c.1745G>A	Substitution	p.Arg582His	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
16431	c.1745G>A	Substitution	p.Arg582His	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
9452	c.1751G>A	Substitution	p.Arg584His	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
11545	c.1751G>A	Substitution	p.Arg584His	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12429	c.1 772G>T	Substitution	p.Arg156Cys	Substitution	1B	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17068	c.1892G>A	Substitution	p.Gly631Asp	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
13575	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
13576	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
13579	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
17461	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	FPLD2	Familial partial lipodystrophy (Dunnigan type)	151660	#
12601	c.1411C>G	Substitution	p.Arg471Gly	Substitution	Tail	FPLD1	Familial partial lipodystrophy (Köbberling)	608600	%
11482	c.[1583C>T; =]+[=; 1748C>T]	Substitution	p.[Thr528Met; =]+[=; Ser583Leu]	Substitution	Tail	FPLD1	Familial partial lipodystrophy (Köbberling)	608600	%
9183	c.1748C>T	Substitution	p.Ser583Leu	Substitution	Tail	FPLD1	Familial partial lipodystrophy (Köbberling)	608600	%
11481	c.1748C>T	Substitution	p.Ser583Leu	Substitution	Tail	FPLD1	Familial partial lipodystrophy (Köbberling)	608600	%

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13490	c.29C>T	Substitution	p.Thr10Ile	Substitution	Head	-	Generalized lipoatrophy syndrome	-	-
8867	c.398G>T	Substitution	p.Arg133Leu	Substitution	1B	-	Generalized lipoatrophy syndrome	-	-
17762	c.1609-12T>G	Substitution	p.Glu536fsX14	Frame shift	Tail	HSS	Hallermann-Streiff syndrome	234100	%
17260	c.1930C>T	Substitution	p.Arg644Cys	Substitution	Tail	HSS	Hallermann-Streiff syndrome	234100	%
13153	c.1609-12T>G	Substitution	p.Glu536fsX14	Frame shift	Tail	-	Heart-hand syndrome, Slovenian Type	610140	%
14097	**c.11C>G**	**Substitution**	**p.Pro4Arg**	**Substitution**	**Head**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14112	**c.11C>G**	**Substitution**	**p.Pro4Arg**	**Substitution**	**Head**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9172	**c.29C>T**	**Substitution**	**p.Thr10Ile**	**Substitution**	**Head**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14108	**c.29C>T**	**Substitution**	**p.Thr10Ile**	**Substitution**	**Head**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14110	**c.29C>T**	**Substitution**	**p.Thr10Ile**	**Substitution**	**Head**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
14101	**c.331G>T**	**Substitution**	**p.Glu111Lys**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14095	**c.406G>C**	**Substitution**	**p.Asp136His**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
17070	**c.412G>A**	**Substitution**	**p.Glu138Lys**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9016	**c.428C>T**	**Substitution**	**p.Ser143Phe**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
12609	**c.428C>T**	**Substitution**	**p.Ser143Phe**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
8871	**c.433G>A**	**Substitution**	**p.Glu145Lys**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14106	**c.475G>A**	**Substitution**	**p.Glu159Lys**	**Substitution**	**1B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
17626	**c.899A>G**	**Substitution**	**p.Asp300Gly**	**Substitution**	**2B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
17903	**c.917T>G**	**Substitution**	**p.Leu306Arg**	**Substitution**	**2B**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14114	**c.1303C>T**	**Substitution**	**p.Arg435Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
9393	**c.1411C>T**	**Substitution**	**p.Arg471Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
12615	**c.1411C>T**	**Substitution**	**p.Arg471Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9394	**c.1579C>T**	**Substitution**	**p.Arg527Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
13119	**c.1579C>T**	**Substitution**	**p.Arg527Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
13651	**c.1579C>T**	**Substitution**	**p.Arg527Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11671	**c.[1583C>T; =]+[=; 1619T>C]**	**Substitution**	**p.[Thr528Met; =]+[=; Met540Thr]**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9013	**c.1626G>C**	**Substitution**	**p.Lys542Asn**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9173	**c.1733A>T**	**Substitution**	**p.Glu578Val**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14099	**c.1762T>C**	**Substitution**	**p.Cys588Arg**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
14104	**c.1762T>C**	**Substitution**	**p.Cys588Arg**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11785	**c.1821G>A**	**Substitution**	**p.=**	**Silent**	**Not affected**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9527	**c.1822G>A**	**Substitution**	**p.Gly608Ser**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
8876	**c.1822G>A**	**Substitution**	**p.Gly608Ser**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9396	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9398	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11428	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11429	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11430	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11431	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11432	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11433	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11434	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11435	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11436	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11437	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11438	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11439	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11440	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11441	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
11442	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11443	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11444	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9395	**c.1824C>T**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11449	**c.1824C>T**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11450	**c.1824C>T**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11451	**c.1824C>T**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11452	**c.1824C>T**	**Substitution**	**p.?**	**Unknown**	**Unknown**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9171	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9017	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9019	**c.1824C>T**	**Substitution**	**p.[=, Val607_Gln656del]**	**Silent, Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
12074	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
12075	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
12076	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
12085	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
12354	c.1824C>T	Substitution	p.=	Silent	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
13533	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
17393	c.1824C>T	Substitution	p.=	Silent	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
17395	c.1824C>T	Substitution	p.=	Silent	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
17564	c.1824C>T	Substitution	p.=	Silent	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#
17729	c.1824C>T	Substitution	p.=	Silent	Not affected	HGPS	Hutchinson-Gilford progeria syndrome	176670	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17818	**c.1824C>T**	**Substitution**	**p.=**	**Silent**	**Not affected**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9009	**c.1868C>G**	**Substitution**	**p.[Thr623Ser, Val622_Gln656del]**	**Deletion, Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
12457	**c.1868C>G**	**Substitution**	**p.[Thr623Ser, Val622_Gln656del]**	**Deletion, Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
9174	**c.1930C>T**	**Substitution**	**p.Arg644Cys**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11615	**c.1960C>T**	**Substitution**	**p.Arg654X**	**Substitution**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
17159	**c.1968G>A**	**Substitution**	**p.=**	**Silent**	**Not affected**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
11784	**c.1968+1G>A**	**Substitution**	**p.Val607-_Gln656del**	**Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
17161	**c.1968+5G>A**	**Substitution**	**p.Val607_Gln656del**	**Deletion**	**Tail**	**HGPS**	**Hutchinson-Gilford progeria syndrome**	**176670**	**#**
12615	**c.1411C>T**	**Substitution**	**p.Arg471Cys**	**Substitution**	**Tail**	**-**	**Lamin-related rigid spine muscular dystrophy**	**-**	**-**
17426	**c.31delC**	**Deletion**	**p.Arg11AlafsX85**	**Frame shift**	**Head**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
14254	**c.73C>G**	**Substitution**	**p.Arg25Gly**	**Substitution**	**Head**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17886	**c.80C>T**	**Substitution**	**p.Thr27Ile**	**Substitution**	**Head**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
18488	**c.80C>T**	**Substitution**	**p.Thr27Ile**	**Substitution**	**Head**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17428	**c.99G>C**	**Substitution**	**p.Glu33Asp**	**Substitution**	**1A**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17429	**c.99G>C**	**Substitution**	**p.Glu33Asp**	**Substitution**	**1A**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17430	**c.99G>C**	**Substitution**	**p.Glu33Asp**	**Substitution**	**1A**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17434	**c.194A>G**	**Substitution**	**p.Glu65Gly**	**Substitution**	**1A**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17435	**c.194A>G**	**Substitution**	**p.Glu65Gly**	**Substitution**	**1A**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
16283	**c.302G>C**	**Substitution**	**p.Arg101Pro**	**Substitution**	**1B**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**
17399	**c.388G>T**	**Substitution**	**p.Ala130Ser**	**Substitution**	**1B**	**LGMD1B**	**Limb-girdle muscular dystrophy type 1B**	**159001**	**#**

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17755	c.388G>T	Substitution	p.Ala130Ser	Substitution	1B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17432	c.471G>A	Substitution	p.=	Silent	Not affected	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9216	c.513G>A	Substitution	p.=	Silent	Not affected	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17575	c.513+1G>A	Substitution	p.?	Unknown	Unknown	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17437	c.565C>T	Substitution	p.Arg189Trp	Substitution	1B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9639	c.622_624delAAG	Deletion	p.Lys208del	Deletion	1B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
12403	c.624_626delGAA	Deletion	p.Lys208del	Deletion	1B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
16145	c.673C>T	Substitution	p.Arg225X	Substitution	L12	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17401	c.673C>T	Substitution	p.Arg225X	Substitution	L12	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17753	c.673C>T	Substitution	p.Arg225X	Substitution	L12	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13555	c.746G>A	Substitution	p.Arg249Gln	Substitution	2A	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9164	c.777T>A	Substitution	p.Tyr259X	Substitution	L2	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
12401	c.777T>A	Substitution	p.Tyr259X	Substitution	L2	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17760	c.777T>A	Substitution	p.Tyr259X	Substitution	L2	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11810	c.855delG	Deletion	p.Ala287LeufsX191	Frame shift	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9021	c.864_867delCCAC	Deletion	p.His289ArgfsX190	Frame shift	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11759	c.864_867delCCAC	Deletion	p.His289ArgfsX190	Frame shift	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
12384	c.908_909delCT	Deletion	p.Ser303CysfsX26	Frame shift	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11811	c.992G>C	Substitution	p.Arg331Pro	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
18309	c.1001_1003delGCC	Deletion	p.Ser334del	Deletion	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
8794	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
8850	c.1130G>T	Substitution	p.Arg377Leu	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9301	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9024	c.1130G>T	Substitution	p.Arg377Leu	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11762	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
13557	c.1130G>A	Substitution	p.Arg377His	Substitution	2B	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11755	c.1146C>T	Substitution	p.=	Silent	Not affected	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11756	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17443	c.1357C>T	Substitution	p.Arg453Trp	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11812	c.1370delA	Deletion	p.Lys457SerfsX21	Frame shift	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17441	c.1380+1G>A	Substitution	p.?	Unknown	Unknown	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
8839	c.1441T>C	Substitution	p.Tyr481His	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
13376	c.1488+5G>A	Substitution	p.?	Unknown	Unknown	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9181	c.1494G>T	Substitution	p.Trp498Cys	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9158	c.1494G>T	Substitution	p.Trp498Cys	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
16442	c.1494G>T	Substitution	p.Trp498Cys	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9159	c.1535T>C	Substitution	p.Leu512Pro	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11763	c.1535T>C	Substitution	p.Leu512Pro	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17449	c.1535T>C	Substitution	p.Leu512Pro	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
17452	c.1535T>C	Substitution	p.Leu512Pro	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11758	c.1583C>A	Substitution	p.Thr528Lys	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
13446	c.1608+1G>A	Substitution	p.?	Unknown	Unknown	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
9412	c.1608+5G>C	Substitution	p.Glu537ValfsX36	Frame shift	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
13151	c.1609-3C>G	Substitution	p.?	Unknown	Unknown	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
11764	c.1718C>T	Substitution	p.Ser573Leu	Substitution	Tail	LGMD1B	Limb-girdle muscular dystrophy type 1B	159001	#
13505	c.373G>A	Substitution	p.Gly125Ser	Substitution	1B	LAF	Lone atrial fibrillation	-	-
13509	c.[373G>A; =]+[=; 1243G>A]	Substitution	p.[Gly125Ser; =]+[=; Val415Ile]	Substitution	1B, Tail	LAF	Lone atrial fibrillation	-	-
13492	c.810+63C>A	Substitution	p.?	Unknown	Unknown	LAF	Lone atrial fibrillation	-	-
13495	c.937-46A>G	Substitution	p.?	Unknown	Unknown	LAF	Lone atrial fibrillation	-	-
13501	c.1149G>A	Substitution	p.=	Silent	Not affected	LAF	Lone atrial fibrillation	-	-
13497	c.1158-44C>T	Substitution	p.?	Unknown	Unknown	LAF	Lone atrial fibrillation	-	-
13499	c.1158-44C>T	Substitution	p.?	Unknown	Unknown	LAF	Lone atrial fibrillation	-	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13507	**c.1243G>A**	**Substitution**	**p.Val415Ile**	**Substitution**	**Tail**	**LAF**	**Lone atrial fibrillation**	**-**	**-**
13511	**c.1462A>C**	**Substitution**	**p.Thr488Pro**	**Substitution**	**Tail**	**LAF**	**Lone atrial fibrillation**	**-**	**-**
13503	**c.1803C>T**	**Substitution**	**p.=**	**Silent**	**Not affected**	**LAF**	**Lone atrial fibrillation**	**-**	**-**
11783	c.176T>G	Substitution	p.Leu59Arg	Substitution	1A	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
18470	c.683A>T	Substitution	p.Glu228Val	Substitution	L12	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
12380	c.[1318G>A; =]+[=; 1580G>A]	Substitution	p.[Val440Met; =]+[=; Arg527His]	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
12615	c.1411C>T	Substitution	p.Arg471Cys	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
13119	c.1579C>T	Substitution	p.Arg527Cys	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
17878	c.1579C>T	Substitution	p.Arg527Cys	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
8851	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
11419	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
11420	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
11421	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
11422	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
8877	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
11453	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
8995	c.1580G>A	Substitution	p.Arg527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
9224	c.1580G>A	Substitution	p.Ala527His	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
17602	c.1580G>T	Substitution	p.Arg527Leu	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
17603	c.1580G>T	Substitution	p.Arg527Leu	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
17747	c.1 580G>T	Substitution	p.Arg527Leu	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#

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Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17748	c.1580G>T	Substitution	p.Arg527Leu	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
12598	c.1585G>A	Substitution	p.Ala529Thr	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
9317	c.1586C>T	Substitution	p.Ala529Val	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
18478	c.1620G>A	Substitution	p.Met540Ile	Substitution	Tail	MADA	Mandibuloacral dysplasia with type A lipodystrophy	248370	#
13054	c.82C>T	Substitution	p.Arg28Trp	Substitution	Head	-	Metabolic syndrome	-	-
13056	c.274C>T	Substitution	p.Leu92Phe	Substitution	1B	-	Metabolic syndrome	-	-
13059	c.1159C>G	Substitution	p.Leu387Val	Substitution	2B	-	Metabolic syndrome	-	-
13061	c.1184C>T	Substitution	p.Ser395Leu	Substitution	Not affected	-	Metabolic syndrome	-	-
13063	c.1196G>A	Substitution	p.Arg399His	Substitution	Tail	-	Metabolic syndrome	-	-
13065	c.1262T>C	Substitution	p.Leu421 Pro	Substitution	Tail	-	Metabolic syndrome	-	-
13067	c.1 315C>T	Substitution	p.Arg439Cys	Substitution	Tail	-	Metabolic syndrome	-	-
13069	c.1516C>G	Substitution	p.His506Asp	Substitution	Tail	-	Metabolic syndrome	-	-
13047	c.1698C>T	Substitution	p.=	Silent	Not affected	-	Metabolic syndrome	-	-

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
13071	c.1961_1962insG	Insertion	p.Thr655AsnfsX49	Frame shift	Tail	-	Metabolic syndrome	-	-
12408	**c.73C>T**	**Substitution**	**p.Arg25Cys**	**Substitution**	**Head**	**-**	**Muscular dystrophy**	**-**	**-**
12410	**c.1130G>T**	**Substitution**	**p.Arg377Leu**	**Substitution**	**2B**	**-**	**Muscular dystrophy**	**-**	**-**
12412	**c.1622G>C**	**Substitution**	**p.Arg541 Pro**	**Substitution**	**Tail**	**-**	**Muscular dystrophy**	**-**	**-**
12414	**c.1045C>T**	**Substitution**	**p.Arg349Trp**	**Substitution**	**2B**	**-**	**Muscular dystrophy and lipodystrophy**	**-**	**-**
17411	**c.1821G>A**	**Substitution**	**p.=**	**Silent**	**Not affected**	**WRS**	**Progeroid syndrome, neonatal**	**264090**	**%**
18482	**c.1940T>G**	**Substitution**	**p.Leu647Arg**	**Substitution**	**Tail**	**WRS**	**Progeroid syndrome, neonatal**	**264090**	**%**
17009	c.1303C>T	Substitution	p.Arg435Cys	Substitution	Tail	RD	Restrictive dermopathy	275210	#
17731	c.1303C>T	Substitution	p.Arg435Cys	Substitution	Tail	RD	Restrictive dermopathy	275210	#
17801	c.1303C>T	Substitution	p.Arg435Cys	Substitution	Tail	RD	Restrictive dermopathy	275210	#
9166	c.1824C>T	Substitution	p.[=, Val607_Gln656del]	Silent, Deletion	Tail	RD	Restrictive dermopathy	275210	#
9208	c.1968+1G>A	Substitution	p.Gly567_Gln656del	Deletion	Tail	RD	Restrictive dermopathy	275210	#

(continued)

Database ID	cDNA Variant	cDNA Variant Types	Protein Variant	Protein Variant Types	Domain	Disease Abbreviation	Disease Name	Omim ID	Omim Symbol
17980	**c.1057C>T**	**Substitution**	**p.Gln353X**	**Substitution**	**2B**	**-**	**Spinal muscular atrophy with cardiac involvment**	**-**	**-**
18424	**c.868G>A**	**Substitution**	**p.Glu290Lys**	**Substitution**	**2B**	**SCD**	**Sudden cardiac death**	**115080**	**#**
17189	**c.908_909delCT**	**Deletion**	**p.Ser303CysfsX26**	**Frame shift**	**2B**	**SCD**	**Sudden cardiac death**	**115080**	**#**
17901	**c.1334T>A**	**Substitution**	**p.Val445Glu**	**Substitution**	**Tail**	**SCD**	**Sudden cardiac death**	**115080**	**#**
9022	c.1804G>A	Substitution	p.Gly602Ser	Substitution	Tail	-	Type A insulin resistance syndrome	-	-

[0072] In some embodiments, the nucleic acid molecule according to any aspect of the present disclosure is for use in treating cardiovascular disease in a subject.

[0073] In some embodiments, the disease or the cardiovascular disease is characterised by the presence of at least one *Lmna* mutation.

[0074] 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.

[0075] According to a third aspect of the present disclosure there is provided an adeno-associated virus vector (AAV) comprising a cardiac troponin T promoter (cTnT), and the transgene according to any aspect of the present disclosure.

[0076] According to a fourth aspect of the present disclosure there is provided a pharmaceutical composition comprising the nucleic acid molecule according to any embodiment of the present disclosure for treating a disease.

[0077] In some embodiments the disease is a laminopathy.

[0078] In some embodiments the pharmaceutical composition comprising the nucleic acid molecule according to the present disclosure is for use in treating a cardiovascular disease in a subject.

[0079] According to a fifth aspect of the present disclosure there is provided 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 embodiment of the present disclosure, or the pharmaceutical composition of the present disclosure.

[0080] In some embodiments of the method of treating a disease in a subject, the disease is characterised by the presence of at least one *Lmna* mutation.

[0081] In some embodiments 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 present disclosure or the nucleic acid molecule of the present disclosure.

[0082] In some embodiments 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.

[0083] In some embodiments 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.

[0084] In some embodiments 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.

[0085] In some embodiments of the method of treating a disease in a subject, the subject is a non-human mammal, such as a mouse, or a human.

[0086] In some embodiments of the method, the mouse is an N195K mouse (Lmna N195K/ N195K), or a *Lmna* conditional knockout (Lmnaflox/flox).

[0087] According to a sixth aspect of the present disclosure there is provided use of the pharmaceutical composition according to the present disclosure or the nucleic acid molecule according to the present disclosure in the manufacture of a medicament for treating a disease caused by one or more *Lmna* mutations.

[0088] In some embodiments the disease is *Lmna* mutation-related cardiovascular disease.

[0089] In some embodiments 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 syn-

drome, Charcot-Marie-Tooth disease type 2, Charcot-Marie-Tooth disease type 2B1 and diseases presented in normal font in Table 1.

[0090] In some embodiments 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.

[0091] According to a seventh aspect of the present disclosure there is provided a method for screening for drug candidates capable of inhibiting or disrupting the LINC complex in a cell.

[0092] Accordingly, in some embodiments 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.

[0093] In some embodiments 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.

[0094] In some embodiments said screening is an in vitro screening.

[0095] In some embodiments said complex is measured by an ELISA method.

[0096] In some embodiments 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.

[0097] In some embodiments 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.

[0098] In some embodiments said complex is measured by a fluorescence anisotropy method.

[0099] In some embodiments the fluorescence anisotropy method employs recombinant SUN and KASH domains. In some embodiments 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.

[0100] In some embodiments 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.

BRIEF DESCRIPTION OF THE FIGURES

[0101]

Figure 1 shows a schematic of the mutations in the lamin A/C gene *LMNA* and the laminopathies resulting from the mutations.

Figure 2 shows a schematic of the positioning of components of the nuclear envelope membrane and lamina.

Figure 3 shows a schematic of the connections between the nucleus and the extracellular matrix via the LINC

complex and how mutations in lamin A/C might result in DCM. The plasma membrane, cytoskeleton and nucleus form a mechanically and physically linked entity. In Lmna mutants, the nucleus is structurally weak. It is much more susceptible to mechanical stress from cytoskeletal forces. This leads to severe damage to the myocyte nuclei that in turn leads to a cascade of events such as apoptosis and fibrosis that results finally in DCM.

Figure 4 shows the effect of microinjection of dextran into the nucleus of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice under low pressure. In the wildtype cells, the dextran stays in the nucleus, while in the Lmna mutant cells the dextran leaks out of the nucleus into the cytoplasm.

Figures 5A - **5B** show schematics of a LINC complex (**Fig. 5A**) and interaction between KASH and SUN (**Fig. 5B**).

Figure 6 shows defects in body weight and longevity in *Lmna*$^{-/-}$ and *Lmna*Δ9 mice are ameliorated in homozygous Sun1 knockout *Lmna*$^{-/-}$*Sun1*$^{-/-}$ and *Lmna*Δ9*Sun1*$^{-/-}$ animals. (**A**) Body weights are averages from mice with the indicated genotypes. The number (n) of animals used is indicated. (**B**) Kaplan-Meier graph showing increased life span of *Lmna*$^{-/-}$*Sun1*$^{-/-}$ compared to *Lmna*$^{-/-}$ mice. Median survival of wild-type or *Sun1*$^{-/-}$ is > 210 days in a 7 month follow up; *Lmna*$^{-/-}$ mice have median survival of 41 days; *Lmna*$^{-/-}$*Sun1*$^{+/-}$ mice have a median survival of 54 days; *Lmna*$^{-/-}$*Sun1*$^{-/-}$ mice have a median survival of 104 days ($p < 0.01$ comparing *Lmna*$^{-/-}$ and *Lmna*$^{-/-}$*Sun1*$^{-/-}$). (**C**) Body weights of *Lmna*Δ9 mice that are wild-type, heterozygous, or homozygous for *Sun1* deficiency. Wild-type and *Sun1*$^{-/-}$ cohorts are graphed for comparison. Values are averages ± SEM from animals in each cohort. Number (n) of animals is indicated ($p < 0.0001$ comparing *Lmna*Δ9*Sun1*$^{+/+}$ and *Lmna*Δ9*Sun1*$^{-/-}$). (**D**) Kaplan-Meier graph showing increased life span of *Lmna*Δ9*Sun1*$^{-/-}$ compared to *Lmna*Δ9*Sun1*$^{+/+}$ mice. *Lmna*Δ9*Sun1*$^{+/-}$ mice are also graphed. ($p < 0.0001$ comparing *Lmna*Δ9*Sun1*$^{+/+}$ and *LmnaΔ9Sun1*$^{-/-}$). (**E**) Cell proliferation of the indicated MEFs. Curves are averages ± SD, representative of > 3 independent isolates from embryos of the indicated genotypes. (**F**) Proliferation curves of MAFs (mouse adult fibroblasts) from WT, *Sun1*$^{-/-}$, *Lmna*Δ9*Sun1*$^{+/+}$ and *Lmna*Δ9*Sun1*$^{-/-}$ mice. MAFs were seeded at a density of 1000 cells per well. Growth was measured, and normalized cell indexes (averages ± SD) are presented.

Figure 7 shows a schematic of the features of the Sun1 protein and the components used to generate a dominant negative Sun1 protein, including a signal sequence, coiled-coil sequence, SUN domain sequence and KDEL sequence.

Figure 8 shows a schematic of a plasmid (SEQ ID NO: 1) used for AAV production.

Figure 9 shows a schematic of a plasmid (SEQ ID NO: 2) comprising sequences from AAV2 and AAV9 for AAV production.

Figure 10 shows a schematic of an AAV expression construct (SEQ ID NO: 3) comprising cardiac-specific promoter and Sun1 dominant negative sequence.

Figure 11 shows a schematic of the features of the dominant negative Sun1 protein, including a signal sequence, coiled-coil sequence, SUN domain sequence and KDEL sequence (SEQ ID NO: 4).

Figure 12 shows a schematic of the features of a dominant negative Sun2 protein, including a signal sequence, lumenal domain sequence and KDEL sequence (SEQ ID NO: 5).

Figure 13 shows a schematic of the region of Sun1 protein used in dominant negative constructs.

Figure 14 shows an alignment of KASH1-KASH5 domain amino acid sequences with conserved residues (SEQ ID Nos: 7, 9, 11, 13 and 15, respectively).

Figure 15 shows a schematic of the LINC complex in wildtype mice, Sun1 KO mice, AAV dominant negative SUN mice and mice with altered KASH domain. The schematic for wildtype mice is obtained from Brian Burke, 2012. The schematic for Sun1 KO mice represents the results from Chen et al., 2012. The AAV dominant negative SUN and the altered KASH domain schematics represent inventor proposals at the priority date on methods for LINC complex disruption to ameliorate laminopathies, based on data obtained at that time.

Figure 16 shows a Kaplan Meier curve of *Lmna* KO mice surviving for an average of 28 days, *Sun1* KO mice living beyond 300 days and cardiac *Lmna* KO/*Sun1* KO mice living beyond 300 days.

Figure 17 shows H&E stained sections of hearts from *Sun1* KO mice, cardiac *Lmna* KO mice and cardiac *Lmna* KO/*Sun1* KO mice, with LmnaKO/Sun1WT hearts showing enlargement of the left ventricle (DCM) compared to WT and LmnaKo/Sun1KO hearts.

Figure 18 shows a schematic of disruption of a LINC complex in a Nesprin-1 ΔKASH mouse. *LmnaKO* Nesprin-1WT mice have a lifespan of about 20 days. *LmnaKO* Nesprin-1-ΔKASH survive about 40 days, which is similar to *Lmna*KO*Sun1*KO mice.

Figures 19A-19B show a schematic of anticipated AAV-cTNT-ON-SUN expression and competition between exogenous ON-SUN and native Sun1 for binding to the KASH domain **(Fig. 19A)** with the ON-SUN shown in 19B (upper panel) and the effect of transfected ON-SUN on native Nesprin2G positioning in cells where the 2 nuclei in the middle panel express the ON-SUN and in the merge panel both show loss of Nesprin2 from the nuclear membranes(**Fig. 19B**).

Figure 20 is a Kaplan Meier curve showing disruption of SUN-KASH interaction in vivo, using AAV9-cTNT-dominant negative Sun1 (DNSun1), extends the longevity of the heart-specific *Lmna* KO in male and female mice.

Figure 21 shows C-terminal amino acids of the KASH domain of Nesprin-2 (KASH2). The 14 or 18 amino acid sequence from KASH2 C-terminus are able to physically interact with the SUN domain of SUN2. Loss of the last 4 amino acids from KASH2 or addition of a single alanine amino acid at the C-terminus of KASH2 is sufficient to disrupt interaction of the KASH2 domain with the SUN domain.

Figure 22 shows a schematic of a screening method for detecting agents that disrupt the LINC complex.

Figure 23 shows a flowchart showing a more detailed screening method for identifying a small molecule to disrupt the LINC complex.

Figure 24 is a Kaplan Meier curve showing that wild type (C57/Bl6) mice with or without a Nesprin-1 KASH-disrupting (C'TΔ8) mutation have a normal lifespan. Mice with a Lmna null/KO mutation (LA-ZP3cre$^{\Delta/\Delta}$) and wildtype (Nesp1$^{+/+}$) or heterozygous (Nesp1$^{+/C'T\Delta8}$) for Nesp1-C'TΔ8 have a median lifespan of 15 or 18 days, which is increased to 38 days in Lmna KO / homozygous Nesp1 mutant (LA-ZP3cre$^{\Delta/\Delta}$; Nesp1 $^{C'T\Delta8/C'T\Delta8}$) mice.

Figure 25 is a Kaplan Meier curve showing that mice with wildtype Lmna (N1$^{CT\Delta8/\,CT\Delta8}$LA$^{+/+}$MCre$^{+/-}$), or floxed alleles of Lmna but lacking a cardiac-specific Cre driver (N1$^{CT\Delta8/\,CT\Delta8}$LA$^{f/f}$MCre$^{+/+}$ and N1$^{WT/WT}$LA$^{f/f}$MCre$^{+/+}$), live for the length of the experiment (~ 80 days at priority filing, which extended to 120 days unchanged). Mice with a cardiomyocyte-specific deletion of Lmna (N1$^{WT/WT}$LA$^{f/f}$MCre$^{+/-}$) have a lifespan of 22-24 days following induction of the Cre/loxP-mediated deletion by tamoxifen (TMX) delivery, which is increased to the length of the experiment in mice with a cardiomyocyte-specific deletion of Lmna induced by TMX and also homozygous mutant for Nesprin-1 (N1$^{CT\Delta8/CT\Delta8}$LA$^{f/f}$MCre$^{+/-}$).

Figures 26A-26D show Kaplan Meier curves of Sun1 loss extending the longevity of *Lmna* mutant mice. (**Fig. 26A**) Wild type (C57/Bl6) mice with or without Sun1 have a normal lifespan, whereas the average postnatal lifespan of the *Lmna$^{Flx/Flx:Zp3}$* mice in which LaminA is deleted in all tissues was 17.5 days (***P=<0.0001; Log-rank test). On a *Sun1$^{-/-}$* background longevity is increased to 32.5 days. (**Fig. 26B**) When *Lmna$^{Flx/Flx}$* was deleted specifically and constitutively in hearts by crossing the mice with the *Cre$^{\alpha MyHC}$* line, the *Lmna$^{Flx/Flx:\alpha MyHc}$* mice lived on average 26.5 days. On a *Sun1$^{-/-}$* background these mice lived for longer than 6 months. (**Fig. 26C**) 3-5 month old *Lmna$^{Flx/Flx}$* were crossed with the Tmx inducible cardiomyocyte specific Cre *Tg(Myh6-cre/Esr1),* (abbreviated to *mcm),* after a single injection of Tmx the mice die within 3-4 weeks. On a *Sun1$^{-/-}$* background these mice lived for more than 1 year. (**Fig. 26D**) *Lmna$^{N195K/N195K}$* mice lived for an average of 78 days compared to *Lmna$^{N195K/N195K}$Sun1$^{-/-}$* mice which had an average lifespan of 111 days. (***P=<0.0001, **P=0.0073 Log-rank test).

Figures 27A-27E show the lifespan and phenotype of *Lmna$^{Flx/Flx:mcm}$* + Tmx mice. (**Fig. 27A**) The average lifespan of the *Lmna$^{Flx/Flx:mcm}$* mice was 27 days after a single Tmx injection (***P=<0.0001; Log-rank test). (**Fig. 27B**) PCR detected the floxed (deleted) *Lmna* gene (arrow head) only in heart tissue after Tmx injection and not in other tissues or when Tmx was not injected (**Fig. 27C**) *Lmna$^{Flx/Flx:mcm}$*+Tmx mice developed kyphosis (arrow head) by 21 days after injection. (**Fig. 27D**) LaminA/C protein, detected by immunofluorescence, were present in control (i, iii), but reduced/absent in cardiomyocyte (CM) nuclei in both isolated CMs (ii second panel) and heart sections (iv) (white arrowheads) with CM nuclei being detected by PCM-1 staining, 21 days after Tmx. (**Fig. 27E**) LaminA/C levels were

quantified by Western analysis of whole heart lysates 21 days after injection. A significant reduction (***P=<0.0001; T-test) in A-type Lamin protein was detected, although Lamin C levels were not reduced as much in the *Lmna$^{Flx/Flx:mcm}$*+Tmx mice compared to *Lmna$^{Flx/Flx:mcm}$*+CTL. (**Fig. 27F**) Quantitative analysis was performed at 21 days post Tmx. The presence of the *LoxP* sites in the *WT-Lmna* gene (*Lmna$^{Flx/Flx}$*) results in a reduction in *Lmna* transcript levels compared to *Lmna$^{Wt/Wt}$* levels, although this had no overt effect on longevity or growth/viability.

Figures 28A-28D show echocardiograms, heart function and histology of *Lmna$^{Flx/Flxmcm}$* + Tmx mice. (**Fig. 28A**) *Lmna$^{Flx/Flx:mcm}$* + Tmx mice show reduced cardiac contractile function. **(Fig. 28B)** *Lmna$^{Flx/Flx:mcm}$* hearts show reduced EF% and FS%, and increased LVID (***P=<0.0001, **P=0.0010; Two way ANOVA). (**Fig. 28C**) Histological analysis of the hearts revealed increased infiltration of nucleated cells and intercellular spaces in *Lmna$^{Flx/Flxmcm}$* hearts (i and ii). Significantly fewer viable (brick-like) CMs were isolated from *Lmna$^{Flx/Flx:mcm}$* hearts compared to *Lmna$^{Flx/Flx:mcm}$* controls (iii). With higher magnification, the isolated cardiomyocytes from *Lmna$^{Flx/Flx:mcm}$* hearts contained large intracellular vacuoles (arrow head, iv). (**Fig. 28D**) The left ventricular lumen in *Lmna $^{Flx/Flx:mcm}$* hearts was enlarged (i) together with increased fibrosis (ii) (**P=0.0007 visible as lighter grey areas in the 28D ii, middle panels and iv left panel) and apoptotic nuclei revealed by TUNEL staining (*P=0.0220; One way ANOVA) (iii and iv right panel). All samples and analyses were performed on hearts 21 days post Tmx injection.

Figures29A-29D shows changes in nuclear morphologies and heart structure with and without Sun1 in the *Lmna$^{Flx/Flx:mcm}$* after Tmx injection. (**Fig. 29A**) CM nuclei with reduced or absent Lamin A/C expression are indicated by white arrow heads (1, 3). CM nuclei (2, 4) with normal Lamin A/C levels are indicated by grey arrowheads. LMNA protein levels, measured by both fluorescence intensity (5) and Western blot (6), were significantly reduced in *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$*+Tmx (***P=0.0009; T-test) and *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$*+Tmx (*P= 0.0359; T-test) compared to *Lmna$^{Flx/Flxmcm}$* Sun1$^{+/+}$ controls (lower graph, 6) (**Fig. 29B**). Left ventricular (LV) enlargement was apparent in the *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$*+Tmx hearts (panel 1) but not in the LV of the *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$*+Tmx hearts (panel 2). The *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$*+Tmx mice had significantly increased fibrosis (panel 3, fibrosis in grey) compared to controls, but there was no significant increase in fibrosis in the *Lmna$^{Flx/Flxmcm}$ Sun1$^{-/-}$*+Tmx hearts (panel 3) compared to controls (panel 4, quantified in panel 5, ***P=0.0001; One way ANOVA). Cardiac papillary muscle active force measurements were significantly reduced from the *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$*+Tmx mice compared to *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$* controls (**P=0.0047; T-test) and *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$* + Tmx (*P=0.0113; T-test) (panel 6). (**Fig. 29C**) CM nuclear morphologies were significantly altered in *L_mna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$*+Tmx mice (Panel 1, solid arrow heads). In the absence of TMX, control heart sections (CTL, panel 2) display few nuclear abnormalities. In the absence of Sun1, *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$*+Tmx cardiomyocytes showed no nuclear abnormalities (Panels 3 and 4). In summary Figure 29C panel 5 reveals that, 70% of CM in *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$*+Tmx mice had NE ruptures/distortions or misshapen nuclei compared to less than 1% of CM nuclei in *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$*+Tmx mice. (Fig. 29D) Echo analyses on TMX-treated and control mice were performed following Tmx induction. Echocardiograms (ECGs) performed at 28 days after Tmx injection on 3-5 month old mice (panel 1). ECGs performed before and after Cre induction revealed a progressive worsening of cardiac contractility in *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$* +Tmx mice (solid black line) compared to *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$* + Tmx mice (panels 2-4). The loss of SUN1 preserved EF (panel 2), FS (panel 3) and Global Longitudinal Strain (GLS, panel 4) in *Lmna$^{Flx/Flx:mcm}$ Sun1$^{-/-}$* + Tmx mice compared to *Lmna$^{Flx/Flx:mcm}$ Sun1$^{+/+}$* +Tmx mice.

Figures 30A-30B show Kaplan Meier graph and heart function effects of deletion of SUN1 on cardiac pathology induced by a missense mutation in the *Lmna* gene (N195K). **(Fig. 30A)** The absence of Sun1 significantly increases the lifespan of *Lma$^{N195K7Flx:mcm}$Sun1$^{-/-}$*+ Tmx mice compared *to Lmna$^{N195K/FlX:mcm}$Sun1$^{+/+}$*+ Tmx mice (*P=0.0101; Log-rank test). Mice with only one copy of the N195K mutation (*Lmna$^{N195K/-:mcm}$Sun1$^{+/+}$* + Tmx) had an average lifespan of 47 days, approximately half the lifespan of mice homozygous i.e. with two copies of the N195K allele. **(Fig. 30B)** Echocardiograms (ECGs) performed before and after Cre induction revealed progressive worsening of cardiac contractility in *Lmna$^{N195K/-:mcm}$Sun1$^{+/+}$* +Tmx mice compared to *Lmna$^{N195K/-:mcm}$Sun1$^{-/-}$* + Tmx mice over time. ECGs images were recorded at 28 days after Tmx injection (left-hand side panels). The loss of SUN1 preserved EF, FS and GLS in *Lmna$^{N195K/Flx:mcm}$ Sun1$^{-/-}$* + Tmx mice compared to *Lmna$^{N195K/Flx:mcm}$ Sun1$^{+/+}$* +Tmx mice (right-hand side bottom 3 panels).

Figures 31A-31G show *Lmna$^{Flxx/Flx:mcm}$*+Tmx mice expressing an AAV transduced DNSun1 exhibit improved cardiac function and increased longevity. **(Fig. 31A)** Protocol for AAV-mediated transduction of the DN-Sun1 miniprotein into *Lmna$^{Flxx/Flx:mcm}$* +Tmx mice. A single Tmx (IP) injection is given at D14 postnatally to induce *Lmna* deletion. AAV9-DNSun1 or AAV9-GFP viral particles are then injected into the chest cavity on D15 postnatally. The experimental endpoint was set at 100 days after Tmx. **(Fig. 31B)** The DNSun1 miniprotein competes with endogenous Sun1 for binding to the KASH domain of the Nesprins (in CMs this is Nesprin1). The miniprotein competes with

endogenous SUN1 in binding to the KASH domain of the Nesprins. As the DNSun1 miniprotein is not anchored in the INM this effectively disconnects the endogenous SUN proteins from binding to the KASH domains so breaking the LINC. **(Fig. 31C)** The presence of the recombined *Lmna* gene following Tmx injection was confirmed by PCR of the heart tissues (upper panel). Robust expression of both AAV9-DNSun1 and AAV9-GFP protein (Dosage: 5x10^10vg/g of mouse) was detected in extracts from whole hearts 99 days post AAV injection (lower panel). **(Fig. 31D)** CMs derived from human iPS stem cells were transduced with the DNSun1 using AVV-OJ as the vector. In CMs expressing high levels of DNSun1, indicated by grey arrows, Nesprin1 localization to the NE is reduced or absent. Nesprin1 localization to the NE is maintained in CMs either not expressing the AVV-OJ-ONSun1 or when expressed at lower levels (white arrow heads) . (**Fig. 31E**) The $Lmna^{Flx/Flx:mcm}$+Tmx+AAV9-GFP mice lived for an average of 34.5d after Tmx induction, whereas $Lmna^{Flx/Flx:mcm}$ +Tmx mice injected with AA9-DNSun1 (5×10^10vg/g/mouse) lived significantly longer (**P=0.0038; Log-rank test) to at least 100D post Tmx, after which the mice were sacrificed for analysis. This set of data was derived from that shown in Fig. 20, adjusted by removing mice that were female and those with a different dose of virus. Fig. E(i) represents male mice and Fig. E(ii) represents female mice. **(Fig. 31F)** At 35d after Tmx, extensive fibrosis (blue in original image, grey here) and ventricular enlargement was detected in $Lmna^{Flx/Flx:mcm}$+Tmx+AAV9-GFP hearts compared to $Lmna^{Flx/Flx:mcm}$ +Tmx + AAV9-DNSun1 hearts. **(Fig. 31G)** ECG analysis confirmed $Lmna^{Flx/Flx:mcm}$ +Tmx+AAV9-DNSun1 hearts had better cardiac function compared to the $Lmna^{Flx/Flx:mcm}$ +Tmx +AAV9-GFP hearts at 35d days after Tmx injection.

Figures 32A-32D shows models of how breaking the LINC by disrupting Sun1 protects cardiomyocytes from contraction induced stress **(Fig. 32A)** Cardiomyocyte nuclei expressing LmnaA/C, are able to withstand mechanical stress and tension forces transmitted via the LINC complex from the cytoplasm to the NE. **(Fig. 32B)** The loss of or introduction of a mutation within the *Lmna* gene results in loss/or incorrect assembly of the nuclear lamina, which weakens the Lamina/NE. The weakened nuclei are damaged due to the tension/stress forces exerted via the LINC complex from the contractile sarcomeres of the cardiomyocytes. **(Figs. 32C**, **D**). In the absence of SUN1 or by disrupting its binding to the KASH domains by expression of ONSun1, the untethered LINC complexes exert less tensional force on the cardiomyocyte nuclei, enabling survival of the *Lmna* mutant cardiomyocytes.

Figure 33: shows the structure of the $Lmna^{Flx/Flx}$ conditional allele. Primer locations for genotyping the *Lmna* gene both before and after Cre recombination are indicated for the $Lmna^{Flx}$ allele (Flox), the *Lmna* deleted allele (Δ) and the wildtype allele [A. S. Wang, et al., Differentiation 89: 11-21 (2015)].

Figure 34 shows a diagram of the recombinant AAV9-DNSun1 and AAV9-GFP miniproteins. The DN-Sun1 includes the Sun domain, an HA tag, a Signal Sequence (SS, for targeting the protein to the ER), and the KDEL (ER retention signal) [M. Crisp et al., J Cell Biol. 172: 41-53 (2006)]. The AAV9-GFP includes the SS and KDEL sequences. GFP was used as a control in place of the Sun1L-KDEL.

Figure 35 shows photomicrographs of cardiomyocyte specific expression of Cre recombinase after Tmx injection. The $Lmna^{Flx/Flx:mcm}$ mice were crossed with the mT/mG (JAX: Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J) reporter mice. In the absence of Cre, RFP is expressed. When Cre is induced, GFP is expressed. Only CMs of $Lmna^{Flx/Flx:mcm}$ mice express GFP upon TMX injection. Heart tissues were analyzed 7 days after Tmx injection.

Figures 36 shows that loss of Sun2 does not rescue loss of *Lmna.* Loss of Sun2 does not extend the lifespan of $Lmna^{\Delta/\Delta}Sun2^{-/-}$ mice.

Figures 37A-37E show the phenotypes of $Lmna^{Flx/Flxmcm}$ $Sun1^{+/+}$ and $Lmna^{Flx/Flxmcm}$ $Sun1^{-/-}$ hearts at 12-14 months after Tmx injection. **(Fig. 37A)** Histological analysis of the aged $Lmna^{FlX/Flx/:mcm}Sun1^{+/+}$ hearts, 12-14 months after the Tmx injection, revealing no significant morphological changes e.g. LV enlargement or **(Fig. 37B)** in fibrosis compared to the controls. (**Fig. 37C**) PCR analysis confirmed the sustained deletion of *Lmna* gene. **(Fig. 37D)** Protein quantification revealed a significant reduction of LMNA levels in $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$+Tmx hearts at 14months after TMX. **(Fig. 37E)** Echocardiograms (left-hand side panel) from the aged mice showed reduced EF and FS (right-hand side panels) in both $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$ +CTL and $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$ + Tmx aged mice.

Figure 38 shows the rescue by AAV9-DNSun1 depends on the dosage of viral particles injected. Lifespan of $Lmna^{Flx/Flx:mcm}$ +TMX mice depends of the dosage of AAV9-DNSun1 with, with a lower concentrations resulting in shorter lifespans. Each dot represents a mouse, horizontal lines indicate mean.

Figures 39A-39C show levels of LaminA/C following Tmx induction and expression of AAV-expressed proteins. **(Fig. 39A)** LaminA/C levels were significantly reduced following Tmx induction, and the presence of either AAV9-

DNSun1 or AAV9-GFP protein did not alter LMNA protein levels (Quantification of LaminA/C immunofluorescence intensity). The amount of LaminA/C, ONSun1 and GFP protein in whole hearts were also quantified by Western analysis (lower 3 graphs). (Analysis performed 35 days after Tmx). **(Fig. 39B)** The expression of both DNSun1 and GFP proteins were dependent on the concentration of viral particles injected. **(Fig. 39C)** Immunofluorescence revealed the majority of CMs were successfully infected and expressed GFP with 5×10^10vg/g of AAV9-GFP (left image) compared to infection with a 10-fold lower (5×10^9 AAV9-GFP, right image) concentration of viral particles.

Figures 40A-40C show CRISPR targeting of Sun1 SUN domain results in loss of Sun1 protein. **(A, B)** Clustal alignment of Sun1 DNA **(Fig. 40A)** and amino acid **(Fig. 40B)** sequence (SEQ ID Nos: 69 and 72, respectively) adjacent to CRISPR-induced mutation in wildtype Sun1, Sun1 with 4 bp insertion (Sun1_plus4; SEQ ID NOs: 70 and 73, respectively) and Sun1 with 7 bp deletion (Sun1_del7; SEQ ID NOs: 71 and 74, respectively). Numbering is of Sun1 coding sequence (A) and protein sequence (B). Bold letters in (B) indicate SUN domain. **(Fig. 40C)** Immunofluorescence staining of mouse adult fibroblasts derived from wildtype and Sun1 mutant mice. Sun1 expression is lost in mutant mice, but Sun2 and Nesprin-1 expression is similar in all 3 genotypes. Scale bar = 10 μm.

Figure 41A-D shows CRISPR targeting of Syne1 C-terminus results in expression of a mutant Nesprin-1 protein. **(A, B)** Clustal alignment of wildtype Nesprin-1 DNA (SEQ ID NO; 75) and Nesprin-1C'TΔ8 (Nesprin1_CTdel8) (SEQ ID NO: 76) **(A)** and amino acid sequence adjacent to CRISPR-induced mutation in wildtype Nesprin-1 (SEQ ID NO: 77) and Nesprin-1C'TΔ8 (Nesprin1_CTdel8) (SEQ ID NO: 78) **(B).** TGA in bold indicates stop codon of Syne1 / Nesprin-1 gene. **(C, D)** Immunoblots of Nesprin-1 from Syne1 / Nesprin-1 wildtype and Syne1 / Nesprin-1C'TΔ8 mutant heart and muscle tissue.

Figure 42A-B are photomicrographs showing CRISPR-induced Syne1 mutation results in mislocalized, "KASH-less" Nesprin-1 protein. Immunofluorescence staining of mouse adult fibroblasts **(A)** and primary myotubes **(B)** derived from wildtype (WT) and Syne1C'TΔ8 mutant mice. Nesprin-1 is mislocalized from the nuclear envelope in the mutant samples. Merged images shows Nesprin-1 and DNA staining. Scale bar = 10 μm.

Figure 43A-C are photomicrographs showing Syne1 mutation does not disrupt localization of certain nuclear envelope proteins. **(A-C)** Immunofluorescence staining of mouse primary myotubes derived from wildtype (WT) and Syne1C'TΔ8 mutant mice. Sun1 (A), Sun2 and emerin (B) and lamin A/C (C) localize normally to the nuclear envelope. Merged images show protein and DNA staining. Arrows indicate examples of normally localized nuclear envelope proteins. Scale bar = 10 μm.

Figure 44A-C are photomicrographs showing Syne1 mutation disrupts localization of nuclear-envelope-localized centrosomal proteins. **(A-C)** Immunofluorescence staining of mouse primary myotubes derived from wildtype (WT) and Syne1C'TΔ8 mutant mice. Pcm1, Pericentrin (Pent), and Akap450, which normally localize to the nuclear envelope in myotubes, are displaced from the nuclear envelope in Syne1C'TΔ8 mutant myotubes. MF20 is an antibody for myosin heavy chain, a myotube marker. Merged images show protein and DNA staining. Arrows indicate typical nuclear envelope staining for these centrosomal proteins. Scale bar = 10 μm.

Figure 45A-C shows Syne1 mutation does not affect mouse phenotype. **(A-B)** Representative images of 12-week-old male (A) and female (B) mice. **(C)** Bodyweight of male and female, wildtype (WT) and Syne1C'TΔ8 mutant, mice over 6 weeks.

Figures 46A-46C shows Syne2 constructs and Syne1/Syne2 double mutant mice experience perinatal lethality. **(Fig. 46A)** Design of IRES-βgal PGK-Neo targeting construct for generating Syne2 mutation. **(Fig. 46B)** Immunofluorescence staining of mouse adult fibroblasts derived from wildtype (WT) and Syne2 mutant mice showing loss of Nesprin-2. **(Fig. 46C)** Images of newborn pups. Top row are of mice with at least 1 wildtype Syne1 or Syne2 allele that appear a healthy pink. Bottom row shows cyanotic double mutant Syne1 $^{C'T\Delta 8/C'T\Delta 8}$:Syne2$^{-/-}$ pups which appear blue and die at birth.

Figure 47 is a Kaplan Meier graph showing a Syne2 mutation does not ameliorate Lmna pathology. Kaplan-Meier survival curve showing that regardless of their Syne2 mutation status (wildtype, heterozygous or mutant), Lmna$^{\Delta/\Delta}$ mice die within 3 weeks of birth.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

[0102] Certain terms employed in the specification, examples and appended claims are collected here for convenience.

[0103] The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

[0104] As used herein, the term "comprising" or "including" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. However, in context with the present disclosure, the term "comprising" or "including" also includes "consisting of". The variations of the word "comprising", such as "comprise" and "comprises", and "including", such as "include" and "includes", have correspondingly varied meanings.

[0105] As used herein, the terms "CRISPR-Cas" and "CRISPR" system are used somewhat interchangeably to refer to a microbial adaptive immune system that uses RNA-guided nucleases to cleave foreign genetic elements. It comprises clustered regularly interspaced short palindromic repeats (CRISPRs), a CRISPR-associated (Cas) endonuclease and a synthetic guide RNA that can be programmed to identify and introduce a double strand break at a specific site within a targeted gene sequence. The palindromic repeats are interspaced by short variable sequences derived from exogenous DNA targets known as protospacers, and together they constitute the CRISPR RNA (crRNA) array. Within the DNA target, each protospacer is always associated with a protospacer adjacent motif (PAM), which can vary depending on the specific CRISPR system. CRISPR-Cas9 is a specific version of the system referring to use of RNA-guided Cas9 nuclease, originally derived from *Streptococcus pyogenes,* whereby the target DNA must immediately precede a 5'-NGG PAM. Variations of the CR!SPR-Cas9 system are known [Ran FA, et al., Nat. Protoc 8, 2281-2308 (2013); Ran FA, et al., Cell 154, 1380-1389 (2013)], including CRISPR-Cpf1, and although CRISPR-Cas9 has been used herein in the Examples, it is not intended that the present disclosure be limited to a particular CRISPR-Cas system.

[0106] As used herein, the term "dominant negative" refers to a mutation whose gene product adversely affects the normal, wild-type gene product within the same cell. This usually occurs if the product can still interact with the same elements as the wild-type product, but block some aspect of its function. In one example, the transgene is expressed as a protein, and said protein that is functional as a dimer. A mutation that removes the functional domain, but retains the dimerization domain would cause a dominant negative phenotype, because some fraction of protein dimers would be missing one of the functional domains.

[0107] As used herein, the term "normal font" in reference to diseases listed in Table 1 refers to those diseases that are in plain text and not in bold text. The term "bold text" has its ordinary meaning.

[0108] As used herein, the term "stabiliser polypeptide" or "stabiliser protein" refers to an inert polypeptide which folds into a discrete domain, thereby ensuring that the remainder of the peptide maintains, for example, the proper topology. In one example, the stabiliser protein ensures that the KASH protein maintains proper topology on the endoplasmic reticulum membrane and the outer nuclear membrane. In another example, the stabiliser polypeptide prevents an attached polypeptide from translocating into, for example, the perinuclear space.

[0109] As used herein, the term "operably linked" means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions. For example, a control sequence which is "operably linked" to a protein coding sequence is ligated thereto, so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences. By way of an example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

[0110] As used herein, there term "extension" refers to one or more amino acids that can be found attached to the N- or the C-terminus of a desired peptide.

[0111] As used herein, the terms "polypeptide", "peptide" or "protein" refer to one or more chains of amino acids, wherein each chain comprises amino acids covalently linked by peptide bonds, and wherein said polypeptide or peptide can comprise a plurality of chains non-covalently and/or covalently linked together by peptide bonds, having the sequence of native proteins, that is, proteins produced by naturally-occurring and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino acid sequence of the native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. A "polypeptide", "peptide" or "protein" can comprise one (termed "a monomer") or a plurality (termed "a multimer") of

amino acid chains.

[0112] The term "subject" is herein defined as vertebrate, particularly mammal, more particularly human. For purposes of research, the subject may particularly be at least one animal model, e.g., a mouse, rat and the like. In particular, for treatment or prophylaxis of a laminopathy, such as DCM, the subject may be a human.

[0113] The term "treatment", as used in the context of the present disclosure refers to ameliorating, therapeutic or curative treatment.

[0114] Without being bound by theory, the inventors submit that the whole basis of the therapy is that disrupting LINC complex function suppresses *Lmna* mutation. It is further noted that the target of the claimed methods is the SUN-KASH interaction in the LINC complex. The endogenous protein levels should not be affected.

[0115] It is also further noted that the transgene (for example, the dominant negative transgene) will not work if the full length SUN domain protein is inserted between the signal sequence and the KDEL, as it will invert the membrane topology of the protein such that the SUN domain is no longer in the perinuclear space/ER lumen. Only the regions following the transmembrane domain can be used, i.e. the luminal domain.

[0116] A person skilled in the art will appreciate that the present disclosure may be practiced without undue experimentation according to the methods given herein. The methods, techniques and chemicals are as described in the references given or from protocols in standard biotechnology and molecular biology text books.

EXAMPLES

Example 1

Materials and Methods

[0117] Mice were maintained at the A*STAR Biological Resource Centre facility and the NUS Animal Facility in accordance with the guidelines of the Institutional Animal Care and Use Committee for each facility. The $Lmna^{Flx/Flx}$ mice were generated and characterized as previously described [A. S. Wang, et al., Differentiation; research in biological diversity, (2015); I. Solovei et al., Cell 152: 584-598 (2013)] (Figure 33). To derive mice with a global deletion *Lmna* ($Lmna^{\Delta/\Delta}$), we crossed the floxed allele ($Lmna^{Flx/Flx}$) to mice in which Cre recombinase is driven by the regulatory sequences of the mouse zona pellucida 3 gene (Zp3;Tg(Zp3-cre)93Knw, JAX stock 003651) [W. N. de Vries et al., Genesis 26: 110-112 (2000)]. To obtain cardiomyocyte-specific deletion of *Lmna* ($Lmna^{Fix/Flx/NIMhc}$), we first crossed the $Lmna^{Flx/Flx}$ mice to mice in which Cre expression was driven by the cardiac-specific murine alpha myosin-heavy chain (Myh6, myosin, heavy polypeptide 6, cardiac muscle, alpha) promoter (MyHC;Tg(Myhca-cre)2182Mds, JAX stock 011038). To obtain a tamoxifen inducible cardiomyocyte-specific deletion of *Lmna* (*LmnaFlx*/*Flx:mcm*), we crossed the $Lmna^{Flx/Flx}$ with mice in which Cre expression was driven by the mouse cardiac-specific alpha-myosin heavy chain promoter (αMHC or alpha-MHC;Myh6) that expressed a tamoxifen-inducible Cre recombinase (MerCreMer) specifically in juvenile and adult cardiac myocytes (mcm;Tg(Myh6-cre/Esr1*)1Jmk, JAX stock 005657). The specificity of mcm Cre expression to cardiomyocytes was confirmed by crossing Cre lines to the mT/mG reporter mice [M. D. Muzumdar, et al., Genesis 45: 593-605 (2007)] (Figure 35). Generation of the $Sun1^{-/-}$mice was previously described [Y. H. Chi et al., Development 136: 965-973 (2009)] as were the $Lmna^{N195K/N195K}$mice [L. C. Mounkes, et al., Hum Mol Genet 14: 2167-2180 (2005)]. The $Lmna^{\Delta/\Delta}$*:*$Sun1^{-/-}$ and $Lmna^{Flx/}$*Flxmcm:*$Sun1^{-/-}$ mice were obtained by crossing the respective Lamin-Cre mice strains with $Sun1^{+/-}$ mice as $Sunt1^{-/-}$ mice are infertile.

[0118] To test for the insertion of loxP sites and conditional deleted allele, genotyping was performed with a duplex PCR protocol with the following primers were used:

FLX/FLX-F1: 5'-CCAGCTTACAGAGCACCGAGCT-3',	SEQ ID NO: 16
FLX/FLX-F2: 5'-TCCTTGCAGTCCCTCTTGCATC-3',	SEQ ID NO: 17
FLX/FLX-R1: 5'-AGGCACCATTGTCACAGGGTC-3'.	SEQ ID NO: 18

[0119] To test for Sun1 deletion, the following primers were used:

Sun1-F: 5'-GGC AAG TGG ATC TCT TGT GAA TTC TTG AC-3'	SEQ ID NO: 19
Sun1-R: 5'-GTA GCA CCC ACC TTG GTG AGC TGG TAC-3'	SEQ ID NO: 20
Sun1-E8: 5'-AGC CAC ATA ACC ACC TGG AG-3'	SEQ ID NO: 21

[0120] To test for the MyHC transgene, the following primers were used:

MyHC-tF: 5'-ATG ACA GAC AGA TCC CTC CTA TCT CC-3'	SEQ ID NO: 22
MyHC-tR: 5'-CTC ATC ACT CGT TGC ATC ATC GAC-3'	SEQ ID NO: 23
MyHC-F: 5'-CAA ATG TTG CTT GTC TGG TG-3'	SEQ ID NO: 24
MyHC-R: 5'-GTC AGT CGA GTG CAC AGT TT-3'	SEQ ID NO: 25

[0121] To test for the presence of mcm transgene, the following primers were used:

mcm-3798t: 5'-AGG TGG ACC TGA TCA TGG AG-3'	SEQ ID NO: 26
mcm-8346t: 5'-ATA CCG GAG ATC ATG CAA GC-3'	SEQ ID NO: 27
mcm-7338: 5'-CTA GGC CAC AGA ATT GAA AGA TCT-3'	SEQ ID NO: 28
mcm-7339: 5'-GTA GGT GGA AAT TCT AGC ATC ATC C-3'	SEQ ID NO: 29

Tamoxifen injection and tissue collection

[0122] Young mice (14 days old) and adult mice (3-5 months old) were injected once with 40mg/kg of Tamoxifen (Sigma) dissolved in Corn Oil (Sigma). Mice were sacrificed by CO_2 euthanasia or anesthetised with a gaseous mixture of 1.5% Isoflurane (BioMac) and $1.5LO_2$ at various time points after tamoxifen injection. Cardiac arrest was induced by injection of 15% KCl, followed by flushing with PBS to remove blood. Hearts for paraffin embedding were additionally flushed with 4% paraformaldehyde (PFA), left in 4% paraformaldehyde (PFA) overnight, dehydrated in 70% ethanol for at least 24hr and embedded in paraffin. Hearts for cryosection were embedded in tragacanth gum (Sigma), frozen in isopentane (BDH-AnalaR) cooled in liquid N_2, cut 9μm sections by cryostat (Leica CM3050), collected onto charged slides and stored at -20°C for histological and immunofluorescence staining. Hearts for protein and RNA extraction were snap frozen in liquid N2 and stored for further processing.

Cardiomyocyte isolation

[0123] Cardiomyocyte isolation was carried out as per standard protocol [M. Ackers-Johnson et al., Circulation Research 119: 909 (2016)]. Briefly, mice were anaesthetised with isoflurane (100% O_2 at 0.5L/min, isoflurane atomiser dial at 4%). Mice hearts were stopped with 15% KCl, descending aorta was cut and hearts were flushed with 7mL of EDTA buffer into the right ventricle. Ascending aorta was clamped using Reynolds forceps, the entire heart removed and placed in a 60mm dish containing fresh EDTA buffer. Hearts were digested by sequential injections of 10mL EDTA buffer, 3mL Perfusion buffer and 30-50mL Collagenase buffer into the left ventricle. Forceps were used to gently pull the digested heart into smaller pieces ~1mm and gentle trituration. Enzymatic activity was inhibited by addition of 5ml of Stop buffer. Cell suspension was passed through a 100um filter, and four sequential rounds of gravity settling to enrich for myocytes, ultimately obtaining a highly pure myocyte fraction. The myocyte pellet was snap frozen in liquid N_2 and stored a -80°C until further processing.

Histological and immunofluorescence microscopy

[0124] For histological studies, sections (9 μm) were stained with standard Hematoxylin and Eosin for cell morphology, Masson's trichrome stain to detect collagen and TUNEL assay to detect apoptotic nuclei. Images were obtained on a Zeiss Axio Imager Microscope. For immunofluorescence on frozen heart sections, sections were warmed to room temperature, rehydrated with PBS, blocked with M.O.M block (Vector Shields) and donkey serum (Sigma-Aldrich), incubated with primary antibodies overnight at 4°C. The slides were then washed in PBS and incubated with secondary antibodies and Hoechst dye (Sigma-Aldrich) for 60mins, washed with PBS and mounted in Prolong-Gold Anti-fade reagent (Invitrogen). Primary antibodies: LMNA/C N-18 (goat, 1:50, Santa Cruz), Sun1 monoclonal (mouse, neat, from B. Burke), PCM-1 (rabbit, 1:200, Sigma) and sarcomere-α-actinin (mouse, 1:100, abcam); Secondary antibodies were: Alexa Fluor 488, 568 and 647 (1:250, Invitrogen). For isolated cardiomyocyte immunofluorescence, myocytes were stained in suspension and spun down gently for each solution change then plated on glass slides for imaging with a Zeiss LSM510 inverted confocal microscope.

Western analysis for LMNA, SUN1, Ha-tag and GFP.

[0125] Whole Hearts and Quadriceps muscles were homogenized in RIPA lysis buffer and spun at 13,200 g, 10 min, 4 °C. Total cell lysates were electrophoresed and transferred to PVDF membrane and blocked with Odyssey Blocking

Buffer (Li-Cor Biosciences). The membrane was incubated with primary antibodies for 2 h at room temperature. After which, membrane was washed in TBST washing solution and incubated in Odyssey IRDye secondary antibodies for 1 h before visualization on the Odyssey Infrared Imaging System (Li-Cor Biosciences). The primary antibodies used for detection of LMNA/C (Rabbit, Cell Signalling) that is specific to an epitope in the first 50 amino acids in LMNA, Sun1 monoclonal (mouse, 1:500, Burke) and control beta-tubulin (rabbit, 1:1000, Abcam).

Active force measurement of cardiac papillary muscle.

[0126] Mouse papillary muscle from mouse left ventricle was prepared according to the methods described before [C. N. Toepfer, et al., J Physiol 594: 5237-5254 (2016)]. Briefly, explanted mouse heart was immediately rinsed with oxygenated ice-cold Krebs-Henseleit solution with 12 unit/mL heparin sodium (EDQM) and 30mM 2,3-Butanedione monoxime (BDM, Sigma) and excess blood was removed. After that, the heart was transferred to ice-cold Krebs-Henseleit solution in a glass petri-dish under a dissection microscope with a cooling stage. Cylindrical papillary (200-300 μm in diameter and 1.5-2mm in length) were excised from the left ventricle. T-shaped aluminium clips with a hole were crimped onto the ends of a papillary preparation and the prepared papillary chunks were fixed using pins onto a glass petri-dish with a layer of PDMS sylgard 184 (Dow Corning). Papillary preparations were immersed in a 2% Triton X-100 solution at 4 °C overnight.

[0127] Force measurement was performed as previously described [C. Toepfer et al., J Biol Chem 288: 13446-13454 (2013)]. The T-shaped aluminium clips at the ends of the papillary preparations were attached to the hooks of a force transducer (AE801, HJK Sensoren+Systeme) and servo-motor in the experimental rig and were glued with shellac in ethanol (Sigma) to minimize the movement during the experiment. Papillary contraction force was measured at 20 °C. The max contraction force was measured in activing solution (100mM TES, 6.5Mm MgCl2, 25mM Ca-EGTA, 5.7mM Na2ATP, 20mM Glutathione, 21.5mM sodium creatine phosphate, pH=7.1, Ionic strength is 150 mmol/L) with 32 μmol/L free Ca2+. The data were collected and processed from the force transducer and DAQ data acquisition device (National Instrument) using a customized software programmed by LabVIEW 2013 (National instrument). At least 5 fibres were tested in each mouse, and at least 3 mice were tested for each experimental group.

AAV9-DN-Sun1 and AAV9-GFP virus

[0128] The DN-Sun1 (SS-HA-Sun1L-KDEL) and GFP (SS-GFP-KDEL) vectors were as described [M. Crisp et al., J Cell Biol. 172: 41-53 (2006)]. Briefly, almost the entire lumenal domain of Sun1 was tagged at its NH_2 terminus with HA (HA-Sun1L). To introduce the HA-Sun1L as a soluble form into the lumen of the ER and PNS, signal sequence and signal peptidase cleavage site of human serum albumin was fused onto the NH_2 terminus of HA-Sun1L to yield SS-HA-Sun1L. To prevent its secretion, a KDEL tetrapeptide was fused to the COOH terminus of SS-HA-Sun1L to form the final SS-HA-Sun1L-KDEL. The HA-Sun1L region was replaced with GFP sequence to generate the SS-GFP-KDEL.

[0129] The DN-Sun1 and GFP fragments were amplified with the primers listed below (same forward primer was used for both fragments) and ligated into pENN-AAV-cTnT-PI-eGFP plasmid (kind gift from Dr J. Jian), digested with NcoI and KpnI, to produce Penn-AAV-cTnT-Sun1DN (Figure 10; SEQ ID NO: 3).

aav Sun1 F	5'-CgagaattcacgcgggccgccATGAAGTGGGTAACCTTTATTTC-3'	SEQ ID NO:30
aav Sun1 R	5'-CgggtcgactctagaggtaccttaCTACAACTCATCTTTCTGGATG-3'	SEQ ID NO:31
aav GFP Sun R	5'-CgggtcgactctagaggtacttaCTACAACTCATCTTTGGATCC-3'	SEQ ID NO:32

[0130] All restriction enzymes were purchased from NEB. PCR reactions were conducted using Q5® Hot Start High-Fidelity 2X Master Mix (NEB, M0494L). Ligations were conducted using isothermal assembly with NEBuilder® HiFi DNA Assembly Master Mix (NEB, E2621L). Primers used for constructing the plasmids were ordered from IDT.

[0131] AAV Viruses were produced as per standard protocol [H. Wakimoto, et al., in Current Protocols in Molecular Biology. (John Wiley & Sons, Inc., 2001)]. Materials supplied by R. Foo: pAAV2/9- the trans-plasmid encoding AAV replicase and capsid gene (SEQ ID NO: 2; available from University of Pennsylvania Penn Vector Core); pAdDeltaF6 - the adenoviral helper plasmid (SEQ ID NO: 1) (available from University of Pennsylvania Penn Vector Core); QIAGEN Plasmid Maxi Kit; HEK293T cells (ATCC); Transfection reagent (polyethylenimine e.g., Polysciences). AAV-DJ capsid was obtained from Cell Biolabs, Inc. The pAAV2/9, AAV-DJ, pAdDeltaF6, DN-Sun1 and GFP plasmids were purified using a QIAGEN Plasmid Maxi Kit. HEK293T cells were transfected with the virus combination of pAAV2/9, pAdDeltaF6 and either DN-Sun1 or GFP plasmids. Cells were collected and virus purified by Iodixanol gradient ultracentrifugation.

[0132] The following timeline was used for infection of the mouse hearts. Mice were genotyped at 10 days postnatally. They were then subjected to 1 IP injection of Tmx (40mg/kg of mouse weight) at 14 days postnatally, followed by a concentration of 5×10^10 vg/g AAV9-DN-Sun1 or AAV9-GFP virus injected into the thoracic cavity at 15 days postnatally.

Adult mice (3-5 months old) were injected IP with a single dose of Tmx (40mg/kg of mouse weight), followed by injection of AAV at a concentration of 5×10^10 vg/g AAV9-DN-Sun1 or AAV9-GFP virus into the thoracic cavity. Young and adult mice were anesthetised with a gaseous mixture of 1.5% Isoflurane (BioMac) and 1.5L O_2 before virus injections.

Plasmid construction and generation of Cas9 mRNA and sgRNAs

[0133] pX330 was obtained from Addgene (#42230, Cambridge, MA, USA). The 20nt Sun1 and Syne1 single guide RNA (sgRNA) sequences were designed with the help of CRISPR Design Tool (crispr.genome-engineering.org). A region of the gene of interest was submitted to the tool to identify suitable target sites. Since off-target mutations are possible in CRISPR/Cas9-mediated targeted mutagenesis in the mouse, the CRISPR Design Tool is able to experimentally assess off-target genomic modifications for each gRNA target sites and provide computationally predicted off-target sites for each intended target, ranking the target sequence according to quantitative specificity analysis on the effects of base-pairing mismatch identity, position and distribution. Complimentary oligonucleotides containing the gRNA target sequences were annealed and cloned into the Bbsl site of pX330. Guide RNA sequences were as follows:

5'-GCACAATAGCCTCGGATGTCG-3' for Sun1ΔSUN,	(SEQ ID NO: 33)
5'-CCGTTGGTATATCTGAGCAT-3' for Syne1-stop,	(SEQ ID NO: 34)
5'-GGTTATGGCCGATAGGTGCAT-3' for Tyrosinase4a	(SEQ ID NO: 35)

[0134] These plasmids (pSun1ΔSUN, pSyne1-stop and pTyrosinase4a) were then sequenced to verify correct insertion of the target sequences. For in vitro transcription, PCR was performed to generate the appropriate transcription templates using a common reverse primer (AAAAGCACCGACTCGGTGCC-3'; SEQ ID NO: 36) and gRNA-specific forward primers that encoded the T7 promoter sequence as follows:

Sun1ΔSUN: 5'-TTAATACGACTCACTATAGCACAATAGCCTCGGATGTCG-3' (SEQ ID NO: 37);

Syne1-stop: 5'-TTAATACGACTCACTATAGCCGTTGGTATATCTGAGCAT-3' (SEQ ID NO: 38);

Tyrosinase4a: 5'-TTAATACGACTCACTATAGGTTATGGCCGATAGGTGCAT-3' (SEQ ID NO: 39)

[0135] The gRNA PCR products were then subjected to agarose gel electrophoresis (1.5% agarose) to confirm successful PCR, gel purified and used as templates for in vitro transcription using the MEGAshortscript T7 kit (Life Technologies). The gRNAs were purified using MEGAclear kit (Life Technologies) and eluted in RNase-free water. A sample of purified gRNAs were then subjected to agarose gel electrophoresis for quality checks before injecting into zygotes.

Generation of mutant mice using CRISPR/Cas9

[0136] 3 to 4 weeks old C57BL/6N females were superovulated with Pregnant Mare Serum gonadotropin (Calbiochem, 36722, 5IU/ml). 48 hours later, the females were injected with human chorionic gonadotropin (Sigma, CG10, 5IU/ml) and were mated with C57BL6 males. The following day, fertilized 0.5dpc embryos were collected from the oviducts. Cas9 mRNA (Sigma, CAS9MRNA, 100ng/ul), Tyrosinase4a gRNA (50ng/ul) and gene-specific gRNA (50 ng/ul) were co-injected into the cytoplasm of the embryos in M2 medium (EmbryoMax® Sigma) using a microinjection system (Nikon). Syne1-stop sgRNA were used to derive Syne1 C'T mutant mice and Sun1ΔSUN sgRNA were used to derive Sun1ΔSUN mutant mice. The injected zygotes were cultured in KSOM with amino acids (EmbryoMax® Sigma) in an incubator maintained at 37°C with 5% CO_2 and 5% O_2 for 2 hours before implanting into 0.5dpc pseudopregnant C3H-ICR females.

DNA extraction for genotyping of CRISPR/Cas9 mice

[0137] Mouse tails were clipped and each placed in a 1.5 ml Eppendorf tube. 80 μl of lysis buffer (25 mM NaOH, 0.2 mM EDTA, pH 12) was dispensed into the tube and heated at 95°C for 60 minutes. After heating, the buffer was neutralized with an equal volume of 40 mM Tris-HCl, pH 5. For certain applications, DNA was extracted and purified from mouse tails using DNeasy Blood and Tissue Kit (QIAGEN).

Genotyping of CRISPR/Cas9 mice

[0138] CRISPR modified mutant mice were genotyped by PCR followed by gel electrophoresis using a high resolution agarose (2% MetaPhor agarose, Lonza).

[0139] Primers for Syne1CT'Δ8 mice were:

Forward: 5'-TGCTCCTGCTGCTGCTTATT-3' SEQ ID NO: 40 and
Reverse: 5'- ACATGGTGGAGCATTTGTCTCC -3' SEQ ID NO: 41

[0140] Primers for Sun1 CRISPR mice were:

Forward: 5'-TGACCTTGAGCTGAAACTGC-3' SEQ ID NO: 42 and
Reverse: 5'-TCAGAACACTGGCACACACA-3' SEQ ID NO: 43

[0141] Lmna mutant mice were genotyped as described in Example 1. To determine sequence of CRISPR-induced mutations, PCR products from mouse tail DNA were subjected to TOPO cloning (Zero BluntTM TOPOTM PCR Cloning Kit, 450245, Thermo Fisher Scientific). Plasmid DNA from at least 10 bacterial colonies were isolated using a mini-prep kit (QIAGEN, QIAprepSpin, Miniprep Kit) and sent for Sanger sequencing.

Derivation of myoblasts, fibroblasts and cell culture for CRISPR/Cas9 study

[0142] To isolate myoblasts, limbs were obtained from euthanized mice and muscles were dissected from bone. Tissue digestion was performed by incubating the muscle tissues in enzyme solution consisting of equal volumes of dispase II (Roche, cat. 04942078001) at a concentration of 2.4 U/ml and 1% collagenase II (GIBCO® Invitrogen, cat 17101-015) in a 37°C water bath for 30 minutes, with occasional mixing at 10 minutes interval. After 30 minutes, enzyme solution was neutralized in D10 media (Dulbeco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum). Mixture is then filtered through 70 μm sterile filter (BD FalconTM, cat 352350) and 40 μm sterile filter (BD FalconTM, cat 352340). The suspension was then centrifuged, supernatant removed and subsequently resuspended in F10 media (GIBCO® Invitrogen, cat. 11550043) supplemented with 10 μg/ml bFGF (GIBCO®, cat PHG0264) and plated in 100 mm plates. Mouse adult fibroblasts were allowed to settle for 2 to 3 hours before collecting the supernatant (with floating myoblasts) and replated into 60 mm plates coated with 0.15% Gelatin (Sigma, cat G1393). D10 media was added to the 100 mm plates with MAFs. To terminally differentiate myoblasts to myotubes, the media was changed to DMEM supplemented with 2% horse serum (Thermo Fisher Scientific GIBCO®, cat 16050122).

Immunoblotting for CRISPR/Cas9 study

[0143] Whole cell lysates were generated using the Lysis-M kit solution (cOmplete; Roche). Cells were washed in ice-cold PBS and lysed with Roche Lysis M buffer, and centrifuged at 14,000g for 10 minutes to remove cell debris. To extract protein from tissue sample, small slices of tissue were rapidly placed into Lysing Matrix D tubes (MP Biomedicals), and snap frozen in liquid nitrogen. After snap freezing, the tubes were either stored at -80°C or used directly for protein analysis. Protein extraction buffer (50 mM Tris (pH7.4), 500 mM NaCl, 0.4% SDS, 5 mM EDTA (pH7.4), 1x Protease inhibitor (cOmpleteTM EDTA-free Protease Inhibitor cocktail, Cat no. 04693159001, Roche), 2% Triton, 1 mM Dithiothreitol, in distilled water) was added to tissues, which were then homogenized using the FastPrepTM-24 Instrument (MP Biomedicals). Samples were then centrifuged at 14,000g for 10 minutes to remove cell debris. Protein concentration was quantified using bicinchoninic acid (BCA) protein kit (Bio-Rad) before loading protein samples onto a polyacrylamide gel to ensure equal amounts were being analyzed. All protein samples were resolved by SDS-PAGE gel analysis and transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore) by wet transfer for 48 hours at 20V at 4°C. Membranes were blocked in TBS containing 0.1% Tween 20 (TBST) supplemented 5% milk powder (Anlene) for 1 hour at room temperature. Western Blot analysis was performed using primary antibodies diluted in 5% milk powder (diluted in TBST). Membranes were incubated for 2 hours at room temperature or overnight at 4°C. For secondary antibodies, horseradish-peroxidase (HRP) (Invitrogen) conjugated antibodies were used for chemiluminescent imaging. The membranes were incubated for 1 hour at room temperature with the secondary antibodies. For immunoblots visualized by chemiluminescence, membranes were incubated in ECL substrate (Pierce) for 1 minute before being exposed to a chemiluminescence sensitive film (Thermo Scientific) and subsequently processed.

Immunofluorescence for CRISPR/Cas9 study

[0144] Cells were grown in 8-well slides (Ibidi) and fixed in ice-cold methanol for 15 minutes at -20°C. They were then rinsed in PBS twice and permeabilized and blocked with 0.1% Triton X, 3% BSA in PBS for 15 minutes at room temperature. The fixed and permeabilized cells were then rinsed in PBS three times. Samples were then incubated with primary

antibodies (Table 2) for 2 hours at room temperature or overnight at 4°C. Samples were then washed with PBS three times and subsequently incubated with secondary antibodies (Life Technologies) and DAPI (Life Technologies) for 1 hour at room temperature. After three washes in PBS, cells were mounted in Anti-fade (1% DABCO, 90% Glycerol, 10% PBS) and inspected using a Zeiss 510 Meta Confocal microscope or Axiovert 200 inverted epifluorescence microscope (Zeiss). Images were recorded and analysed using Zeiss ZEN, Metamorph or Image J (NIH) software.

Table 2: Antibodies used for immunofluorescence study.

Antibody	Type and Source	.Concentration
Akap450, HPA-026109	Polyclonal, Sigma	.1:500
MF20	Monoclonal, DSHB	1:25.
Nesp1 (MANNES1A)	Monoclonal, Glenn Morris	1:1000 (Western) 1:50 (IF)
Nesp1-C'T	Monoclonal, Brian Burke	Undiluted supernatant
LaminA, ab8984	Monoclonal, Abeam	1:200
LaminA, SSD	Monoclonal, Brian Burke	1:200
Pcm-1, HPA-023374	Polyclonal, Sigma	1:100
Pcnt, ab4448	Polyclonal, Abcam	1:100
Sun1-9F10	Monoclonal, Brian Burke	1:200
Sun2-3.1E	Monoclonal, Brian Burke	1:500
Nesprin-2	Polyclonal, MyBiosource.com	1:500

Mouse genetics

[0145] Lmna mice and tamoxifen injection were described in Example 1. To obtain $Lmna^{\Delta/\Delta}$:$Syne1^{C'T\Delta8/C'T\Delta8}$ and $Lmna^{Fix/Flxmcm}$:$Syne1^{C'T\Delta8/C'T\Delta8}$ double mutant mice, $Lmna^{\Delta/+}$ or *Lmna*$^{Flx/Flxmcm}$ mice were intercrossed with $Syne1^{C'T\Delta8/C'T\Delta8}$ mice. In the Syne2 mouse model, a IRES-β-gal neomycin selectable cassette (PgkNeo) flanked by loxP sites was inserted into the Syne2 gene, resulting in deletion of part of exon 102 and all of exons 103-104. The neomycin cassette was subsequently removed by crossing with Cre recombinase mice. $Syne1^{C'T\Delta8/+}$ or $Syne1^{C'T\Delta8/C'T\Delta8}$ mice were crossed with $Syne2^{+/-}$ or $Syne2^{-/-}$ mice to obtain mice with mutant Syne1 and Syne2 alleles, which were intercrossed to obtain double mutant mice. Kaplan-Meier method was used to draw the survival curves.

Human guide RNA sequences

[0146] Potential guide RNA sequences to disrupt human SYNE1 KASH domain or SUN1 SUN domain were determined using CRISPR tool in Benchling software (Benchling Inc. USA) and are shown in Table 3.

Table 3: Potential guide RNA sequences to target final exons in human SYNE1 (Nesprin-1) or SUN1 genes

Gene Name	ENSEMBL gene ID	Chromosome	Position	Strand	Sequence	PAM	CRISPR enzyme	SEQ ID NO:
SYNE1	ENSG00000131018	6	515330	-	TCGTGTATCTGAGCATGGGG	TGGAAT	saCas9	44
SYNE1	ENSG00000131018	6	515335	-	GCCATTCGTGTATCTGAGCA	TGGGGT	saCas9	45
SYNE1	ENSG00000131018	6	515340	+	TCCACCCCATGCTCAGATAC	ACGAAT	saCas9	46
SYNE1	ENSG00000131018	6	515320	-	GAGCATGGGGTGGAATGACC	GGG	spCas9	47
SYNE1	ENSG00000131018	6	515321	-	TGAGCATGGGGTGGAATGAC	CGG	spCas9	48
SYNE1	ENSG00000131018	6	515330	-	TCGTGTATCTGAGCATGGGG	TGG	spCas9	49
SYNE1	ENSG00000131018	6	515333	-	CATTCGTGTATCTGAGCATG	GGG	spCas9	50
SYNE1	ENSG00000131018	6	515334	-	CCATTCGTGTATCTGAGCAT	GGG	spCas9	51
SYNE1	ENSG00000131018	6	515335	-	GCCATTCGTGTATCTGAGCA	TGG	spCas9	52
SYNE1	ENSG00000131018	6	515345	+	CCCATGCTCAGATACACGAA	TGG	spCas9	53
SYNE1	ENSG00000131018	6	515333	+	CCCGGTCATTCCACCCCATG	TTTG	Cpf1	54
SUN1	ENSG00000164828	7	873276	+	TTTTTCTAACTGGGGCCATC	CTGAGT	saCas9	55
SUN1	ENSG00000164828	7	873285	-	CCGATACAGACAGGTATACT	CAGGAT	saCas9	56
SUN1	ENSG00000164828	7	873266	+	AACTTCGGATTTTTTCTAAC	TGG	spCas9	57
SUN1	ENSG00000164828	7	873267	+	ACTTCGGATTTTTTCTAACT	GGG	spCas9	58
SUN1	ENSG00000164828	7	873268	+	CTTCGGATTTTTTCTAACTG	GGG	spCas9	59
SUN1	ENSG00000164828	7	873280	-	ACAGACAGGTATACTCAGGA	TGG	spCas9	60
SUN1	ENSG00000164828	7	873296	+	CTGAGTATACCTGTCTGTAT	CGG	spCas9	61
SUN1	ENSG00000164828	7	873281	+	TTCTAACTGGGGCCATCCTG	TTTT	Cpf1	62
SUN1	ENSG00000164828	7	873282	+	TCTAACTGGGGCCATCCTGA	TTTT	Cpf1	63
SUN1	ENSG00000164828	7	873283	+	CTAACTGGGGCCATCCTGAG	TTTT	Cpf1	64
SUN1	ENSG00000164828	7	873284	+	TAACTGGGGCCATCCTGAGT	TTTC	Cpf1	65

PAM, protospacer adjacent motif; saCas9, *Staphylococcus aureus* Cas9; *spCas9,_Streptococcus pyogenes* Cas9; Cpf1, CRISPR from *Prevotella* and *Francisella 1.*

Statistical analysis

[0147] All statistical analysis was performed using Graphpad Prism software.

Example 2

Cardiomyocyte specific loss of *Lmna* results in the rapid onset of heart failure

[0148] To further define the interaction between *Sun1* and *Lmna* in postnatal pathology in mice, we specifically ablated the *Lmna* gene in different tissues by using a conditional $Lmna^{Flx/Flx}$ line of mice (Figure 33), that when recombined by Cre activation, results in the complete loss of LaminA/C protein [A. S. Wang, et al., Differentiation; research in biological diversity, (2015); I. Solovei et al., Cell 152: 584-598 (2013)]. When $Lmna^{Flx/Flx}$ was constitutively deleted in all tissues by crossing the $Lmna^{Flx/Flx}$ mice with *Zp3-Cre* mice [W. N. de Vries et al., Genesis 26: 110-112 (2000)], the mean postnatal lifespan was 17.5 days (Fig 26A). When the same deletion was induced in the absence of *Sun1,* the $Lmna^{\Delta/\Delta}$*:Sun1*$^{-/-}$ mice lived to a mean of 32.5 days, almost a doubling in longevity (Fig 26A). Performing the same *Lmna* deletion on a *Sun2* null background did not extend the longevity of $Lmna^{\Delta/\Delta}$ mice, revealing the longevity extension is specific to the loss of *Sun1* (Fig 36). Since the A-type lamins are widely expressed in almost all adult tissues, we then determined to what extent, *Lmna* deletion, specifically in cardiomyocytes, contributes to the early postnatal death of $Lmna^{\Delta/\Delta}$ mice. Furthermore, the inventors wished to ascertain whether loss of *Sun1* would increase longevity in these mice harbouring *Lmna* deficient cardiomyocytes. We first crossed the $Lmna^{Flx/Flx}$ with a constitutive myh6 Cre [R. Agah et al., J Clin Invest 100: 169-179 (1997)], in which Cre expression, though constitutive, is restricted to cardiomyocytes but commences during embryogenesis. These mice survived slightly longer than the $Lmna^{\Delta/\Delta}$ to an average of 26.5 days postnatally (Figure 26C). When the same cardiomyocyte specific deletion was performed on a *Sun1*$^{-/-}$ background, this resulted in a significant increase in longevity to at least 6 months and beyond after birth (Figure 26C). To further define the loss of *Lmna* and its effect in postnatal/adult cardiomyocytes we derived mice homozygous for the $Lmna^{Flx/Flx}$ allele carrying the inducible cardiomyocyte specific Cre Tg(Myh6-cre/Esr1), (here abbreviated to *mcm)* in which Cre is induced by a single injection of tamoxifen (Tmx) [D. S. Sohal et al., Circ Res 89: 20-25 (2001)]. From this cross, the average lifespan of 3-5 month old $Lmna^{Flx/Flx:mcm}$ mice, following Cre induction was 27 days (Figure 27A). Controls were unaffected by Tmx injection. PCR and immunofluorescence analysis confirmed the *Lmna* deletion was specific to the $Lmna^{Flx/Flx:mcm}$ cardiomyocytes, with no detectable recombination occurring in the brain, diaphragm, lung, liver and skeletal muscle, or in wild-type control animals (Figure 27B). By 21 days post injection, $Lmna^{Flx/Flx:mcm}$ mice showed laboured breathing, a dishevelled, ungroomed appearance, increased lethargy and kyphosis (Figure 27C). Immunofluorescence analysis of isolated cardiomyocytes (CM) and sections of $Lmna^{Flx/Flx:mcm}$ hearts showed reduced levels of LaminA protein and cardiomyocyte nuclei without any LaminA expression (Figure 27D). LaminA protein levels were decreased 3.5 fold in $Lmna^{Flx/Flx:mcm}$ hearts after Cre induction compared to uninduced $Lmna^{Flx/Flx:mcm}$ and $Lmna^{+/+/\,mcm}$ hearts (Figure 27E). By sampling $Lmna^{Flx/Flx:mcm}$ mice at specific time points after Tmx injection, it was estimated that it takes 7-14 days after Cre induction for LMNA protein levels to fall by 50% (data not shown), a rate consistent with a study using siRNA *LMNA* knockdown in human fibroblasts by 1.3-fold after 48 hrs and a further 4-fold reduction after 10.5 days [A. Buchwalter and M. W. Hetzer, Nature communications 8: 328 (2017); T. Sieprath et al., Nucleus 6: 236-246 (2015)]. Echocardiograms (ECGs) performed at 21 days after Cre induction revealed poor cardiac contractility in $Lmna^{Flx/Flx:mcm}$ mice compared to $Lmna^{Flx/Flx:mcm}$ controls (Figure 28A). There was a significant reduction in the Ejection Fraction (EF%) and Fractional shortening (FS%) ($P<0.0001$) (Figure 28B). The left systolic and diastolic ventricular internal diameters (LVID) were enlarged, compared to $Lmna^{Flx/Flx:mcm}$ controls (Figure 28B). Significantly fewer viable (brick-like) cardiomyocytes were isolated from $Lmna^{Flx/Flx:mcm}$ +Tmx hearts compared to $Lmna^{Flx/Flx:mcm}$ controls (Figure 28C). Visual analysis revealed the isolated cardiomyocytes from $Lmna^{Flx/Flx:mcm}$ +Tmx hearts contained large intracellular vacuoles (Figure 28C). Histological analysis of $Lmna^{Flx/Flx:mcm}$ +Tmx hearts, revealed infiltration of nucleated cells and increased intercellular spaces between cardiomyocytes compared to $Lmna^{Flx/Flx:mcm}$ control hearts (Figure 28D). The left ventricular lumen in $Lmna^{Flx/Flx:mcm}$ +Tmx hearts was significantly enlarged, together with significantly increased levels ($P=0.0098$) of fibrosis were noted in $Lmna^{Flx/Flx:mcm}$ +Tmx hearts compared to in $Lmna^{Flx/Flx:mcm}$ controls (Figure 28D). Increased numbers of apoptotic cells were also identified in $Lmna^{Flx/Flx:mcm}$ +Tmx hearts compared to control hearts (Figure 28D). However there was no evidence of extensive DNA damage detectable in the cardiomyocytes, as assessed by Rad51, MRE11, H2AX phosphor-Ser and 53BP1 immunostaining (Data not shown).

Example 3

Deletion of *Sun1* ameliorates cardiac pathology induced by *Lmna* loss

[0149] Mice with *Lmna* mutations show a significant increase in longevity and health in the absence of *Sun1* [C. Y.

Chen et al., Cell 149: 565-577 (2012)]. As described, induced deletion of *Lmna* in cardiomyocytes ($Lmna^{Flx/Flx:mcm}$ +Tmx) results in death within 1 month post Cre induction (Figure 26C). Strikingly, when the same deletion was induced on a *Sun1* null background the mice survived for more than 1 year after Cre induction (Figure 26C). Hearts from $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$+Tmx mice, 3 weeks after induction, were compared to those from $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$+Tmx to determine the extent to which SUN1 loss ameliorated the pathological changes induced by *Lmna* loss in cardiomyocytes. Immunofluorescent imaging for Lamin A/C identified many elongated and distorted nuclei. In some of these, residual Lamin A/C was displaced to one pole of the nucleus (Figure 29A panel 1 and insert) in the $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$+Tmx hearts. In contrast in the $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$ +Tmx hearts, while there were many elongated nuclei, these showed few if any distortions, even when there was no Lamin A/C staining (Figure 29A panel 3 yellow arrow heads). Western analysis of whole hearts revealed a significant reduction in Lamin A/C in the $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$ +Tmx hearts (P=0.0359) lysates compared to $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$ controls (Figure 29A lower panels). The $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$+Tmx cardiomyocyte nuclei exhibited increased longitudinal length, together with a segmented appearance, with the segments connected by narrow bridges (Figure 29A and C, panel 1 arrows). However, in the absence of *Sun1,* $Lmna^{FlxlFlxmcm}Sun1^{-/-}$ cardiomyocyte nuclei exhibited no abnormalities or segmentation (Figure 29C panels 3 and 4). In total, 70% of cardiomyocytes in $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$ mice had ruptured or misshapen nuclei compared to fewer than 1% of the cardiomyocytes from the $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$ (Figure 29C panel 5).

[0150] Clear enlargement of the left ventricle (LV) was evident in the $Lmna^{Flx/Flx:mcm}Sun1^{+/+}$ mice but not in the LVs of the $Lmna^{Flx/Flxmx:mcm}Sun1^{-/-}$ +Tmx hearts (Figure 29B, panels 1 and 2). The $Lmna^{Flx/Flx:mcm}$ $Sun1^{+/+}$ hearts exhibited significantly increased levels of fibrosis (P<0.0001) compared to controls, whereas there was no significant fibrosis in the $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$ hearts (Figure 29B, panels 3-5).

[0151] As a model for ventricular muscle mechanics we measured the active force in cardiac papillary muscle. The active force was significantly reduced by 66% in $Lmna^{Flx/Flx:mcm;}Sun1^{+/+}$+Tmx papillary muscle (P=0.0028) compared to $Lmna^{Flx/Flx:mcm}$ $Sun7^{+/+}$+CTL. In the absence of SUN1, $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$+Tmx cardiac papillary active force was maintained at levels not significantly different from those of controls (Figure 29B panel 6).

[0152] Echocardiograms performed before and after Cre induction revealed progressive worsening of cardiac contractility in the $Lmna^{Flx/Flx:mcm}$ $Sun1^{+/+}$ +Tmx mice compared to $Lmna^{Flx/Fl:mcm}Sun1^{-/-}$+Tmx mice (Figure 29D). Loss of SUN1 preserved both EF, FS and Global Longitudinal Strain (GLS) (GLS is a separate parameter used to assess myocardial contractility, and is a better predictor of heart failure) in $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$ + Tmx mice compared to $Lmna^{Flx/Flx:mcm}$ $Sun1^{+/+}$ +Tmx mice.

[0153] PCR analysis of the aged $Lmna^{Flx/Flx:mcm}Sun1^{-/-}$+Tmx hearts 12-14 months after Tmx injection confirmed the sustained deletion of *Lmna* gene (Figure 37C), while protein quantification revealed a significant reduction of LMNA levels in $Lmna^{Flx/Flx:mcm}$ $Sun1^{-/-}$+ Tmx hearts 12-14 months after Tmx (Figure 37D). Histological analysis of the 12-14 month $Lmna^{Flx/Flx:mcm}$ $Sunt1^{-/-}$+ Tmx hearts revealed no significant increase in fibrosis compared to controls (Figures 37A and B). However, echocardiograms on these aged mice showed reduced EF and FS in both $Lmna^{Flx/Flx:mcm}$ $Sun1^{+/+}$ + CTL and $Lmna^{Flx/Flxmcm}Sun1^{-/-}$ + Tmx mice (Figure 37E), although the average lifespan of $Lmna^{Flx/Flx}$ mice is 13-14 months (Figure 26C and so the reduced contractile function may have been due to ageing. Together these findings demonstrate that loss of *Lmna,* in adult (2-3 month old) cardiomyocytes is sufficient to result in cardiac failure within 3-4 weeks after Cre activation, but the pathology is strikingly reduced by deleting *Sun1,* with this reduction being sustained for a year.

Example 4

Loss of SUN1 extends longevity of *Lmna* missense mutants

[0154] As most cases of *LMNA* induced DCM result from missense mutations, we determined what effect loss of SUN1 had on the longevity and cardiac function of a previously described *Lmna* mutant mouse line carrying the N195K missense mutation that dies from DCM [L. C. Mounkes, et al., Hum Mol Genet 14: 2167-2180 (2005)], with this mutation having been identified in 2 unrelated patients diagnosed with AD-EDMD [D. Fatkin et al., N Engl J Med 341: 1715-1724 (1999); J. P. van Tintelen et al., Am Heart J 154: 1130-1139 (2007)]. Here too, we found that the absence of SUN1 significantly extended the lifespan of this mutant mouse line with improved cardiac function (Figure 26D). We extended these findings by deriving mice heterozygous for the N195K mutation, with the *WT-Lmna* allele being floxed i.e. $Lmna^{N195K/Flx}\times Sun1^{+/+}$. Inducing the Tmx inducible cardiomyocyte Cre allele in these mice ($Lmna^{N195K/Fix:mcm}$ +Tmx) resulted in the deletion of the WT floxed *Lmna* allele making the cardiomyocytes hemizygous for the $Lmna^{N195K/-}$ mutation. These mice had a mean lifespan of less than 50 days, a longevity half that of the original $Lmna^{N195K/N195K}$ homozygotes (Figure 30A). When the $Lmna^{N195K/Flx:mcm}$ +Tmx mutation was induced on a *Sun1* null background longevity was significantly extended from <50days to >200days (Figure 30A), revealing that loss of *Sun1* is also effective at preventing DCM caused by *Lmna* missense mutations specifically in cardiomyocytes.

[0155] Echocardiograms performed before and after Cre induction revealed progressive worsening of cardiac con-

tractility in the *Lmna*$^{N195K/Flx:mcm}$ *Sun1*$^{+/+}$ mice compared to *Lmna*$^{N195K/Fix:mcm}$ *Sun1*$^{-/-}$ mice (Figure 30B). Loss of SUN1 preserved both EF, FS and Global Longitudinal Strain (GLS) in *Lmna*$^{N195K/-:mcm}$*Sun1*$^{-/-}$ mice compared to *Lmna*$^{N195K/-:mcm}$ *Sun1*$^{+/+}$ mice (Figure 30B).

Example 5

AAV9 mediated transduction and expression of a DNSun1 prolongs the lifespan of the *Lmna*$^{Flx/Flx:mcm}$+Tmx mice.

[0156] The above results demonstrated that genetically ablating SUN1's functions or reducing SUN1 levels could be of therapeutic value in treating DCM. We then tested whether this was due to the complete ablation of SUN1's functions to overcome its toxic over-abundance versus leaving its levels untouched and specifically disrupting its LINC complex-associated role in tethering KASH-domain proteins in the ONM, thereby tethering the nucleus to components of the cytoskeleton. To distinguish between these 2 possibilities, Adenovirus Associated Virus (AAV) was utilized to transduce and express, specifically in cardiomyocytes, a dominant negative SUN1 minigene whose protein product would compete with both SUN1- and SUN2-KASH binding in the cardiomyocyte perinuclear space [M. Crisp et al., J Cell Biol 172: 41-53 (2006)]. A region corresponding to the entire luminal domain of the *Sun1* gene was tagged at its N terminus with an HA (HA-Sun1L) epitope. To localize the resulting protein product to the endoplasmic reticulum (ER) and perinuclear space (between the INM and ONM - PNS), the signal sequence and signal peptidase cleavage site of human serum albumin was fused to the N terminus of HA-Sun1L to yield SS-HA-Sun1L. To prevent the miniprotein's secretion, a KDEL tetrapeptide was linked to the C- terminus of SS-HA-Sun1L, forming SS-HA-Sun1L-KOEL (Figure 34). The signal sequence would ensure the HA-Sun1KDEL accumulates intracellularly within the contiguous peripheral ER and PNS lumen. The cDNA sequence encoding the minigene was fused to the chicken cardiotroponin promoter (cTnT) to ensure the minigene is only transcribed in cardiomyocytes [K. M. Prasad, et al., Gene Ther 18: 43-52 (2011)]. A diagram of how SS-HA-Sun1L (DN-Sun1) displaces the KASH domain proteins from the LINC complex in the PNS to the ER is presented in Figure 15 (third panel) and Figure 31B.

[0157] To verify that the ON-Sun1 functioned in cardiomyocytes (CM) we initially transduced human CMs derived from iPS stem cells using the AAV-DJ system [D. Grimm, et al., J Virol. 82(12):5887-911 (2008)] that provides for a higher infectivity rate in cultured cells than the AAV9 serotype used to transduce the ON-Sun1, under transcriptional control of the cTnT promoter, in the mouse hearts. The ON-Sun1 was effective at displacing Nesprin-1 from the nuclear envelopes in the CMs that were expressing the ON-Sun1 as shown in Figure 31D. Cells expressing high levels and low levels of ON-Sun1 are indicated by grey and white arrowheads respectively. High levels of ON-Sun1 expression resulted in the displacement of Nesprin-1 from the nuclear envelope. This confirmed that the ON-Sun1 was effective at disrupting the LINC complex in CMs.

[0158] We used AAV (serotype 9) to transduce and express the ON-Sun1 minigene in the hearts of postnatal mice by intrathoracic injection. The procedure is summarized in Figure 31A and all mice were sacrificed at 100 days after Tmx injection for analysis. Detection by PCR of the *Lmna* deletion in the hearts confirmed Cre induction by Tmx injection (Figure 31C). To determine the localization and expression levels of the ON-Sun1 minigene, total protein was extracted from half the heart. Western analysis revealed robust expression of both AAV9-DNSun1 and AAV9-GFP control protein (Dose injected: 5×10^10vg/g of mouse) 99 days after AAV injection (Figure 31C) with the expression levels of both proteins being dependent on the dose of viral particles injected (Figure 38). The expression of either AAV9-DNSun1 or AAV9-GFP proteins did not affect LMNA protein levels (Figure 39A). Immunofluorescence analysis revealed that a larger percentage of cardiomyocytes were expressing GFP with 5×10^10vg/g of AAV9-GFP compared to the levels resulting from a 10-fold lower dose of viral particles (5×10^9 AAV9-GFP) (Fig. 39B and Fig. 39C).

[0159] The *Lmna*$^{Fix/Fix:mcm}$ +Tmx mice, injected with AAV9-GFP control, lived an average of 34.5 days after Tmx, whereas *Lmna*$^{Flx/Flx:mcm}$+Tmx mice injected with AA9-DNSun1 (5×10^10 vg/g of mouse) lived significantly longer with the majority surviving at least 100 days after Tmx, before their termination for analysis (P=0.0002) (Figure 20 shows early time period results with male and female mice; Figure 31E shows results at 100 days with separate graphs for male and female mice and mice with different virus injection titre removed). Echo analysis confirmed *Lmna*$^{Flx/Flx:mcm}$ +Tmx+AAV9-DNSun1 hearts were functioning better than *Lmna*$^{Flx/Flx:mcm}$ +Tmx +AAV9-GFP hearts at 35 days post Tmx (Figure 31G). Although the *Lmna*$^{Flx/Flx:mcm}$ +Tmx+AAV9-DNSun1 mice were alive at 100 days after induction both EF% and FS% were significantly lower compared to control *Lmna*$^{Flx/FlxWT}$+Tmx mice (Figure 31G). At 35 days post Tmx, increased fibrosis was detected in both the *Lmna*$^{Flx/Flx:mcm}$ +Tmx+AAV9-DNSun1 and *Lmna*$^{Flxx/Flxxmcm}$+Tmx+AAV9-GFP hearts (Figure 31F), although fibrosis in the *Lmna*$^{Flx/Flx:mcm}$ +Tmx+AAV9-DNSun1 hearts was significantly lower than in *Lmna*$^{Flxx/Flxxmcm}$+Tmx+AAV9-GFP hearts (Figure 31F lower panels).

Example 6

Disruption of the LINC complex in mice using CRISPR/Cas9

[0160] Mice harboring a variety of Lmna mutations, both global and cardiac-specific, show a significant increase in longevity and health in the absence of Sun1 [(Chen et al., Cell 149: 565-577 (2012) and Examples 2-4]. Prior to the findings described in Examples 2-5, the mechanism of this rescue was unclear, but was speculated to be due to the toxic effects of excess Sun1 in Lmna mutants [Chen et al., Cell 149: 565-577 (2012)]. AAV-mediated expression of a dominant negative LINC-complex-disrupting transgene ameliorates the pathology associated with *Lmna* mutation [Example 5]. The findings in Examples 2-5 are consistent with the idea that LINC complex function, rather than excess Sun1, is the molecular driver of Lmna pathology. This was surprising, as genetic disruption of the LINC complex via loss of Sun1 and Sun2 [K. Lei et al., Proc Natl Acad Sci USA 106: 10207-10212 (2009)], or cardiac-specific disruption of Nesprin-1 and Nesprin-2 [Banerjee et al., PLOS Genet 10(2): e1004114 (2014)], in mice, resulted in various pathologies.

[0161] To develop alternative means of disrupting the LINC complex *in vivo,* the possibility of using CRISPR/Cas9 genome editing to disable the SUN and KASH domains of the proteins constituting the LINC complex was examined. As both the SUN domain and the KASH domain are located at the C-termini of their respective proteins, we hypothesized that a CRISPR guide RNA targeted to the 3' end of the genes encoding SUN or KASH domain proteins would result in a premature stop codon 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. In Example 2, we found that loss of Sun2 did not ameliorate Lmna-associated pathologies. Thus we chose to target the Sun1 SUN domain using CRISPR as Sun1 appears to be the dominant SUN domain protein mediating *Lmna* pathology. Of the KASH domain proteins, only Nesprin-1, Nesprin-2 and Nesprin-3 are broadly expressed [H. F. Horn, Current topics in developmental biology 109: 287-321 (2014)). Nesprin-1 and Nesprin-2 are close paralogues that are functionally redundant. They interact with the actin and microtubule cytoskeleton, whereas Nesprin-3 appears to interact specifically with intermediate filaments [Kim et al., Biol. Chem. 396: 295-310 (2015)]. As we already had Nesprin-2 and Nesprin-3 mutant mouse strains derived by conventional gene targeting available in the laboratory, we chose to target the KASH domain of Nesprin-1 using CRISPR to test the possibility of using CRISPR/Cas9 *in vivo* for treatment of laminopathies.

[0162] The Sun1 gene and the Syne1 gene encoding Nesprin-1 protein were directly targeted *in vivo* by microinjecting C57/Bl6 mouse zygotes with Cas9 mRNA and gRNA targeting the SUN1 (5'-GCACAATAGCCTCGGATGTCG-3'; SEQ ID NO: 66) or KASH1 (5'-CCGTTGGTATATCTGAGCAT-3' SEQ ID NO: 67) domains, followed by implantation into surrogate mothers. Note the SUN1 gRNA targeted Sun1 upstream of the SUN domain so as to ablate the SUN domain. A gRNA (5'-GGTTATGGCCGATAGGTGCAT-3'; SEQ ID NO: 68) targeting the tyrosinase gene was co-injected - progeny that had undergone CRISPR genome editing would have white or mosaic coat color resulting from tyrosinase disruption. These pups were genotyped to confirm successful gene disruption and used as founder animals to establish Sun1 or Nesprin-1 mutant colonies.

Characterization of mutant mice

[0163] Following Sanger sequencing of founder animals and F1 progeny, we focused on characterizing Sun1 mutant alleles (Figure 40A) with a 7 bp deletion (Sun1_del7, or Sun1Δ7; SEQ ID NO: 71) and 4 bp insertion (Sun1_plus4; SEQ ID NO: 70), and a Syne1 (Nesprin-1) mutant allele (Figure 41A) with a 8 bp deletion (Syne1_CTdel8, or Syne1 C'TΔ8; SEQ ID NO: 76). The Sun1 mutant alleles were predicted to produce mRNA with premature stop codons resulting in a truncated Sun1 protein lacking a SUN domain (Figure 40B). Tail tip fibroblasts were isolated from Sun1 homozygous mutant animals. Immunofluorescence staining revealed loss of Sun1 protein (Figure 40C), suggesting that the indels generated by CRISPR caused nonsense-mediated decay of Sun1 mRNA. It is unclear whether the site of mutation, being outside the SUN domain rather than inside the SUN domain, had an effect on the expression of the mutated gene. As we were unable to obtain Sun1 mutant alleles that produced Sun1 protein lacking the SUN domain, instead obtaining essentially Sun1 null animals, we did not further characterize these mutant lines.

[0164] The Syne1 C'TΔ8 allele is predicted to produce a protein where the final 11 amino acids in the wildtype sequence (SEQ ID NO: 77) are mutated and are followed by an additional 50 amino acids encoded by an alternate reading frame (Figure 41B; SEQ ID NO: 78). Immunoblotting performed on Syne1WT and Syne1C'TΔ8 heart and muscle tissues revealed a ~120kDa band in WT corresponding to the striated muscle-enriched Nesprin-1α isoform of the Syne1 gene (Figure 41C, D). In the C'TΔ8 heart and muscle tissues, the presumptive Nesprin-1α polypeptide appeared to be less abundant and of lower electrophoretic mobility than in the wildtype (Figure 41C, D). This is consistent with the 8bp deletion in the Syne1C'TΔ8 allele introducing a novel stop codon downstream, resulting in a protein of higher molecular weight. In addition, a ~1 MOa band likely corresponding to Nesprin-1Giant was observed in heart tissue from both Syne1WT and Syne1C'TΔ8 mice.

[0165] Immunofluorescence analysis of mouse adult fibroblasts (MAFs) derived from 12 week old mice revealed that Nesprin-1 was mis-localized from the nuclear envelope to the cytoplasm in the Syne1C'TΔ8 MAFs (Figure 42A). Similarly, in myotubes, Nesp-1 redistributes to the cytoplasm in the Syne1C'TΔ8 myotubes as compared to Syne1WT (Figure 42B). Other LINC complex and NE proteins such as SUN1, SUN2, Emerin and LaminA remained localized to the NE (Figure 43A-C). Consistent with previous reports [Gimpel et al., Curr. Biol. 27: 2999-3009.e9. (2017)], disruption of Nesprin-1 in myotubes led to mislocalization of centrosomal proteins PCM1, Pent and Akap450 from the myotube nuclear envelope (Figure 44A-C). Mislocalization of Nesprin-1 from the nuclear envelope is consistent with disruption of the Nesprin-1 KASH domain, preventing Nesprin-1C'TΔ8 mutant protein from interacting with the SUN domains of Sun1 and Sun2, which would normally restrict Nesprin-1 to the nuclear envelope. As the transmembrane region is not disrupted, it is likely that Nesprin-1 is mislocalized to the endoplasmic reticulum (ER) in the C'TΔ8 mutant, as the ER and the perinuclear space form a contiguous membrane system.

[0166] Similar to one previously reported Nesprin-1 mouse model [Zhang et al., Development 134(5): 901-8 (2007)], and in contrast to two other models [Puckelwartz et al., Hum Mol Genet 18: 607-620 (2009); Zhang et al., Hum Mol Genet 19: 329-341 (2010)], the disrupted KASH domain of Nesprin-1 results in no overt phenotypic differences between the Syne1 wildtype (WT) and Syne1C'TΔ8 mutant (Figure 45A-B). Both male and female homozygous mutants were fertile with no significant differences in body weight between the Syne1WT and Syne1C'TΔ8 mice (Figure 45C). Syne1C'TΔ8 mice also did not exhibit any growth retardation or obvious muscle dystrophy, nor did they display any difficulty in movement or grooming, which can be indications of muscle deterioration.

[0167] In order to probe the role of other KASH domain proteins in *Lmna* pathology, mice mutant for Syne2, encoding Nesprin-2, were generated by conventional gene targeting (Fig. 46A). To characterize the mutation, immunofluorescence microscopy of tail tip fibroblasts was carried out. Syne2$^{-/-}$ homozygous mutant fibroblasts expressed little to no Nesprin-2 (Fig. 46B). Consistent with previous findings [Zhang et al., Development 134(5): 901-8 (2007)], while Syne2$^{-/-}$ mice were overtly normal, with no growth retardation or infertility, Nesprin-1/2 double mutant mice (Syne1$^{C'T\Delta8/C'T\Delta8}$:Syne2$^{-/-}$) were perinatal lethals (Fig. 46C).

Disruption of Nesprin-1 KASH domain ameliorates Lmna pathologies

[0168] Even though Nesprin-1 was still expressed, Nesprin-1-containing LINC complexes would not be formed in Syne1$^{C'T\Delta8/C'T\Delta8}$ cells and animals. Since AAV-mediated disruption of the LINC complex using dominant negative Sun1 *in vivo* rescues *Lmna* pathologies (Example 5), we reasoned that the "KASH-less" Nesprin-1 mutant allele we generated might also rescue *Lmna* pathology. To test this hypothesis, mice heterozygous for a Lmna null (Lmna$^{\Delta/\Delta}$) allele (Example 1) were intercrossed with Syne1C'TΔ8 mice to obtain Lmna$^{\Delta/\Delta}$:Syne1$^{C'T\Delta8/}$ C'TΔ8 double mutant mice. While Lmna$^{\Delta/\Delta}$ mice lived for 15-17 days, Lmna$^{\Delta/\Delta}$:Syne1$^{C'T\Delta8/}$C'TΔ8 double mutant mice lived for up to 42 days (Figure 24). Lmna null mice heterozygous for the Syne1$^{C'T\Delta8}$ allele did not experience any lifespan extension. Lmna$^{\Delta/\Delta}$ mice on a Syne2$^{-/-}$ homozygous mutant background also did not experience lifespan extension (Figure 47), indicating that Lmna pathology is mediated primarily by Nesprin-1/Sun1 LINC complexes.

[0169] To examine the effect of the Syne1$^{C'T\Delta8/C'T\Delta8}$ allele in mice with cardiac-specific loss of Lmna, mice homozygous for a conditional Lmna$^{Flx/Flx}$ allele carrying the inducible cardiomyocyte specific Cre Tg(Myh6-cre/Esr1) (here abbreviated to mcm), in which Cre is induced by a single injection of tamoxifen (Tmx), were used as described in Examples 1-2. Cardiac-specific deletion of Lmna results in death within a month, but mice with the same deletion induced on a homozygous Syne1$^{C'T\Delta8/C'T\Delta8}$ background lived for at least 120 days after Tmx induction (Figure 25; no change from day 80-120).

Example 7

Method for screening small molecules that block SUN-KASH interactions

[0170] Crystallographic studies of human SUN2 reveal that the SUN domain is assembled as a clover-like trimeric structure [Sosa et al., Cell 149(5): 1035-47 (2012)]. Trimerization is mediated by a triple-helical coiled-coil, with an estimated length of 40-45nm. This is sufficient to bridge the perinuclear space (PNS), allowing SUN and KASH domains to directly interact [Sosa et al., Cell 149(5): 1035-47 (2012)]. The KASH binding site is formed primarily within a groove formed at the interface between adjacent SUN domains (Figure 5B; Figure 21 left panel). This groove accommodates part of the KASH domain, about 18 residues, in an extended conformation. However, it is the C-terminal tetrapeptide of the KASH domain, featuring three proline residues followed by a terminal aliphatic reside, Leu or Thr (for Nesp1 and Nesp2 respectively), that is crucial for the SUN-KASH interaction (Figure 21 right panel, adapted from Figure 1 of Sosa et al, Cell 149(5): 1035-47 (2012). The significance of this tetrapeptide is that it is situated in a well-defined pocket formed within a single SUN monomer. Modification of this peptide in any way, including the addition of a single residue (an Ala) at the C-terminus, completely eliminates the SUN-KASH association over the entire SUN-KASH contact region (Sosa

et al., Cell 149(5): 1035-47 (2012) and Figure 22 left panel). The conclusion is that while stable binding of the KASH domain requires 18-20 residues, it is the C-terminal tetrapeptide that actually initiates binding. Thus, blocking the tetrapeptide binding-pocket within the SUN monomer will abolish SUN-KASH association. We have described in this disclosure an AAV-based gene therapy strategy to break endogenous SUN-KASH interactions as a treatment for laminopathies, including dilated cardiomyopathy. Alternatively, a small molecule that blocks the SUN-KASH interaction at the SUN binding pocket would disrupt LINC complexes and similarly treat laminopathies. A variety of standard methods exist to screen for small molecule drugs *in vitro.*

[0171] An *in vitro* screen can be set up employing recombinant SUN and KASH domains or KASH peptide, for which methods of production have been previously published [Sosa et al., Cell 149(5): 1035-47 (2012)]. One such screen involves an assay technique analogous to an enzyme-linked immunosorbent assay (Figure 22 right panel, similar to Lepourcelet et al., Cancer Cell. 5(1): 91-102 (2004)). Recombinant SUN domain is immobilized on a solid surface, typically in 96-well plates, and then complexed with recombinant KASH domain linked to an enzyme that can generate a colorimetric or chemiluminescent readout. One method for enabling this linkage is to synthesize a biotinylated KASH peptide, which can then be linked with commercially available streptavidin-horseradish peroxidase (HRP) conjugate. Candidate compounds are obtained from appropriate suppliers and screened for their ability to inhibit KASH-SUN associations *in vitro.* Compounds that fail to inhibit the SUN-KASH interaction will result in a well in the plate where the recombinant SUN binds to the enzyme-linked KASH domain. Following wash steps and incubation with colorimetric or chemiluminescent HRP substrates, the presence of the SUN-KASH interaction is detected in standard plate readers. If the compound can inhibit SUN-KASH interaction then, following the wash step, the KASH domain is removed and there would be reduced or no enzymatic reaction in the well.

[0172] Alternatively, fluorescence anisotropy or polarization can be used to screen for small molecule inhibitors of SUN-KASH interactions *in vitro* [Lea, W.A., and Simeonov, A.. Expert Opin Drug Discov 6: 17-32 (2011)]. This assay also employs recombinant SUN and KASH domains. The KASH domain is fluorescently labeled; for example a chemically synthesized KASH peptide could be readily functionalized with a fluorescein moiety. Fluorescence anisotropy of the interacting KASH domain interacting with SUN domain can be measured using standard equipment such as a plate reader. A small molecule inhibitor that disrupts the SUN-KASH interaction can be readily detected as the fluorescence anisotropy of the fluorescent KASH will change if it is not bound to SUN.

[0173] As is typical in drug screening campaigns, the compounds which successfully pass the *in vitro* primary screen will then be subjected to cell-based secondary screens (Figure 23). In this case, immunofluorescence microscopy will be employed to identify those compounds that can dissociate LINC complexes. This is manifest as dispersal of the KASH component to the peripheral endoplasmic reticulum while the cognate SUN protein is retained in the inner nuclear membrane. This microscopy-based assay can be performed first on HeLa cells. Active compounds are then evaluated on cultured cells from disease-relevant tissue, such as cardiac cells. An additional secondary screen may include the ability of the identified compound to rescue proliferation defects in Lmna knockout cells. Following hit-to-lead optimization of the identified compound using standard methods, the compound can be tested in mouse models of laminopathies such as those described herein for *Lmna* dilated cardiomyopathy. Efficacy of the leads can be evaluated using lifespan of the mutant mice and echocardiograms, as described herein, to assess heart function.

DISCUSSION

[0174] DCM caused by *LMNA* is regarded as being aggressive, and often leads to premature death or cardiac transplantation [M. Pasotti et al., J Am Coll Cardiol 52: 1250-1260 (2008); M. R. Taylor et al., J Am Coll Cardiol 41: 771-780 (2003)]. By 60 years, 55% of *LMNA* mutation carriers die of cardiovascular failure or receive a heart transplant, compared with 11% of patients with idiopathic cardiomyopathy. Attempts to ameliorate DCM by fitting a pacemaker have been at best of transient benefit. Consequently it is necessary develop new therapeutic avenues to treat DCM caused by *LMNA* mutations.

[0175] The majority of *LMNA* mutations causing DCM are dominant negative missense. Treatment by conventional gene therapy to repair each mutation would be daunting and removal of the mutated allele, leaving the patient hemizygous for the remaining normal WT allele may also result in heart failure [G. Bonne et al., Nature genetics 21: 285-288 (1999)]. Various other routes downstream of the Lamin gene have been explored for potential therapeutic intervention, and have included mTOR inhibition with rapamycin/rapalogues [J. C. Choi et al., Science translational medicine 4: 144ra102 (2012); F. J. Ramos et al., Science translational medicine 4: 144ra103 (2012)] and inhibition of the MEK1/2 kinase pathway [W. Wu, et al., Circulation 123: 53-61 (2011)]. Both avenues, resulted in improved ventricular function and increased longevity (10-40%) but the extent and long-term efficacy was significantly less than that we observed with the loss of *Sun1.*

[0176] The molecular mechanisms underlying the varied phenotypes of the laminopathies are still not well understood, though two alternative hypotheses have been proposed to explain the tissue-specific pathologies. The first "gene regulation hypothesis" proposes that *LMNA* mutations/loss disrupt the equilibrium of various molecular pathways due to

the mutations altering interactions with NE proteins and chromatin, which in turn alter gene expression. Evidence in support of this hypothesis comes from studies reporting changes in signalling pathways including the AKT-MTOR pathway [J. C. Choi et al., Science translational medicine 4: 144ra102 (2012)], WNT/β-catenin pathway [L. Hernandez et al., Dev Cell 19: 413-425 (2010); C. Le Dour et al., Hum Mol Genet 26: 333-343 (2017)], TGF-β/Smad [J. H. Van Berlo et al., Hum Mol Genet 14: 2839-2849 (2005); T. V. Cohen et al., Hum Mol Genet 22: 2852-2869 (2013)], MAP Kinase pathway [A. Brull, et al., Front Physiol 9: 1533 (2018)] and the ERK1/2 - CTGF/CCN2 pathway [M. Chatzifrangkeskou et al., Hum Mol Genet 25: 2220-2233 (2016)]. While these changes have been documented, none has clearly established whether these changes are not a secondary compensatory effect of a diseased tissue. Sun1 also fits into this rubric of disrupted expression levels as Sun1 protein, but not mRNA, is upregulated in laminopathies, leading to the proposal that laminopathy phenotypes are caused by toxicity from excess Sun1 [C. Y. Chen et al., Cell 149: 565-577 (2012)].

[0177] The second hypothesis suggested *Lmna* loss or mutation leads to increased nuclear fragility. As a result mechanical stress and tension forces transmitted via the LINC complex from the cytoplasm to the NE causes damage to the NE [J. Lammerding et al., J Clin Invest 113: 370-378 (2004)]. This hypothesis is similar to that proposed for Duchenne muscular dystrophy (DMD), where loss of dystrophin increases the fragility of the muscle cell membrane and when tension-stress forces are applied during muscle contraction this results muscle cell rupture and death [D. J. Blake, et al., Physiol Rev 82: 291-329 (2002)]. *Lmna* mutant fibroblasts show nuclear deformation, defective mechanotransduction, and reduced viability when subjected to mechanical strain, together with increased nuclear rupture at low and moderate pressures when compared to WT nuclei [J. Lammerding et al., J Clin Invest 113: 370-378 (2004); J. Lammerding et al., J Cell Biol 170: 781-791 (2005); J. Lammerding et al., J Biol Chem 281: 25768-25780 (2006)]. In contracting mouse cardiomyocytes, mechanical stress and tension forces caused by 500-600 contractions per minute are transmitted to the NE via the LINC complex, resulting in nuclear distortion, damage and eventual death/loss as described in Figures 28 and 29. Presumably, such forces would cause significant damage to the fragile NE of *Lmna* null cardiomyocytes, resulting in CM death. If the tension-stress hypothesis is damaging to the NE, then unlinking the LINC complex, by disrupting SUN1, would reduce the tension-stress on the CM nuclei, and prevent CM cell death in the mutant CMs (Figure 32A-C). One caveat here is that complete disruption of the LINC complex, as would be the case following overexpression of ON-Sun1, could potentially be deleterious rather than therapeutic. At the cellular level, multiple mechanical phenomena including intracellular force transmission, cell polarization and migration, were impacted following LINC complex disruption by dominant negative SUN and KASH constructs [Lombardi et al., J Biol Chem 286(30):26743-53 (2011)]. In animal models, Sun1/Sun2 [Lei et al., Proc Natl Acad Sci 106(25):10207-12 (2009)] and Nesprin-1/Nesprin-2 [Zhang et al., Development 134(5):901-8 (2007)] double mutant mice experience perinatal lethality and cardiac-specific disruption of the KASH domains of Nesprin-1 and Nesprin-2 using an embryonic cardiac Cre driver (Nkx2.5-Cre) results in early onset cardiomyopathy [Banerjee et al., PLOS Genet 10(2):e1004114 (2014)].

[0178] We attempted to distinguish the tension-stress hypothesis from the expression level hypothesis in cardiomyocytes, using a ON-Sun1 construct to compete with endogenous Sun1 and Sun2 proteins for KASH-domain-binding and so unlink the LINC complex without directly altering Sun1 levels (Figures 32D & 34). The AAV9 vector, which has a high affinity for CM, was used to deliver ON-Sun1 under the cTnT promoter to CMs [C. Zincarelli, et al., Mol Ther 16: 1073-1080 (2008)]. Our results showed the successful delivery of GFP to cardiomyocytes (Figure 31C), and robust expression of both the control GFP and ON-Sun1 proteins (Figure 31C) with the latter resulting in the dispersal of the KASH domain proteins from the cardiomyocyte nuclei (Figure 31D). Surprisingly, not only did AAV-DN-Sun1 ameliorate the pathology in mice with depleted cardiac Lmna levels, it also had no discernible effect on the cardiac health of wildtype mice, which would be expected to also experience complete LINC complex disruption in their hearts (Figures 31E & G). This suggests that an intact LINC complex may be required in embryonic development, but not postnatally.

[0179] In addition, using CRISPR/Cas9 in mice, we generated a Syne1 mutant allele (C'TΔ8) that gave rise to a truncated Nesprin-1 protein with a disrupted, non-functional, KASH domain. Mice lacking Lmna globally or in the heart have a shortened lifespan, but the presence of a homozygous $Syne1^{C'T\Delta8/C'T\Delta8}$ mutation resulted in significant lifespan extension. Loss of Sun1 or AAV-mediated disruption of the LINC complex by dominant negative transgenes *in vivo* resulted in similar rescue of *Lmna* pathology (Example 2-5), while Sun2 and Nesprin-2 mutations did not. Taken together, these data suggest that LINC complexes comprised of Sun1 and Nesprin-1 drive the pathology in *Lmna* mutant cells and animals.

[0180] There have been a number of reports on the use of AAV to deliver CRISPR/Cas components *in vivo* for treating diseases. Our results predict that AAV-mediated CRISPR/Cas, such as CRISPR/Cas9, delivery to target the Nesprin-1 KASH domain in disease-affected tissue can be used to treat laminopathies, including dilated cardiomyopathy. For instance, cardiotropic AAVs (e.g. AAV9) can be used to deliver transgene cassette(s) containing a cardiac-specific promoter (e.g. cTnT) driving Cas endonuclease enzyme expression and an appropriate promoter (e.g. U6) driving gRNA expression to treat *LMNA* DCM. Since the packaging capacity of AAV is limited to 4.7 kb, a smaller Cas9 derived from *Staphylococcus aureus* (saCas9) rather than the larger, more commonly used, *Streptococcus pyogenes* Cas9 may be preferred. Alternatively, other CRISPR enzymes such as Cpf1, which is small enough for AAV packaging and has a more commonly found protospacer adjacent motif (PAM) than saCas9, could be used [Zetsche, B., et al., Cpf1 Is a

Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. Cell 163, 759-771 (2015)].

[0181] Guide RNAs would target the 3' region of the Nesprin-1 gene encoding the KASH domain (Table 3). While we have targeted the region adjacent to the stop codon, in principle any gene region encoding the KASH domain could be targeted as indels generated by CRISPR would likely result in frameshift mutations that disable the KASH domain. However, as the final 4 amino acids in the KASH domain are known to be absolutely required for SUN domain interaction and hence LINC complex formation [Sosa et al., Cell 149(5):1035-47 (2012)], it is prudent to select gRNA in the vicinity of the stop codon as even indels that do not result in a frameshift could still mutate the relevant KASH amino acids required for the SUN-KASH interaction. Furthermore, it should be noted that because the Syne1 gene encoding Nesprin-1 is very large and has multiple splice isoforms and alternative start sites, guide RNAs targeted outside the KASH domain, while giving rise to some mutant Nesprin-1 isoforms, may not perturb expression of other isoforms of Nesprin-1 protein, including KASH-containing isoforms. This would result in formation of functional or partially functional Nesprin-1/Sun1 LINC complexes that would still be able to drive pathology in Lmna mutants. This CRISPR/Cas9 strategy likely cannot be extended to the KASH domain of Nesprin-2, since $Lmna^{\Delta/\Delta}$:$Syne2^{-/-}$ mice are phenotypically indistinguishable from $Lmna^{\Delta/\Delta}$ mice.

[0182] We did not further investigate the Sun1 mutant mice generated in this study as instead of mice with Sun1 lacking the SUN domain, we essentially obtained Sun1 null mice, which have already been well characterized. We suspect that inducing CRISPR mutation in Sun1 resulted in nonsense-mediated decay (NMD) of Sun1 transcript. Occurrence of a premature termination codon (PTC) 50-55 nucleotides upstream of a exon-exon junction is a trigger for NMO [Popp, M.W., and Maquat, L.E. Cell 165: 1319-1322 (2016)]. Also, PTCs occurring in the middle of a transcript are more likely to result in NMO [Eberle et al., PLOS Biology 6: e92 (2008); Reber et al., MBoC 29: 75-83 (2018)]. In the Sun1_plus4 mutant, the PTC is more than 55 nucleotides upstream of the exon-exon junction and is likely to trigger NMD. For the Sun1Δ7 mutant, the PTC is less than 50 nucleotides from the exon-exon junction. However for both mutants, since we targeted upstream of the sizeable SUN domain, the PTCs are roughly 2/3 of the way along the length of the transcript, and hence also likely to trigger NMD. In order to specifically disrupt the SUN domain in Sun1 without inducing a null mutation, we can adopt a similar strategy as for Nesprin-1 - directing the guide RNA at the very 3' end of the coding region of the transcript (Table 3). Earlier work demonstrated that mutation of a tyrosine residue to phenylalanine at the C-terminus of SUN2 (Y707F) abolished KASH binding [Sosa et al., Cell 149(5):1035-47 (2012)]. This critical tyrosine residue is conserved in SUN1 (Y812 in Uniprot E9PHI4) and present in the final coding exon of the SUN1 transcript. Selection of a gRNA 5' proximal to the codon for Y812 would produce indel mutations that cause a frameshift mutation that would mutate Y812 and disrupt KASH binding. As the gRNA would be in the final coding exon, the likelihood of triggering NMO would be low. One can thus envision a CRISPR/Cas9-based strategy to treat laminopathies by targeting a critical residue required for KASH-binding in the SUN1 SUN domain. AAVs could be used to deliver CRISPR enzyme and gRNA targeting SUN1 in appropriate disease tissue, such as the heart. Incapacitation of SUN1 KASH binding would then ameliorate the deleterious effects of *Lmna* mutations.

[0183] From these results we propose that the loss of or mutations within *Lmna* causes instability in the CM nuclei due to loss or incorrect assembly of the nuclear lamina. This makes the nuclei susceptible to the tension/stress forces exerted via the LINC complex from the contractile sarcomeres of the CMs. In the absence of SUN1, or following mutation of Nesprin-1 KASH domain, the untethered LINC complexes exert less tensional force on the CM nuclei, enabling survival of the lamin deficient cardiomyocyte.

[0184] These results provide an opportunity to use the AAV-mediated delivery of DN-Sun, ON-KASH, or direct mutation of endogenous SUN or KASH proteins as potential therapeutics for laminopathy-related DCM in patients. The AAV system, as a therapeutic delivery route in patients is established and has been approved by the FDA for treating some diseases. It is becoming more widely used with multiple on-going clinical trials, including the introduction into patients with heart disease. However, even though tension-stress may be the primary cause for *Lmna* deficient CM death, disrupting SUN1 may not be effective in preventing *LMNA* mutation induced cell death in skeletal muscle, as $Lmna^{\Delta/\Delta}$:$Sun^{-/-}$ die at an earlier age than those mice where *Lmna* was specifically deleted in the CMs. Which muscle groups (or even other tissues lacking *Lmna)* result in the early lethality remain to be identified. However in most of the *LMNA* DCM patients it is heart failure that is the cause of death, and our results show that disrupting the LINC complex in CMs could be effective at preventing heart failure for an extended period.

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Claims

1. A nucleic acid molecule comprising an adeno-associated virus expression vector (AAV vector) for use in a method of treating a disease caused by a mutation to *LMNA,* wherein the AAV vector comprises an operably linked transgene, 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, and wherein the 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.

2. The nucleic acid molecule for 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 nucleic acid molecule for use according to claim 1 or claim 2, 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.

4. The nucleic acid molecule for use according to any one of claims 1 to 3, wherein the SUN domain-containing protein is SUN1 or SUN2.

5. The nucleic acid molecule for use according to any one of claims 1 to 4, wherein the C-terminal targeting peptide sequence is a KDEL sequence.

6. The nucleic acid molecule for use according to any one of claims 1 to 5, wherein the AAV vector is a cardiac- or cardiomyocyte-specific expression vector.

7. The nucleic acid molecule for use according to any one of claims 1 to 6, wherein the AAV vector is selected from the group consisting of: AAV9, AAV1, AAV6, AAV8, AAV2i8, and AAV9.45.

8. The nucleic acid molecule for use according to any one of claims 1 to 7, wherein the AAV vector comprises a cardiac-

or cardiomyocyte-specific promoter.

9. The nucleic acid molecule for use according to any one of claims 1 to 8, wherein the AAV 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.

10. The nucleic acid molecule for use according to any one of claims 1 to 9, 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.

11. The nucleic acid molecule for use according to claim 10, 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.

Patentansprüche

1. Nucleinsäuremolekül, das einen Adeno-assoziierten Virusexpressionsvektor (AAV-Vektor) umfasst, zur Verwendung in einem Verfahren zur Behandlung einer Erkrankung, die durch eine Mutation an LMNA verursacht wird, wobei der AAV-Vektor ein operabel gebundenes Transgen umfasst, wobei die Expression des Transgens in einer transfizierten Zelle zum Aufbruch eines Linker-of-Nucleoskeleton-and-Cytoskeleton- (LINC-) Komplexes in der transfizierten Zelle führt und wobei das Transgen Nucleinsäuresequenzen zum Exprimieren der SUN-Domäne eines eine SUN-Domäne enthaltenden Proteins, eine N-terminale Signalsequenz, eine Signalpeptidase-Spaltstelle und eine C-terminale Targeting-Peptidsequenz umfasst, die die Sekretion eines aus dem Transgen exprimierten Peptids verhindert.

2. Nucleinsäuremolekül zur Verwendung nach Anspruch 1, wobei die Expression des Transgens zum Aufbruch der Protein-Protein-Wechselwirkung zwischen SUN und KASH des LINC-Komplexes führt.

3. Nucleinsäuremolekül zur Verwendung nach Anspruch 1 oder Anspruch 2, wobei das Transgen Nucleinsäuresequenzen zum Exprimieren der Lumendomäne des eine SUN-Domäne enthaltenden Proteins umfasst, wobei die Lumendomäne eine Coiled-Coil-Domäne und eine SUN-Domäne umfasst.

4. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 3, wobei das eine SUN-Domäne enthaltende Protein SUN1 oder SUN2 ist.

5. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 4, wobei die C-terminale Targeting-Peptidsequenz eine KDEL-Sequenz ist.

6. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 5, wobei der AAV-Vektor ein Herz- oder Kardiomyozyt-spezifischer Expressionsvektor ist.

7. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 6, wobei der AAV-Vektor aus der aus AAV9, AAV1, AAV6, AAV8, AAV2i8 und AAV9.45 bestehenden Gruppe ausgewählt ist.

8. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 7, wobei der AAV-Vektor einen Herz- oder Kardiomyozyt-spezifischen Promotor umfasst.

9. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 8, wobei der AAV-Vektor einen Herz- oder Kardiomyozyt-spezifischen Promotor umfasst, der aus der aus einem Herztroponin-T-Promotor (cTnT), einem α-Myosin-Schwerketten- (α-MHC-) Promotor und einem Myosin-Leichtketten- (MLC2v-) Promotor bestehenden Gruppe ausgewählt ist.

10. Nucleinsäuremolekül zur Verwendung nach einem der Ansprüche 1 bis 9, wobei die Erkrankung aus der Gruppe ausgewählt ist, die aus Folgendem besteht: einer Herz-Kreislauf-Erkrankung, restriktiver Dermopathie, familiärer partieller Lipodystrophie, mandibuloakraler Dysplasie mit Typ-A-Lipodystrophie, metabolischem Syndrom, Charcot-Marie-Tooth-Krankheit Typ 2, Charcot-Marie-Tooth-Krankheit Typ 2B1 und Erkrankungen, die in normaler Schrift in Tabelle 1 angeführt sind.

11. Nucleinsäuremolekül zur Verwendung nach Anspruch 10, wobei die Herz-Kreislauf-Erkrankung aus der Gruppe ausgewählt ist, die aus Folgendem besteht: Laminopathie, Kardiomyopathie, dilatativer Kardiomyopathie (DCM), dilatativer Kardiomyopathie 1A, dilatativer Kardiomyopathie mit Reizleitungssystemdefekten, Kardiomyopathie mit fortgeschrittenem AV-Block und Arrhythmie, einsamem Vorhofflimmern; Muskeldystrophie, Kardiomyopathie im Zusammenhang mit Emery-Dreifuss-Muskeldystrophie (autosomal dominant), Kardiomyopathie im Zusammenhang mit Emery-Dreifuss-Muskeldystrophie (autosomal rezessiv), Kardiomyopathie im Zusammenhang mit Limb-Girdle-Muskeldystrophie Typ 1B, Kardiomyopathie im Zusammenhang mit kongenitaler Muskeldystrophie; Syndromen des vorzeitigen Alterns, Kardiomyopathie im Zusammenhang mit dem atypischen Werner-Syndrom, Kardiomyopathie im Zusammenhang mit dem Hutchinson-Gilford-Progerie-Syndrom und Erkrankungen, die fettgedruckt in Tabelle 1 angeführt sind.

Revendications

1. Molécule d'acide nucléique comprenant un vecteur d'expression de virus adéno-associé (vecteur AAV) à utiliser dans un procédé de traitement d'une maladie provoquée par une mutation de LMNA, dans laquelle le vecteur AAV comprend un transgène lié de manière fonctionnelle, dans laquelle une expression du transgène dans une cellule transfectée entraîne une perturbation d'un complexe de lieur de nucléosquelette et de cytosquelette (LINC) dans la cellule transfectée, et dans laquelle le transgène comprend des séquences d'acide nucléique pour exprimer le domaine SUN d'une protéine contenant un domaine SUN, une séquence de signal d'extrémité N-terminale, un site de clivage de peptidase de signal et une séquence de peptide de ciblage d'extrémité C-terminale empêchant la sécrétion d'un peptide exprimé à partir du transgène.

2. Molécule d'acide nucléique à utiliser selon la revendication 1, dans laquelle l'expression du transgène entraîne la perturbation de l'interaction protéine-protéine entre SUN et KASH du complexe LINC.

3. Molécule d'acide nucléique à utiliser selon la revendication 1 ou la revendication 2, dans laquelle le transgène comprend des séquences d'acide nucléique pour exprimer le domaine luminal de la protéine contenant un domaine SUN, dans laquelle le domaine luminal comprend un domaine de bobine enroulée et un domaine SUN.

4. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 3, dans laquelle la protéine contenant le domaine SUN est SUN1 ou SUN2.

5. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 4, dans laquelle la séquence peptidique de ciblage d'extrémité C-terminale est une séquence KDEL.

6. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 5, dans laquelle le vecteur AAV est un vecteur d'expression spécifique au coeur ou aux cardiomyocytes.

7. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 6, dans laquelle le vecteur AAV est choisi dans le groupe comprenant : AAV9, AAV1, AAV6, AAV8, AAV2i8 et AAV9.45.

8. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 7, dans laquelle le vecteur AAV comprend un promoteur spécifique au coeur ou aux cardiomyocytes.

9. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 8, dans laquelle le vecteur AAV comprend un promoteur spécifique au coeur ou aux cardiomyocytes choisi dans le groupe comprenant un promoteur de troponine T cardiaque (cTnT), un promoteur de chaîne lourde d'α-myosine (α-MHC) et un promoteur de chaîne légère de myosine (MLC2v).

10. Molécule d'acide nucléique à utiliser selon l'une quelconque des revendications 1 à 9, dans laquelle la maladie est choisie dans le groupe comprenant : une maladie cardiovasculaire, une dermopathie restrictive, une lipodystrophie

partielle familiale, une dysplasie mandibuloacrale avec une lipodystrophie de type A, un syndrome métabolique, une maladie de Charcot-Marie-Tooth de type 2, une maladie de Charcot-Marie-Tooth de type 2B1 et des maladies présentées en caractères normaux dans le tableau 1.

11. Molécule d'acide nucléique à utiliser selon la revendication 10, dans laquelle la maladie cardiovasculaire est choisie dans le groupe comprenant : laminopathie, cardiomyopathie, cardiomyopathie dilatée (DCM), cardiomyopathie dilatée 1A, cardiomyopathie dilatée avec anomalies du système de conduction, cardiomyopathie avec bloc AV avancé et arythmie, fibrillation auriculaire isolée ; dystrophie musculaire, cardiomyopathie associée à la dystrophie musculaire d'Emery-Dreifuss (autosomique dominante), cardiomyopathie associée à la dystrophie musculaire d'Emery-Dreifuss (autosomique récessive), cardiomyopathie associée à la dystrophie musculaire des ceintures de type 1B, cardiomyopathie associée à la dystrophie musculaire congénitale ; syndromes de vieillissement prématuré, cardiomyopathie associée au syndrome atypique de Werner, cardiomyopathie associée au syndrome de Hutchinson-Gilford et maladies présentées en caractères gras dans le tableau 1.

Fig. 1

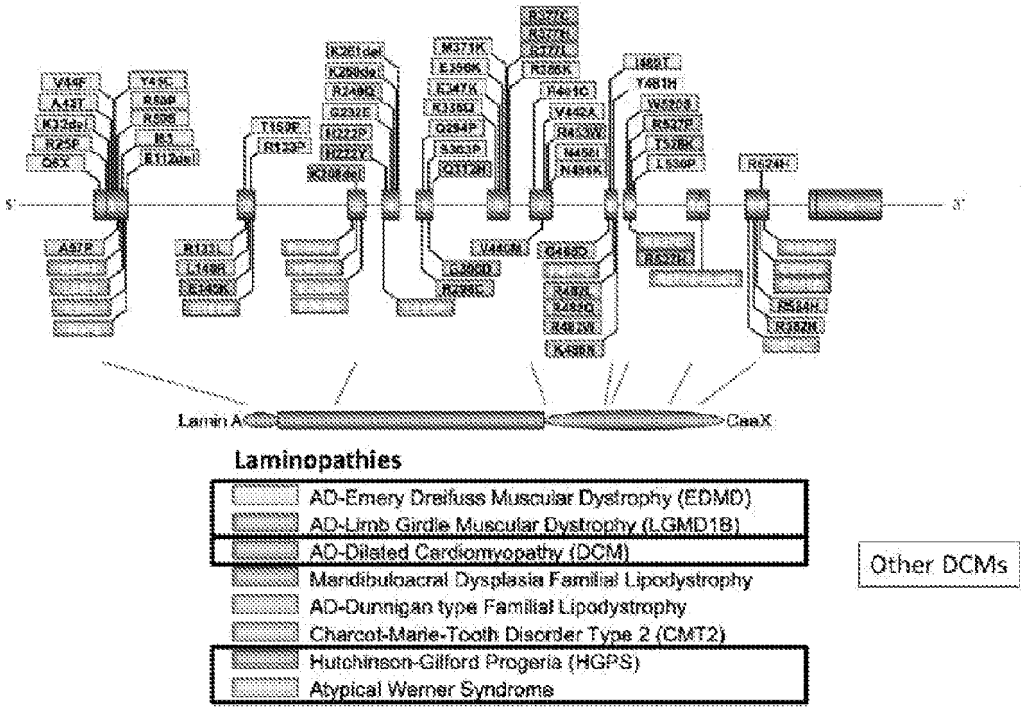

Lamin A
CaaX
Laminopathies
AD-Emery Dreifuss Muscular Dystrophy (EDMD)
AD-Limb Girdle Muscular Dystrophy (LGMD1B)
AD-Dilated Cardiomyopathy (DCM)
Mandibuloacral Dysplasia Familial Lipodystrophy
AD-Dunnigan type Familial Lipodystrophy
Charcot-Marie-Tooth Disorder Type 2 (CMT2)
Hutchinson-Gilford Progeria (HGPS)
Atypical Werner Syndrome
Other DCMs

Fig. 2

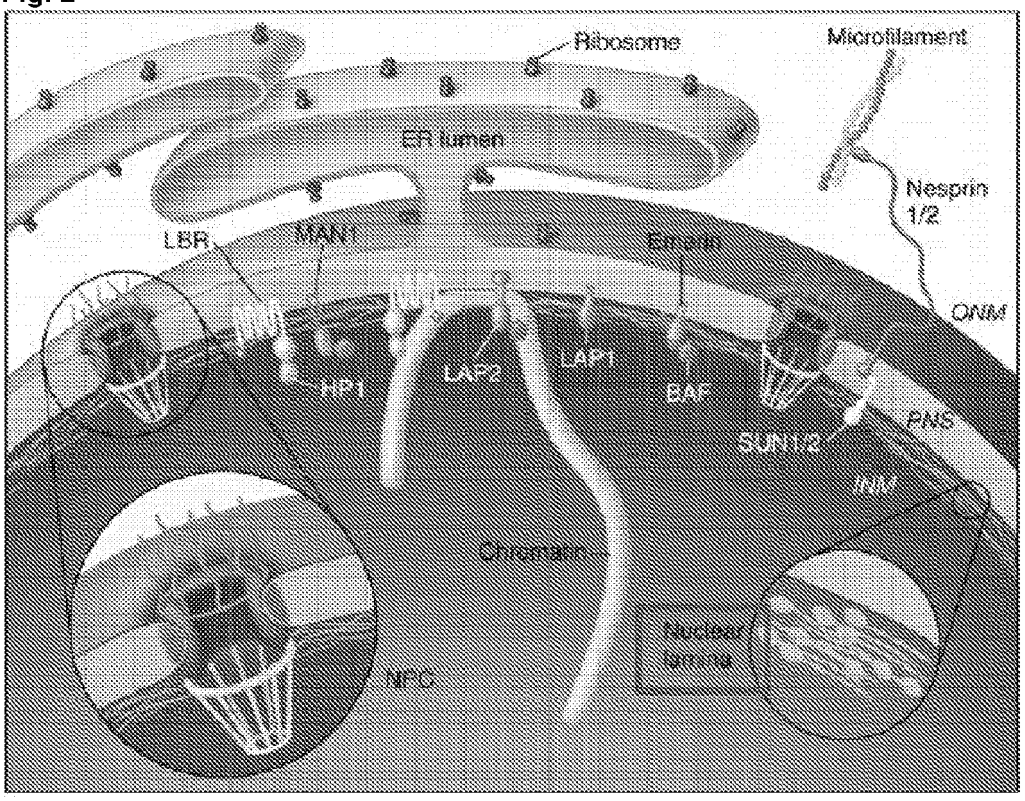

Ribosome
Microfilament
ER lumen
Nesprin 1/2
LBR
MAN1
Emerin
ONM
HP1
LAP2
LAP1
BAF
SUN1/2
PNS
INM
Chromatin
NPC
Nuclear lamina

Fig. 3

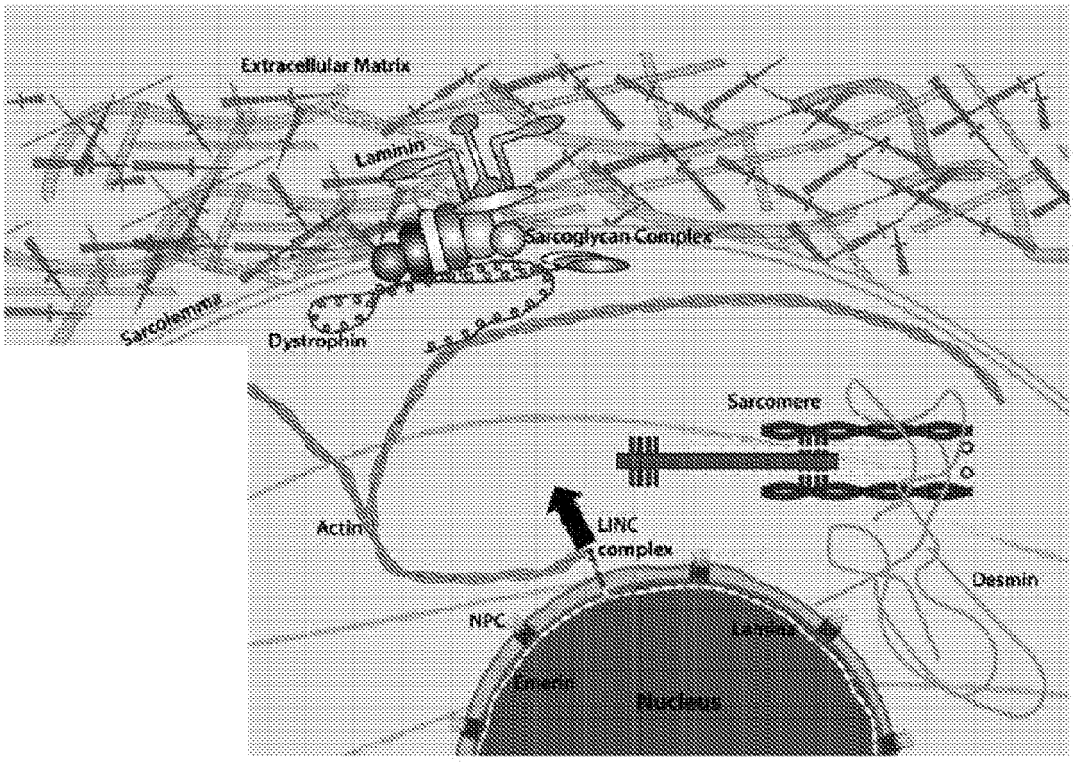

Extracellular Matrix
Laminin
Sarcoglycan Complex
Sarcolemma
Dystrophin
Sarcomere
Actin
LINC complex
Desmin
NPC
Lamina
Nucleus

Fig. 4

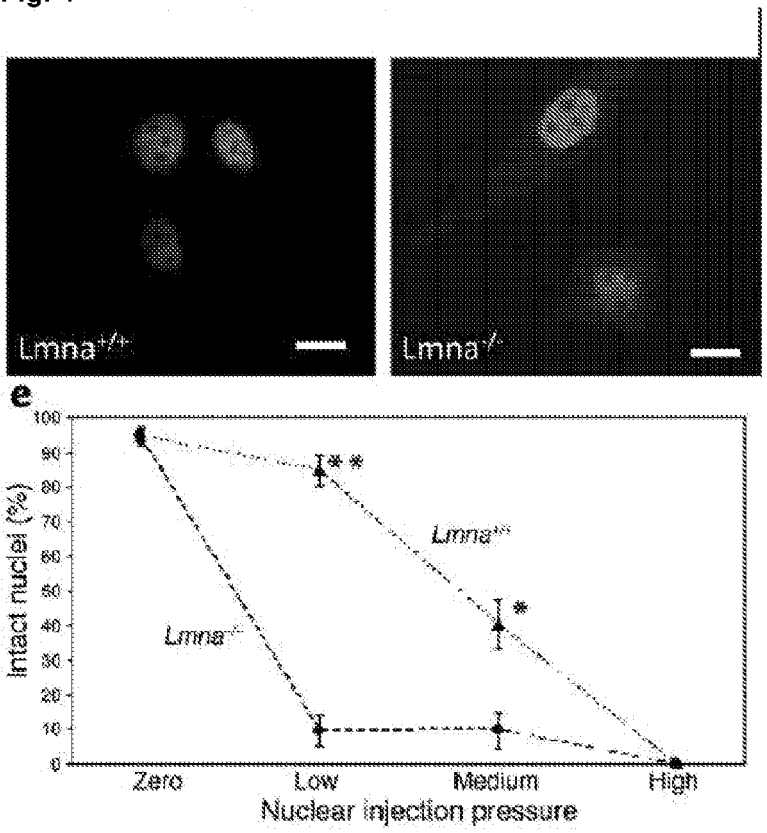

Lmna+/+
Lmna-/-
e
Intact nuclei (%)
Lmna+/+
Lmna-/-
Zero
Low
Medium
High
Nuclear injection pressure

Fig. 5A

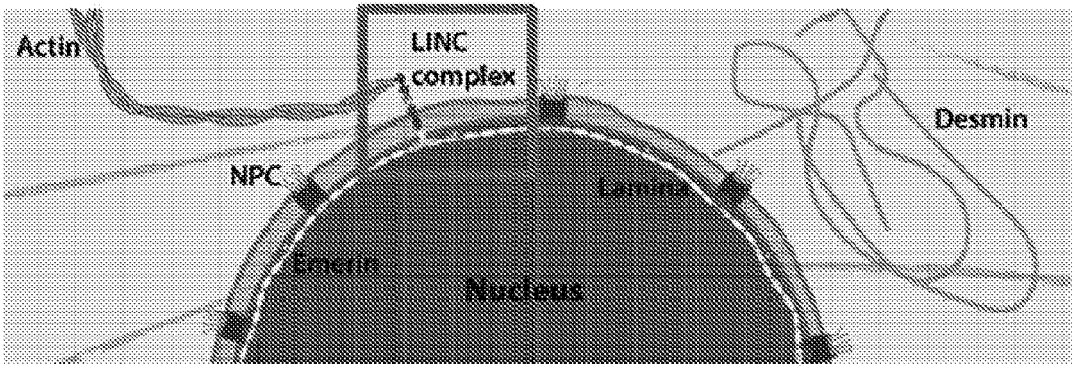

Actin
LINC complex
Desmin
NPC
Lamina
Emerin
Nucleus

Fig. 5B

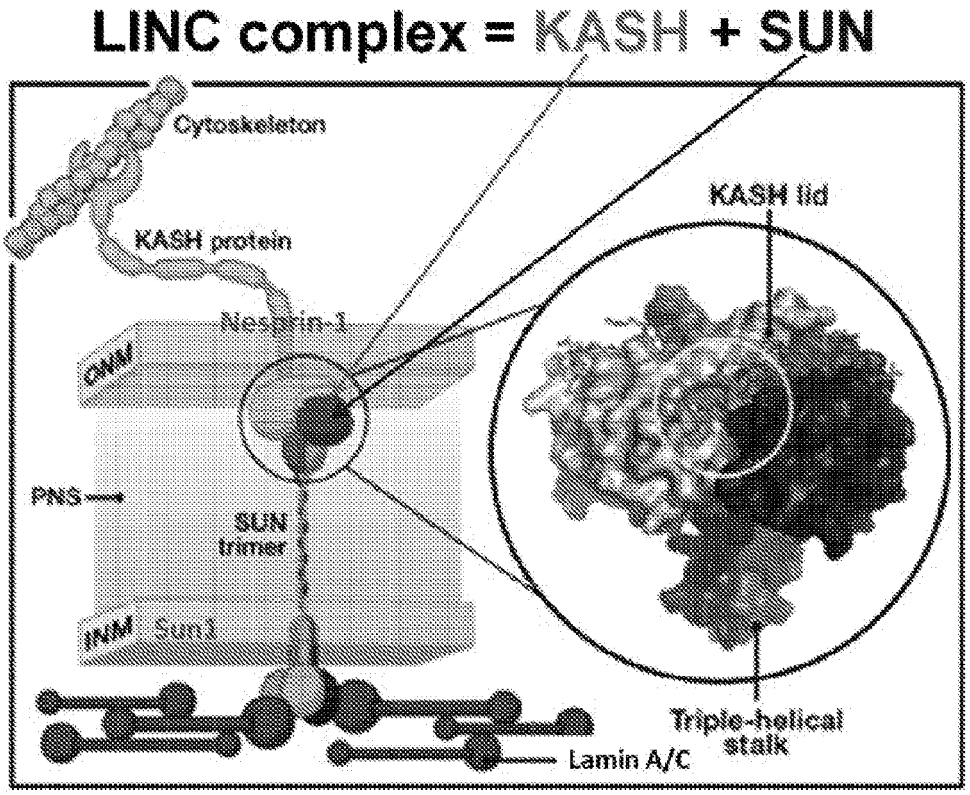

LINC complex = KASH + SUN
Cytoskeleton
KASH protein
Nesprin-1
ONM
PNS
SUN trimer
INM Sun1
KASH lid
Triple-helical stalk
Lamin A/C

Fig. 6A

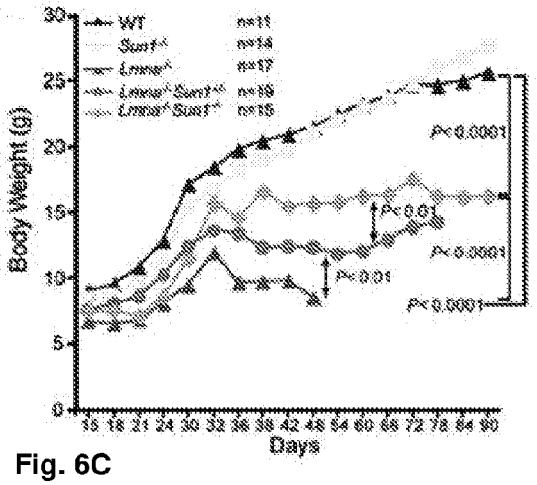


Fig. 6B

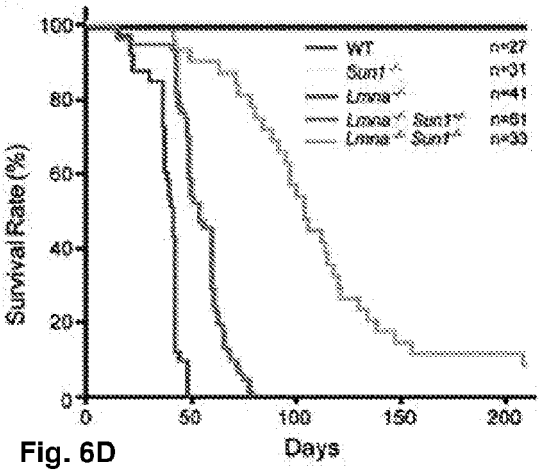


Fig. 6C

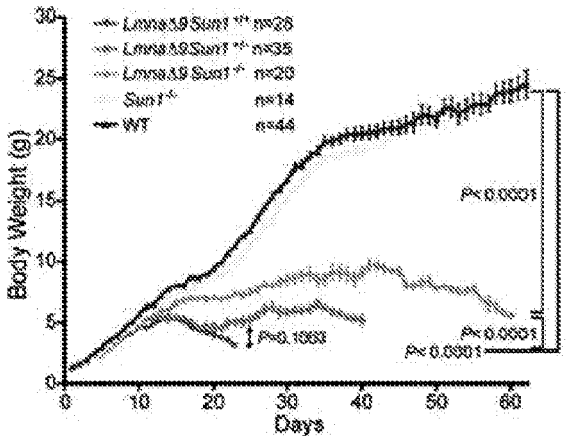


Fig. 6D

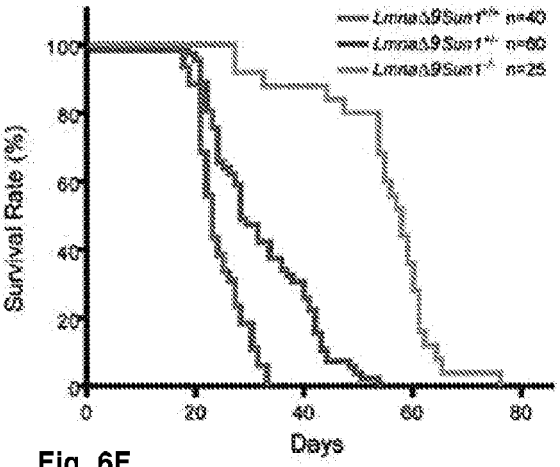


Fig. 6E

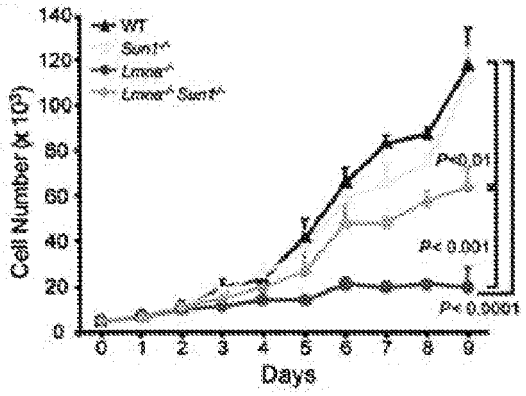


Fig. 6F

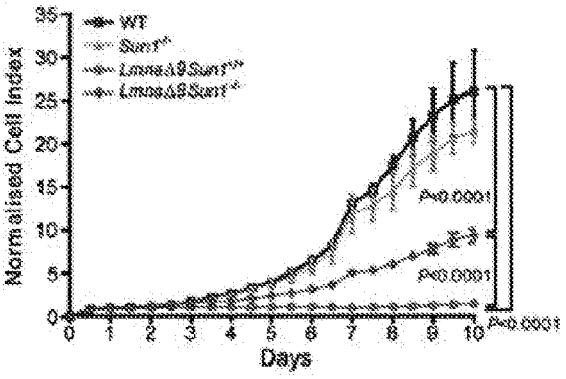


Fig. 7

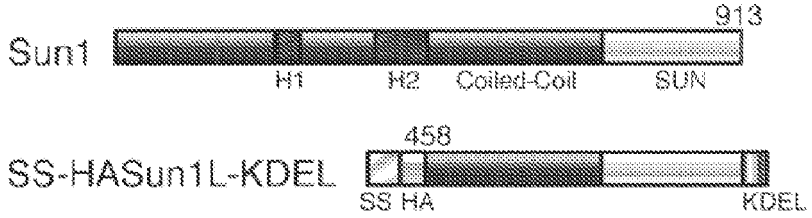

913
Sun1
H1
H2
Coiled-Coil
SUN
458
SS-HASun1L-KDEL
SS HA
KDEL

Fig. 8

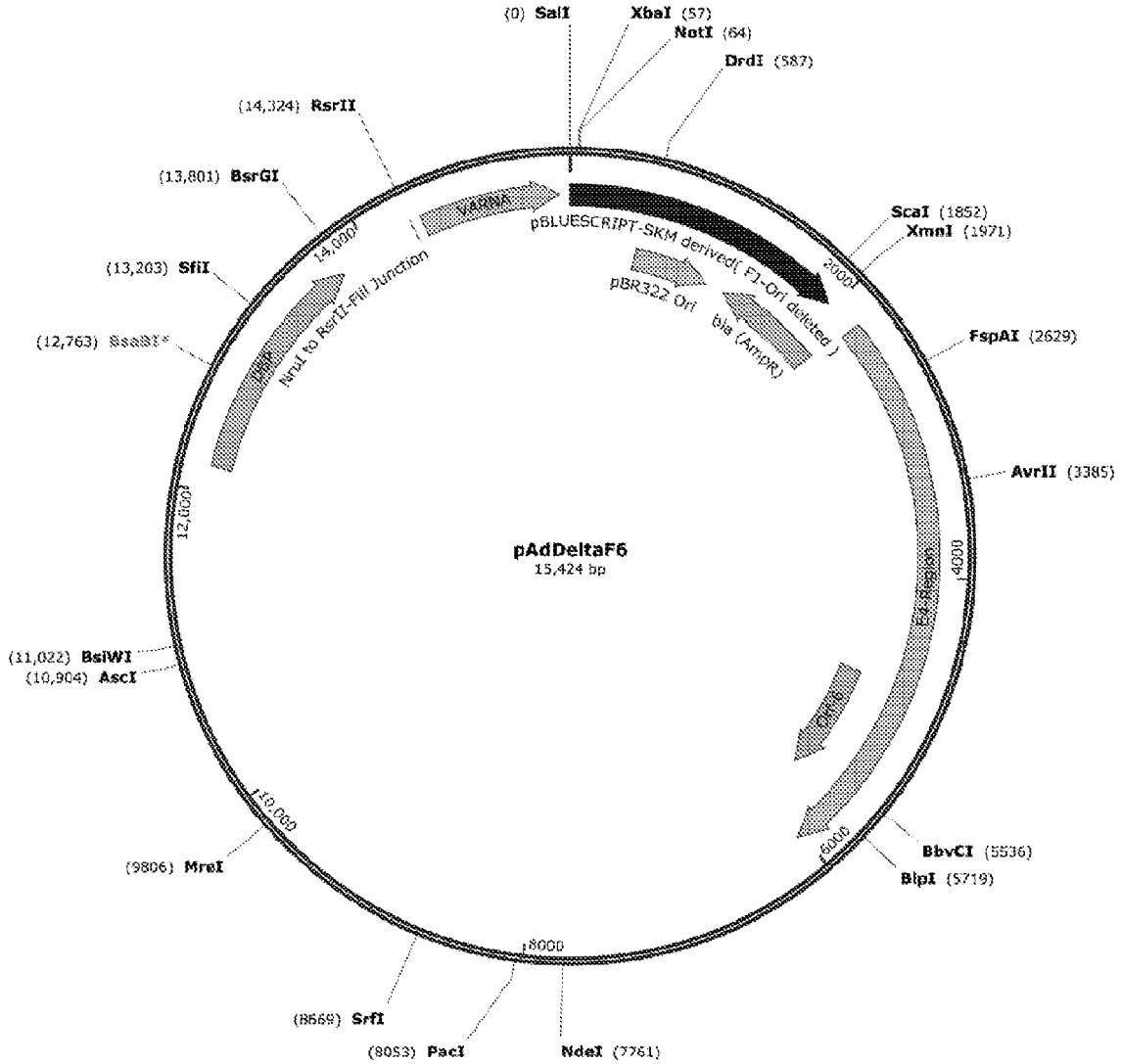

(0) SalI
XbaI (57)
NotI (64)
DrdI (587)
(14,324) RsrII
(13,801) BsrGI
(13,203) SfiI
(12,763) SspBI*
14,000
VA RNA
pBLUESCRIPT-SKM derived(f1-Ori deleted)
pBR322 Ori
bla (AmpR)
2000
ScaI (1852)
XmnI (1971)
FspAI (2629)
AvrII (3385)
4000
E4 Region
6000
BbvCI (5536)
BlpI (5719)
8000
(8669) SrfI
(8053) PacI
NdeI (7761)
10,000
(9806) MreI
12,000
(11,022) BslWI
(10,904) AscI
NruI to RsrII-Fill Junction
pAdDeltaF6
15,424 bp

Fig. 9


(6983) NaeI
(6981) NgoMIV
NcoI (372)
SgrAI (706)
BciT* (713)
(6218) ScaI
BstBI (1371)
BstEII (1447)
ZraI (1617)
AatII (1619)
HindIII (1629)
pAAV 2/9 (p0008) Q
7330 bp
LacZ promoter
PasI (2788)
BsiWI (3007)
(4398) EcoRV
(4241) SbfI
(4232) PmeI
(4029) AfeI
(3972) XcmI

Fig. 10


5 prime ITR
cTnT Gene Promo
Intron 1
Signal Sequence
HA tag
Insert
2000
KDEL
Rabbit Globin poly A
3000
f1 ori
AP (R)
Vector
4000
5000
1000
pENN-AAV-cTnT-Sun1DN
5721 bp

Fig. 11

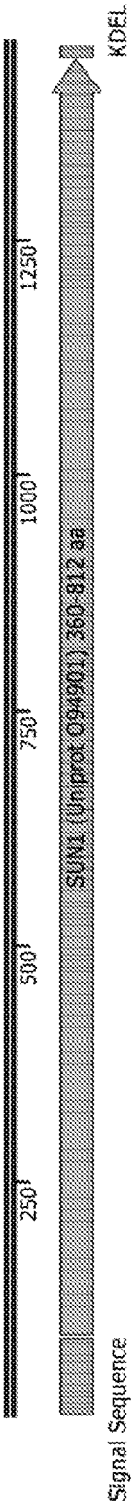

250
500
750
1000
1250
SUN1 (Uniprot Q9H9Q1) 360-812 aa
KDEL
Signal Sequence

Fig. 12

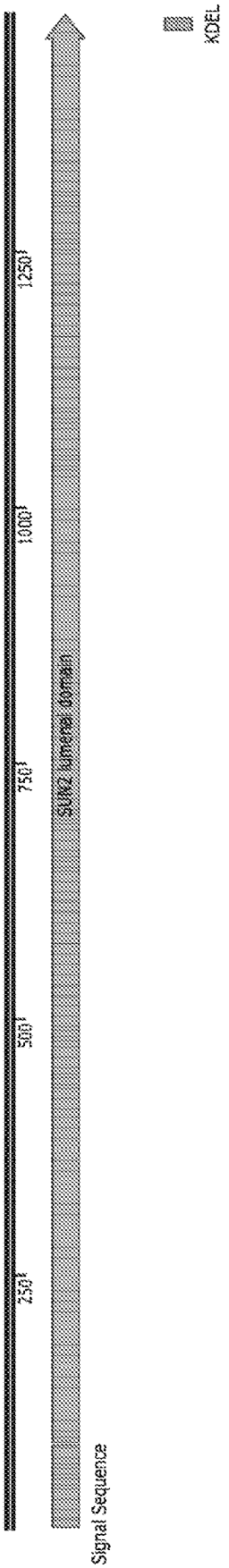

250
500
750
1000
1250
SUN2 luminal domain
KDEL
Signal Sequence

Fig. 13

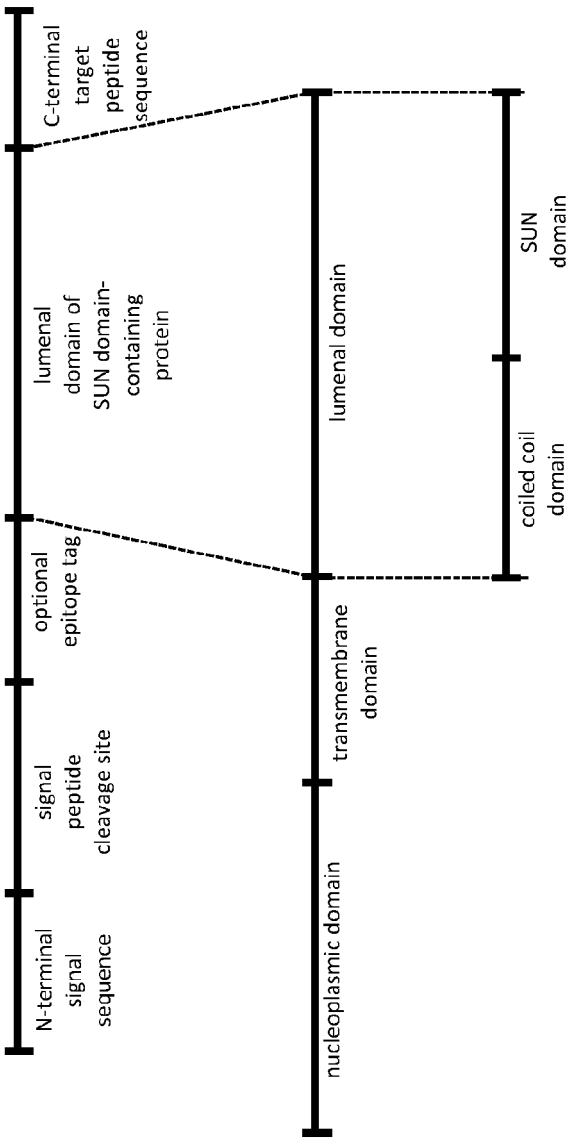


Fig. 14

```
SEQ ID NO:7  KASH1_aminoacid      RGFLFRVLRA-ALP---LQLLLLLIGLACLVPMSEEDYSCALSNNFARSFHPKLRYTNG
SEQ ID NO:9  KASH2_aminoacid      RSFLSRVVRA-ALP---LQLLLLLLLLACLLPSSEEDYSCTQANNFARSFYPNLRYTNG
SEQ ID NO:11 KASH3_aminoacid      GSLFRRACCV-ALP---LQLLLLFLLLFLLPIREEDRSCTLANNFARSFTLNLRYNGP
SEQ ID NO:13 KASH4_aminoacid      ----DPASRQPLTFLLLILFLLFLLLVCAMFLLP--ASGGPCCSHARIPRTPYLVLSYVNG
SEQ ID NO:15 KASH5_aminoacid      ----LRVTRHPLIPAPVLGLLLLLLLSVLLGPSPPPTWP----------HLQLCYLQP
                                        . * **:**::   * *                    * *

SEQ ID NO:7  KASH1_aminoacid PPPL
SEQ ID NO:9  KASH2_aminoacid PPPT
SEQ ID NO:11 KASH3_aminoacid PPT-
SEQ ID NO:13 KASH4_aminoacid LPPV
SEQ ID NO:15 KASH5_aminoacid PPV-
                             *
```

Fig. 15

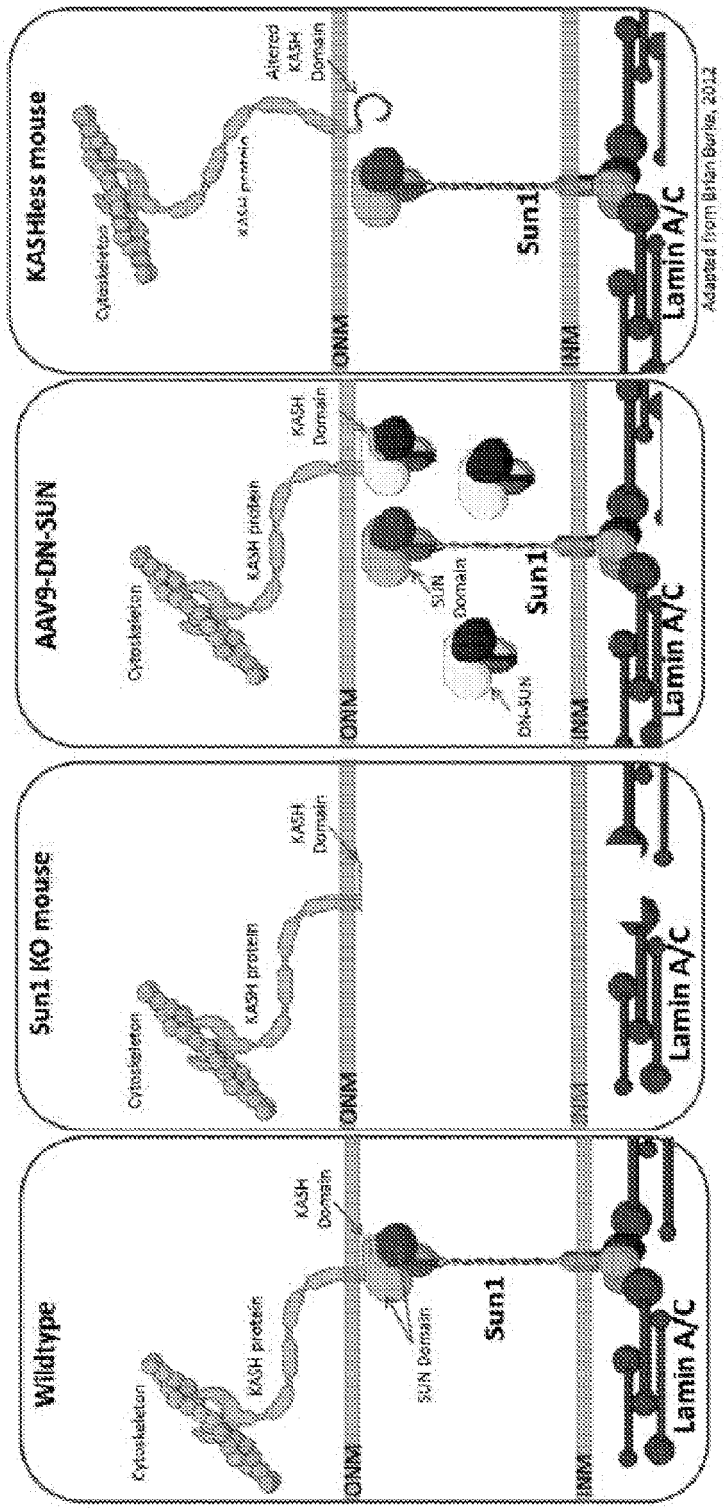

Wildtype
Cytoskeleton
KASH protein
KASH Domain
SUN Domain
Sun1
ONM
INM
Lamin A/C
Sun1 KO mouse
AAV9-DN-SUN
DN-SUN
KASHless mouse
Altered KASH Domain
Adapted from Brian Burke, 2014

Fig. 16

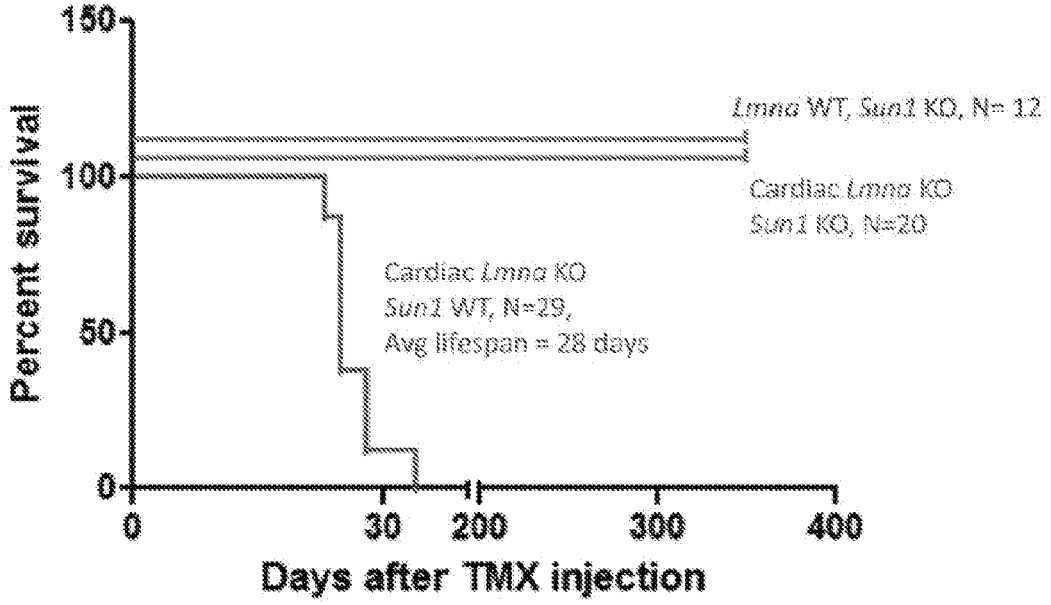

150
100
50
0
Percent survival
Lmna WT, Sun1 KO, N= 12
Cardiac Lmna KO
Sun1 KO, N=20
Cardiac Lmna KO
Sun1 WT, N=29,
Avg lifespan = 28 days
0
30
200
300
400
Days after TMX injection

Fig. 17

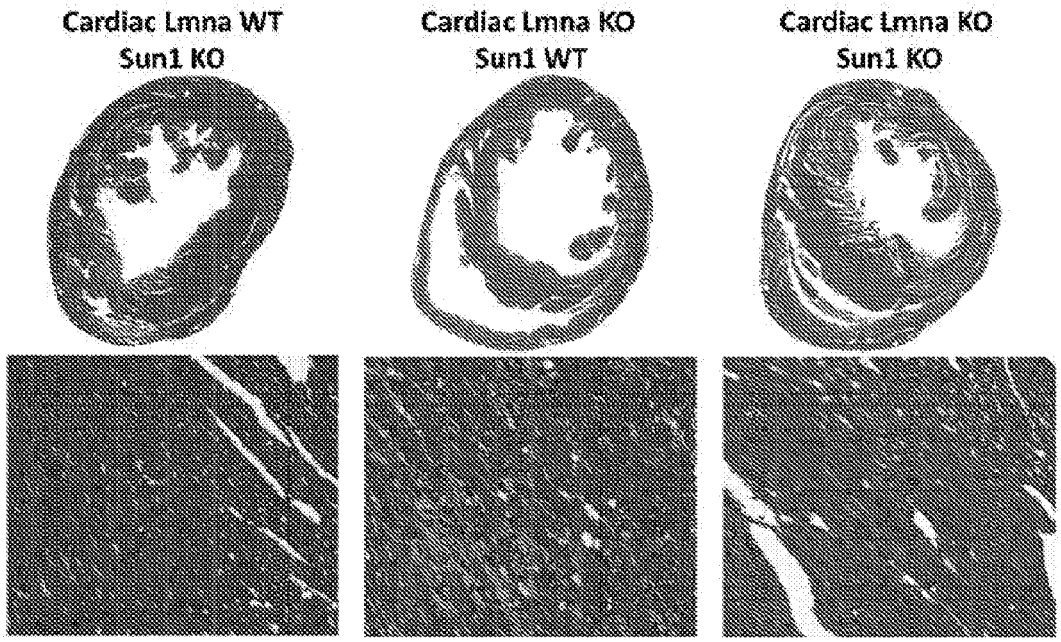

Cardiac Lmna WT
Sun1 KO
Cardiac Lmna KO
Sun1 WT
Cardiac Lmna KO
Sun1 KO

Fig. 18

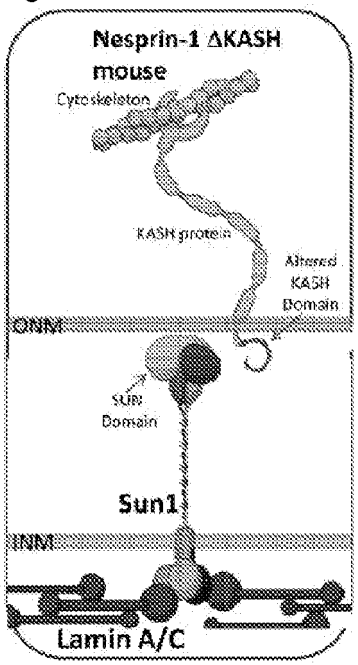


Fig. 19A

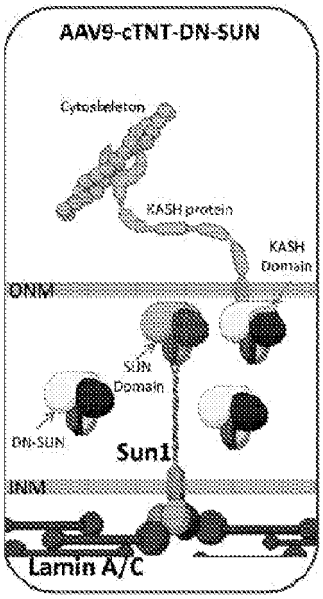


Fig. 19B

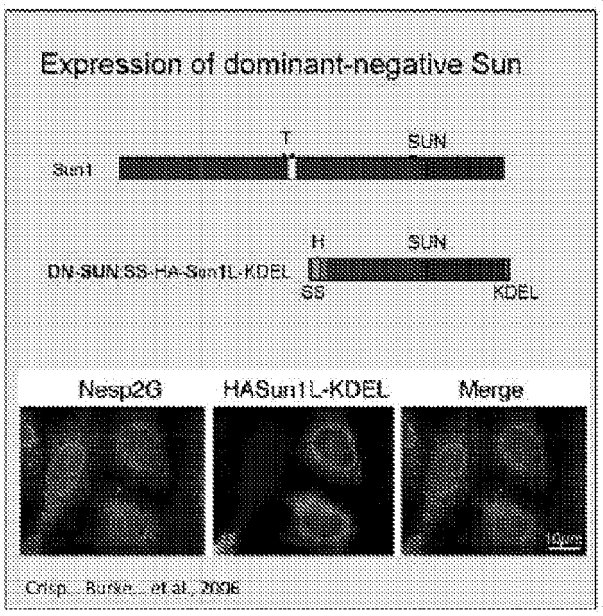


Fig. 20

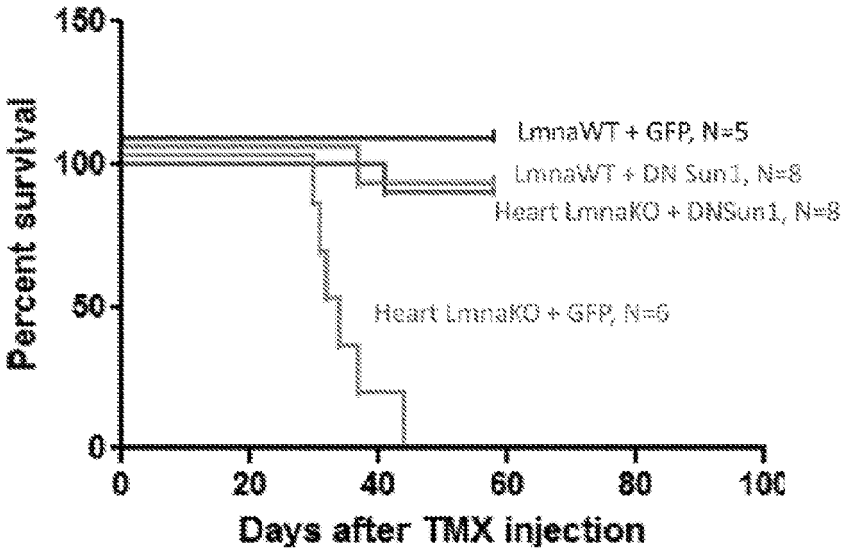

150
100
50
0
Percent survival
0
20
40
60
80
100
Days after TMX injection
LmnaWT + GFP, N=5
LmnaWT + DN Sun1, N=8
Heart LmnaKO + DNSun1, N=8
Heart LmnaKO + GFP, N=6

Fig. 21

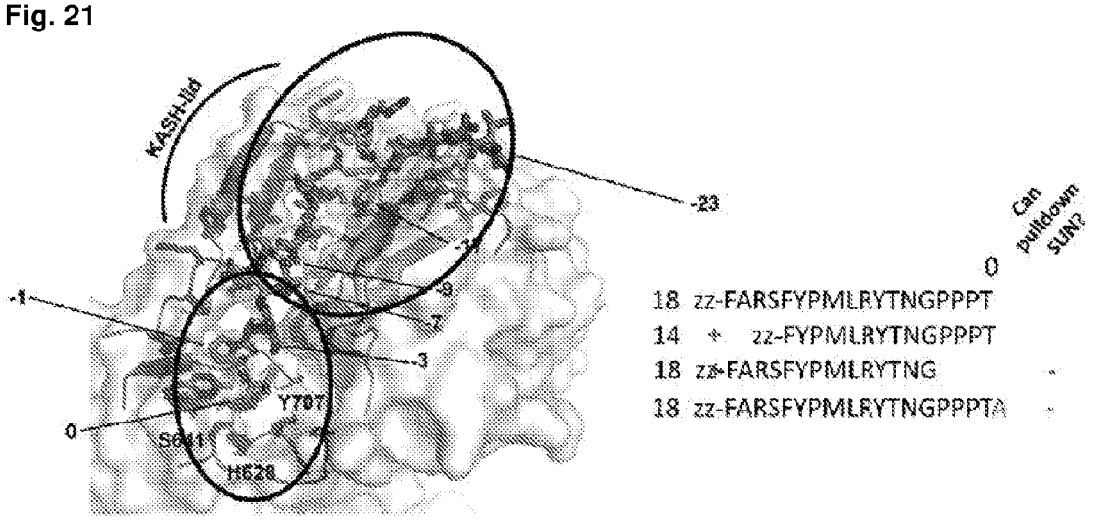

KASH-lid
-23
-1
0
Y707
S611
H626
Can pulldown SUN2
18 zz-FARSFYPMLRYTNGPPPT
14 zz-FYPMLRYTNGPPPT
18 zz-FARSFYPMLRYTNG
18 zz-FARSFYPMLRYTNGPPPTA

Fig. 22

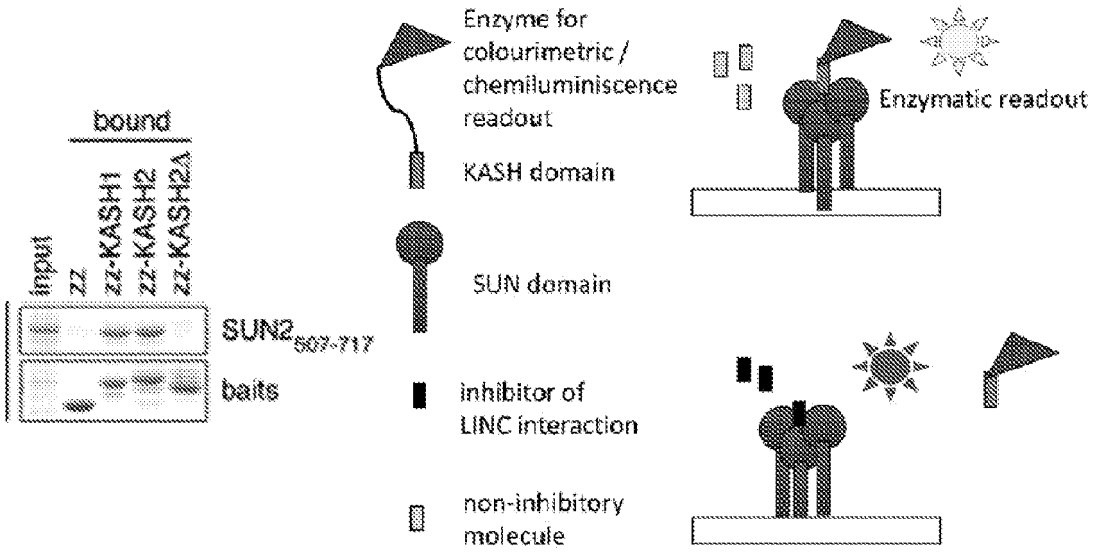

bound
input
zz
zz-KASH1
zz-KASH2
zz-KASH2Δ
SUN2507-717
baits
Enzyme for colourimetric / chemiluminiscence readout
KASH domain
SUN domain
inhibitor of LINC interaction
non-inhibitory molecule
Enzymatic readout

Fig. 23

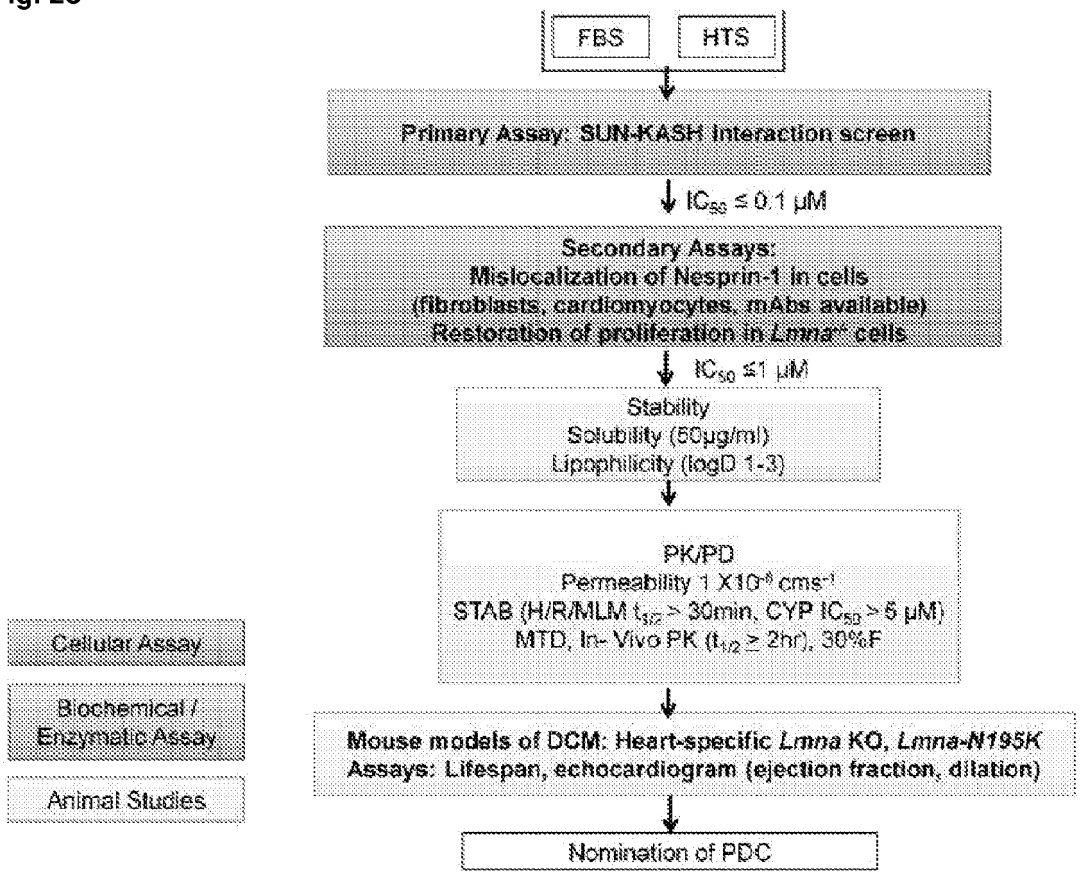

FBS
HTS
Primary Assay: SUN-KASH interaction screen
IC50 ≤ 0.1 µM
Secondary Assays:
Mislocalization of Nesprin-1 in cells (fibroblasts, cardiomyocytes, mAbs available)
Restoration of proliferation in Lmna-/- cells
IC50 ≤1 µM
Stability
Solubility (60µg/ml)
Lipophilicity (logD 1-3)
PK/PD
Permeability 1 X10-6 cms-1
STAB (H/R/MLM t1/2 > 30min, CYP IC50 > 5 µM)
MTD, In- Vivo PK (t1/2 ≥ 2hr), 30%F
Mouse models of DCM: Heart-specific Lmna KO, Lmna-N195K
Assays: Lifespan, echocardiogram (ejection fraction, dilation)
Nomination of PDC
Cellular Assay
Biochemical / Enzymatic Assay
Animal Studies

Fig. 24

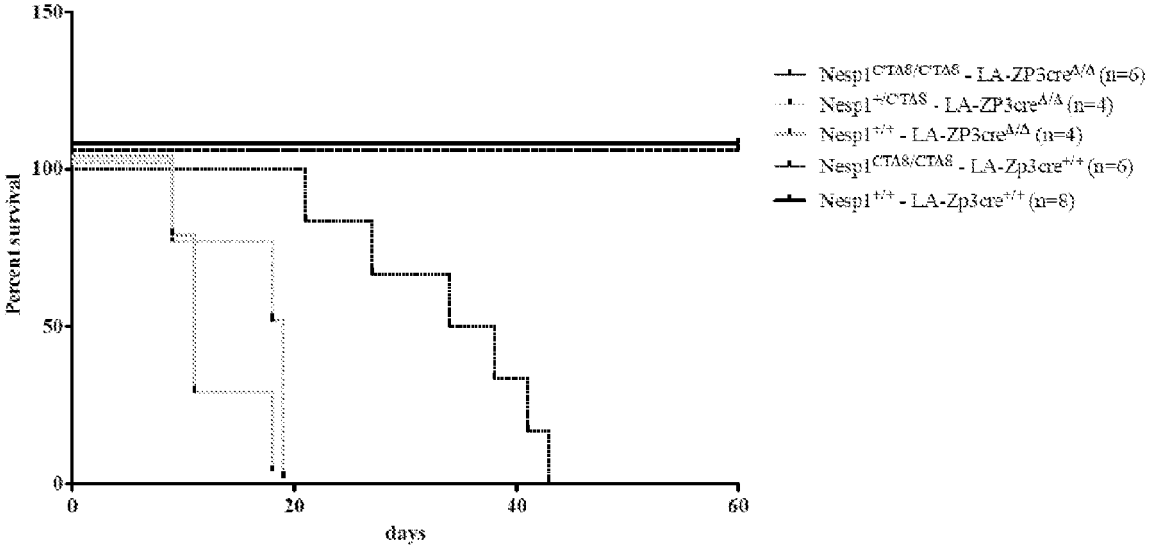

150
100
50
0
Percent survival
0
20
40
60
days
Nesp1CTΔ8/CTΔ8 - LA-ZP3creΔ/Δ (n=6)
Nesp1+/CTΔ8 - LA-ZP3creΔ/Δ (n=4)
Nesp1+/+ - LA-ZP3creΔ/Δ (n=4)
Nesp1CTΔ8/CTΔ8 - LA-Zp3cre+/+ (n=6)
Nesp1+/+ - LA-Zp3cre+/+ (n=8)

Fig. 25

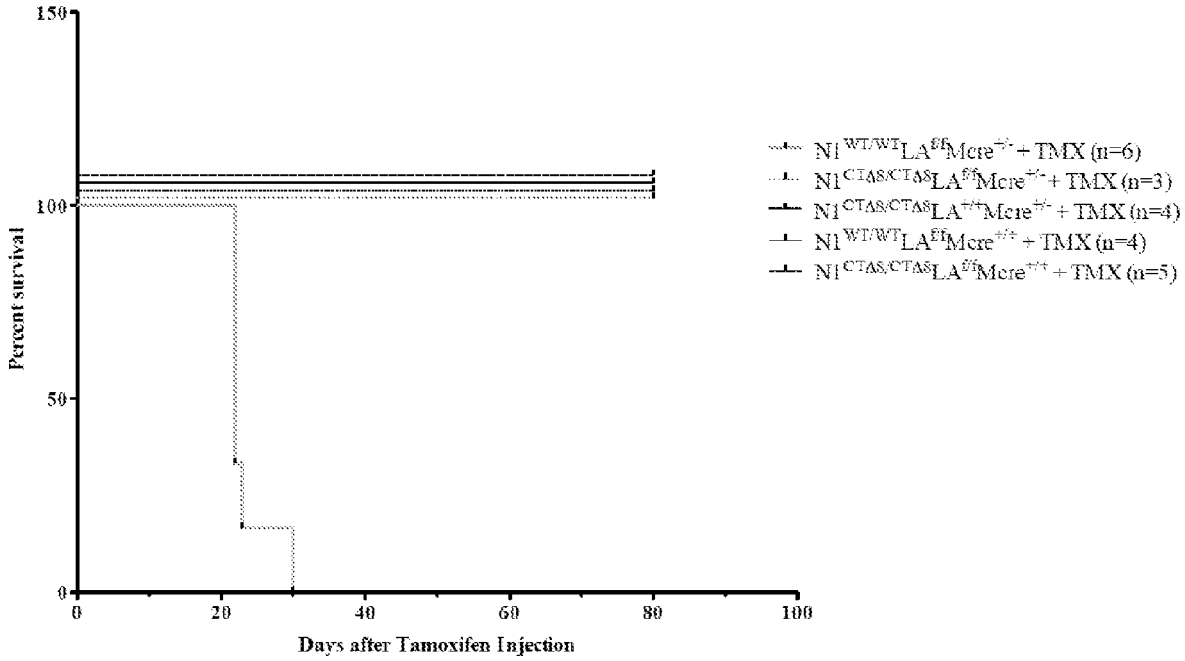

150
100
50
0
Percent survival
0
20
40
60
80
100
Days after Tamoxifen Injection
N1WT/WTLAfl/flMcre+/- + TMX (n=6)
N1CTΔS/CTΔSLAfl/flMcre+/- + TMX (n=3)
N1CTΔS/CTΔSLA+/+Mcre+/- + TMX (n=4)
N1WT/WTLAfl/flMcre+/+ + TMX (n=4)
N1CTΔS/CTΔSLAfl/flMcre+/+ + TMX (n=5)

Fig. 26A

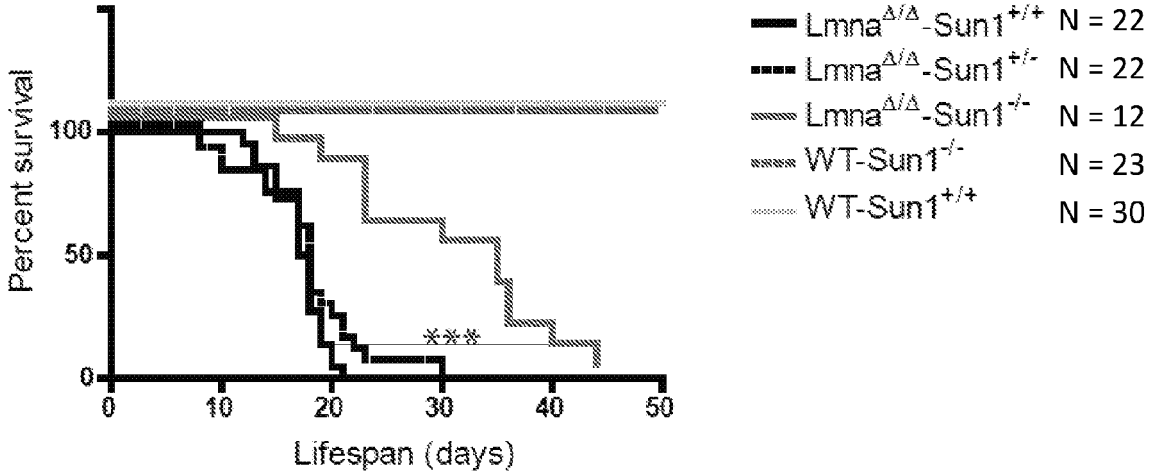


Fig. 26B

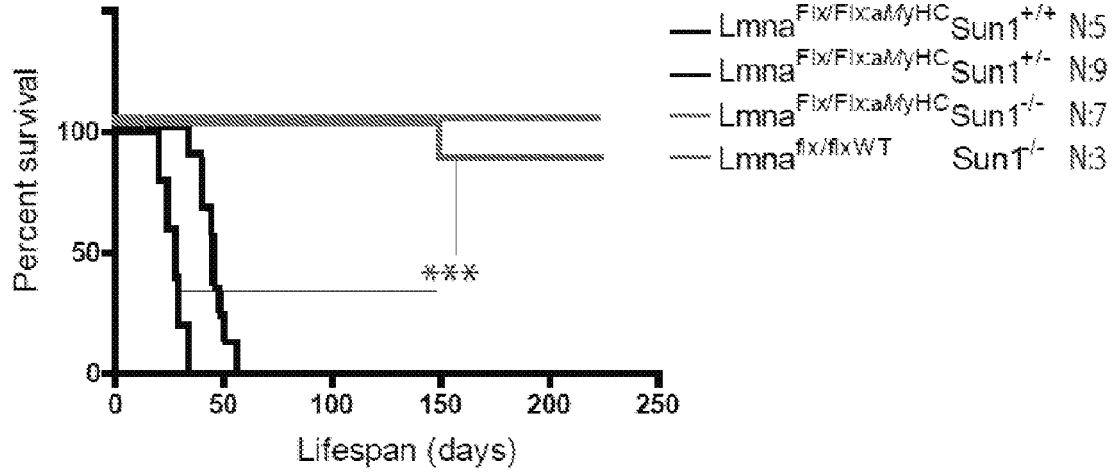


Fig. 26C

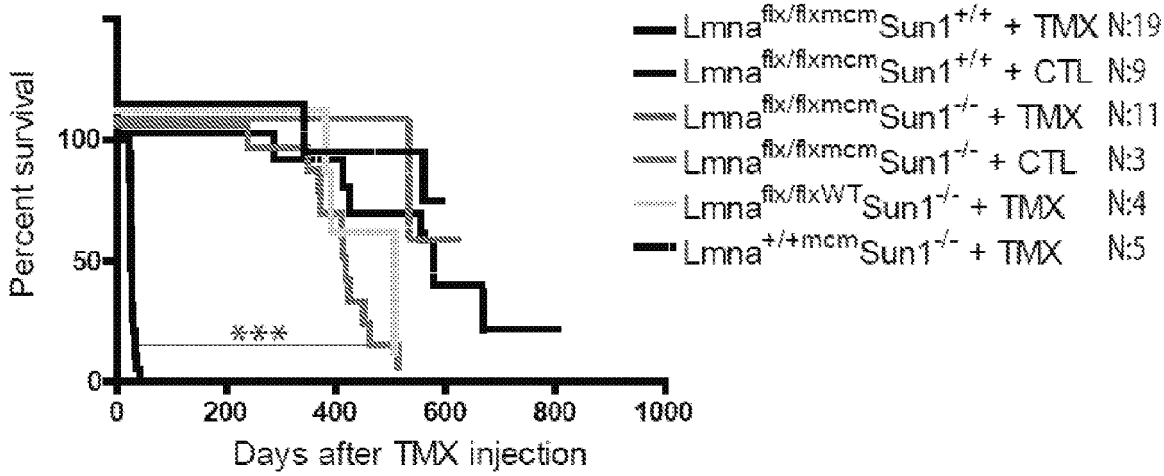


Fig. 26D

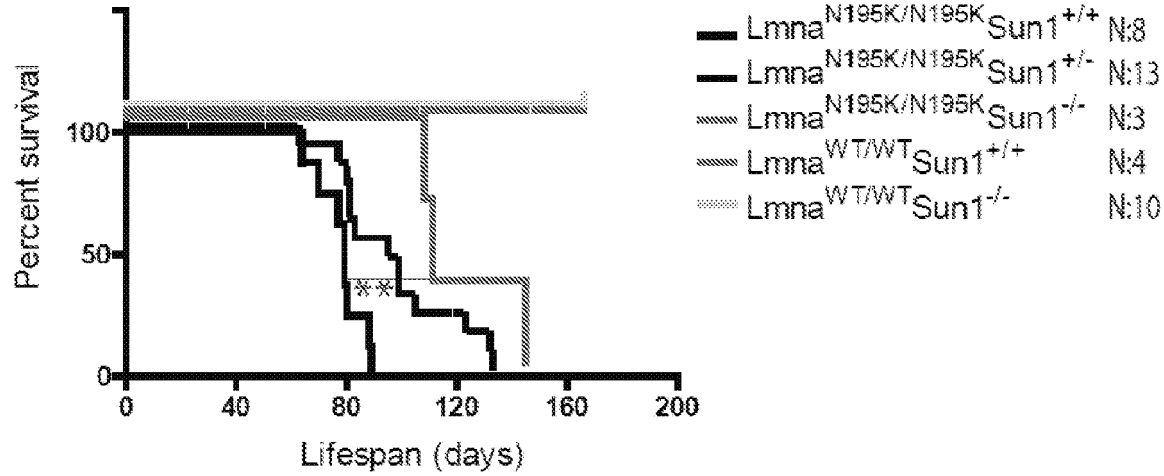


Fig. 27A

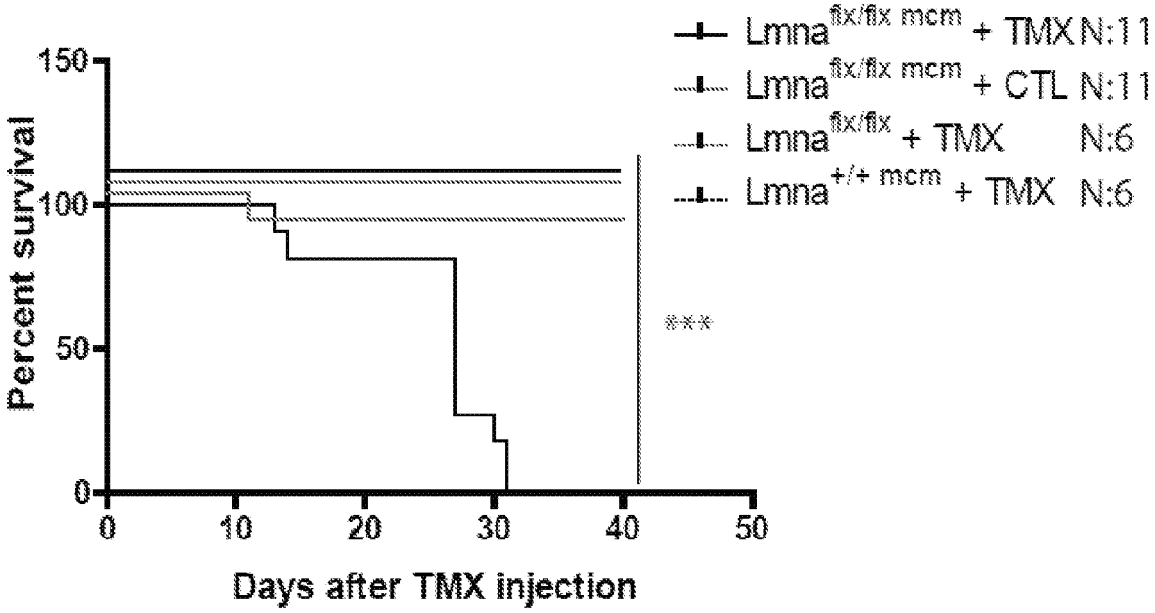


Fig. 27B

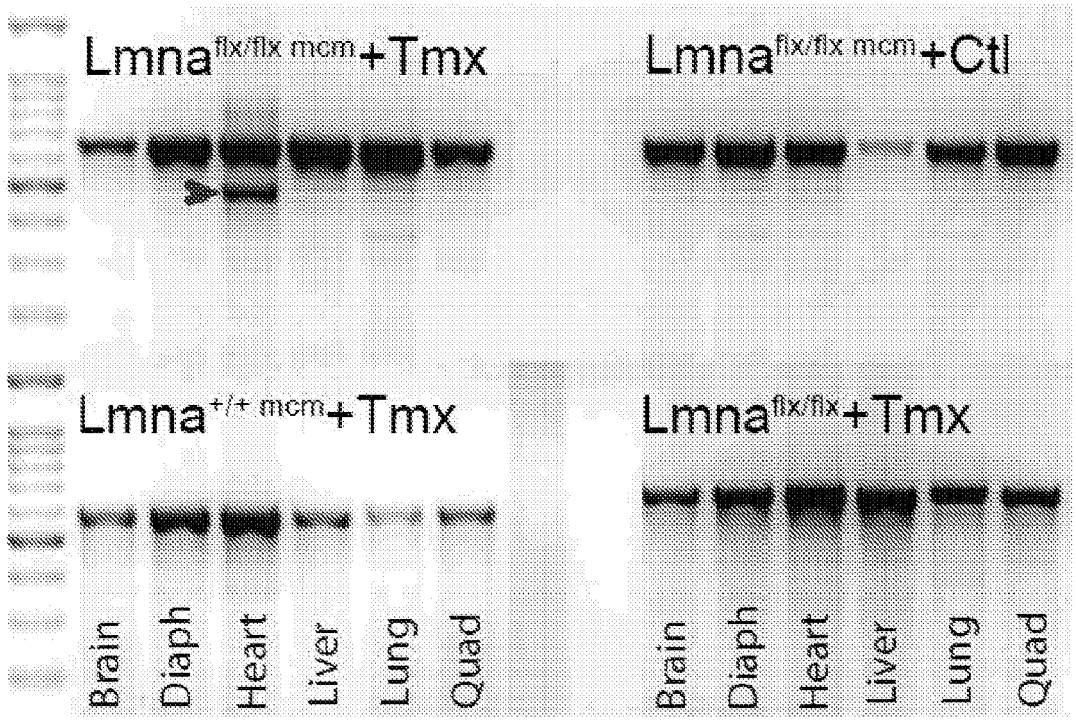


Fig. 27C

Fig. 27D

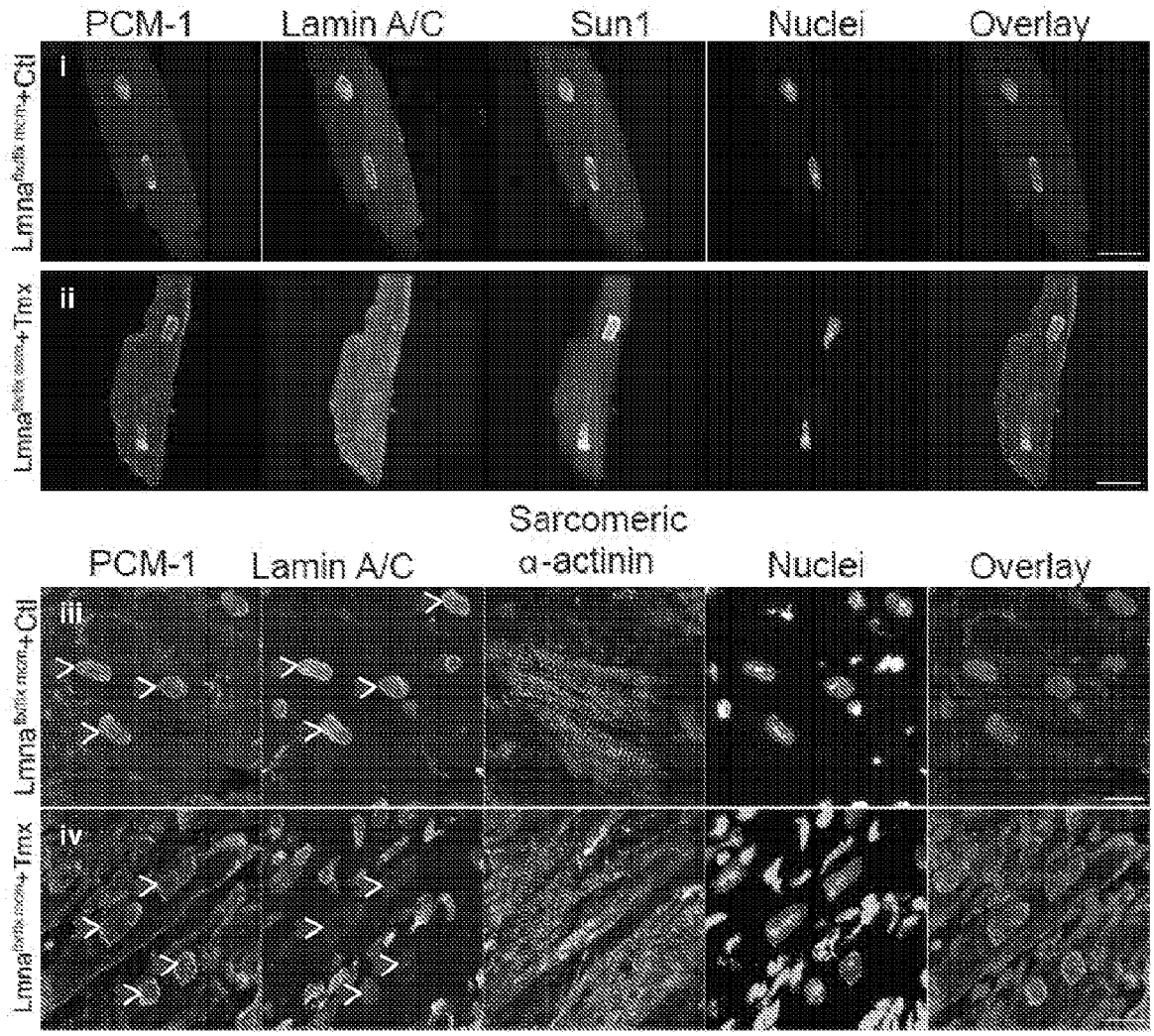

PCM-1
Lamin A/C
Sun1
Nuclei
Overlay
i
ii
+Ctl
+Tmx
Sarcomeric
α-actinin
iii
iv

Fig. 27E

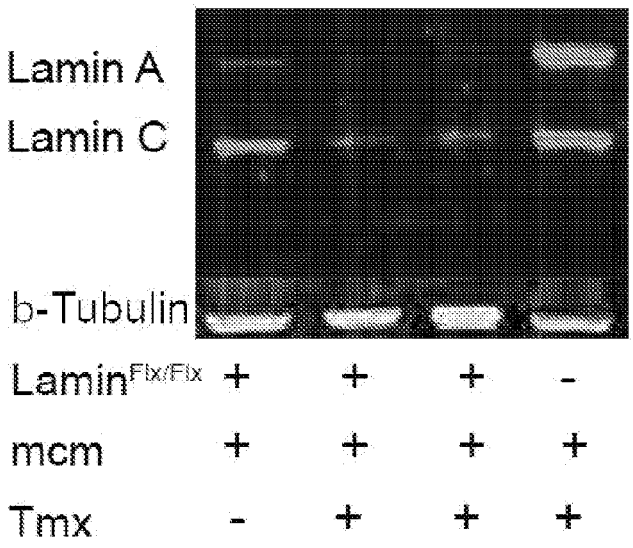


Fig. 27F

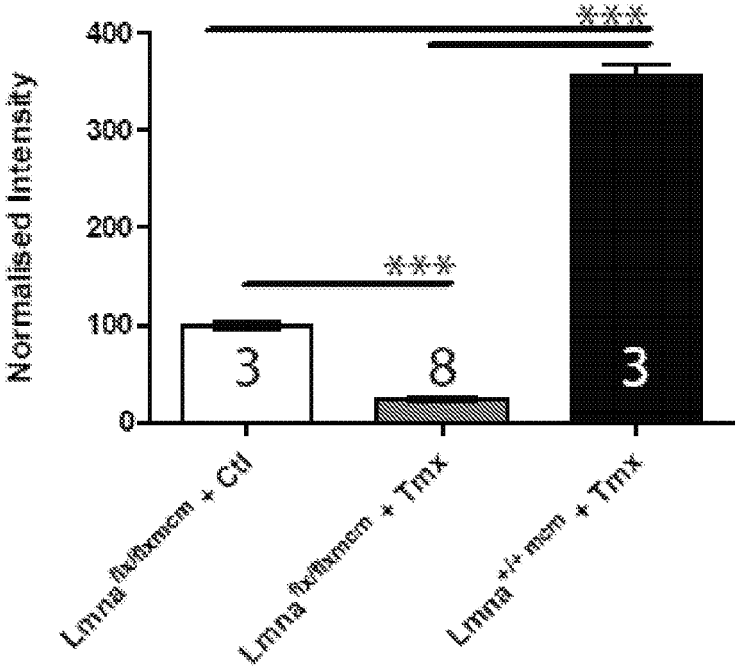


Fig. 28A

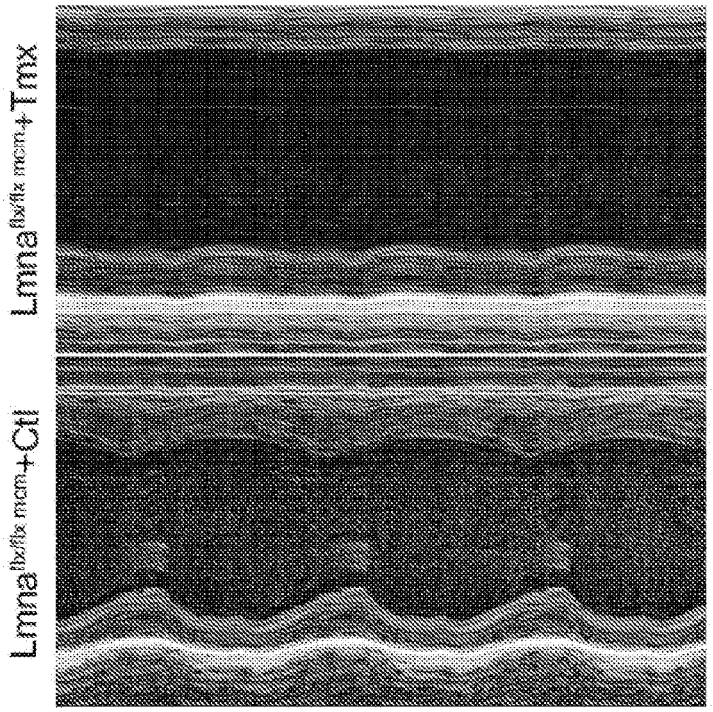


Fig. 28B

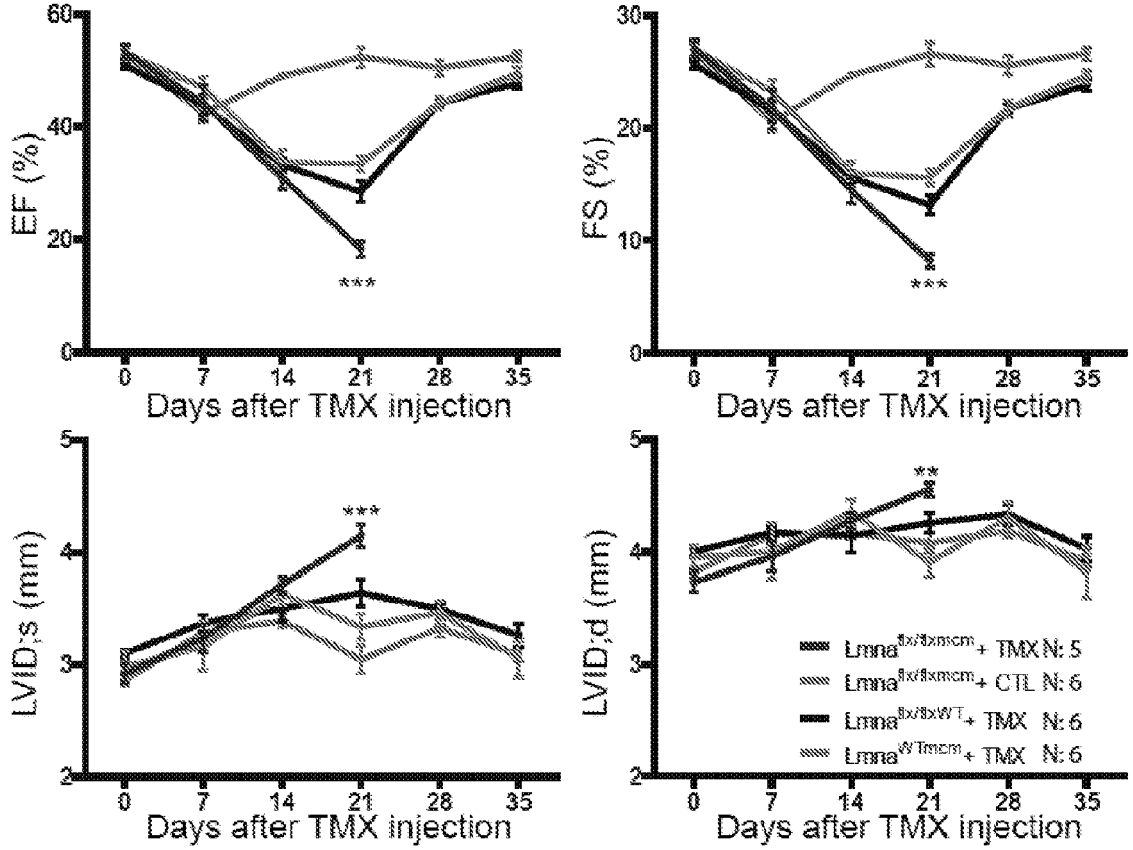


Fig. 28C

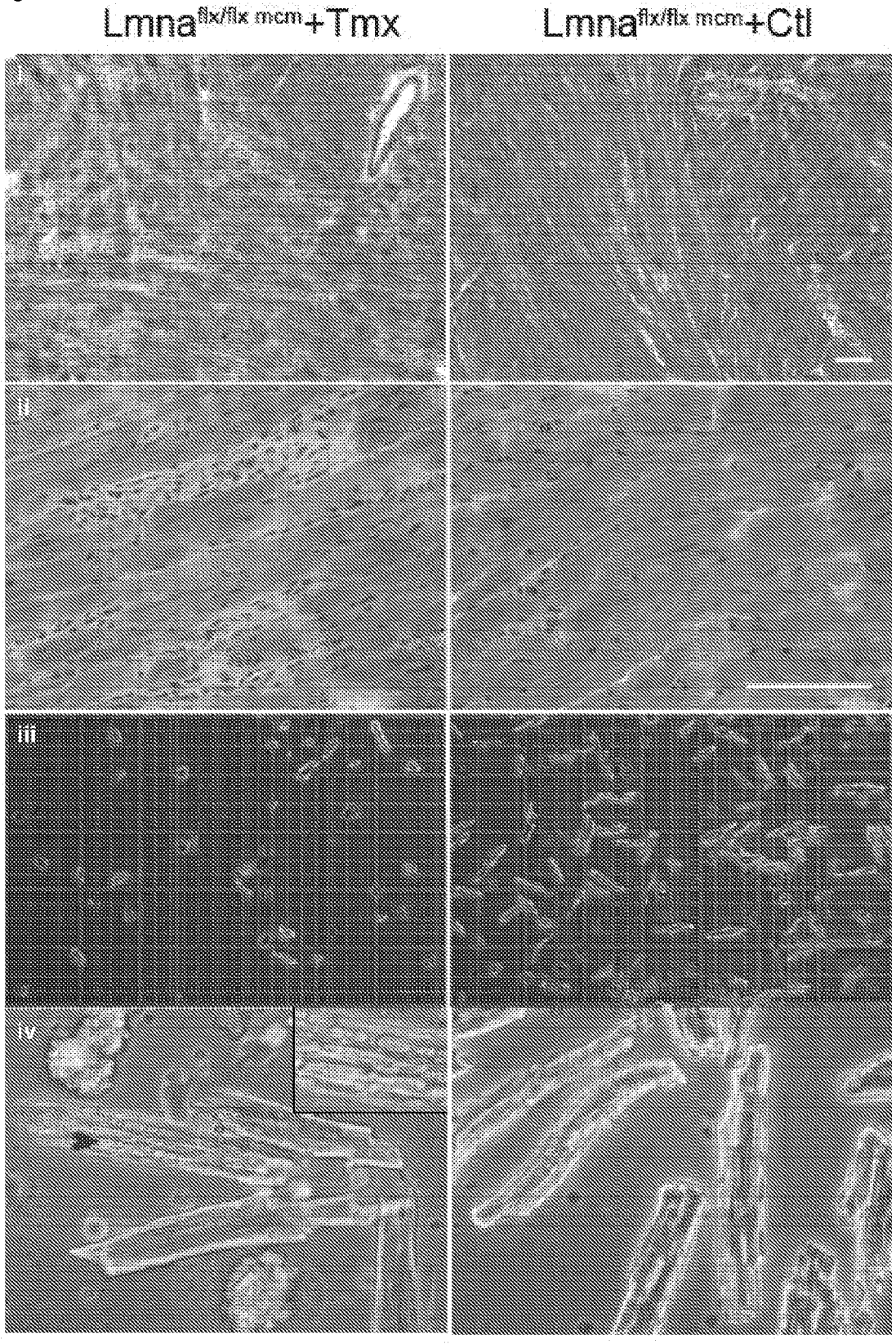


Fig. 28D

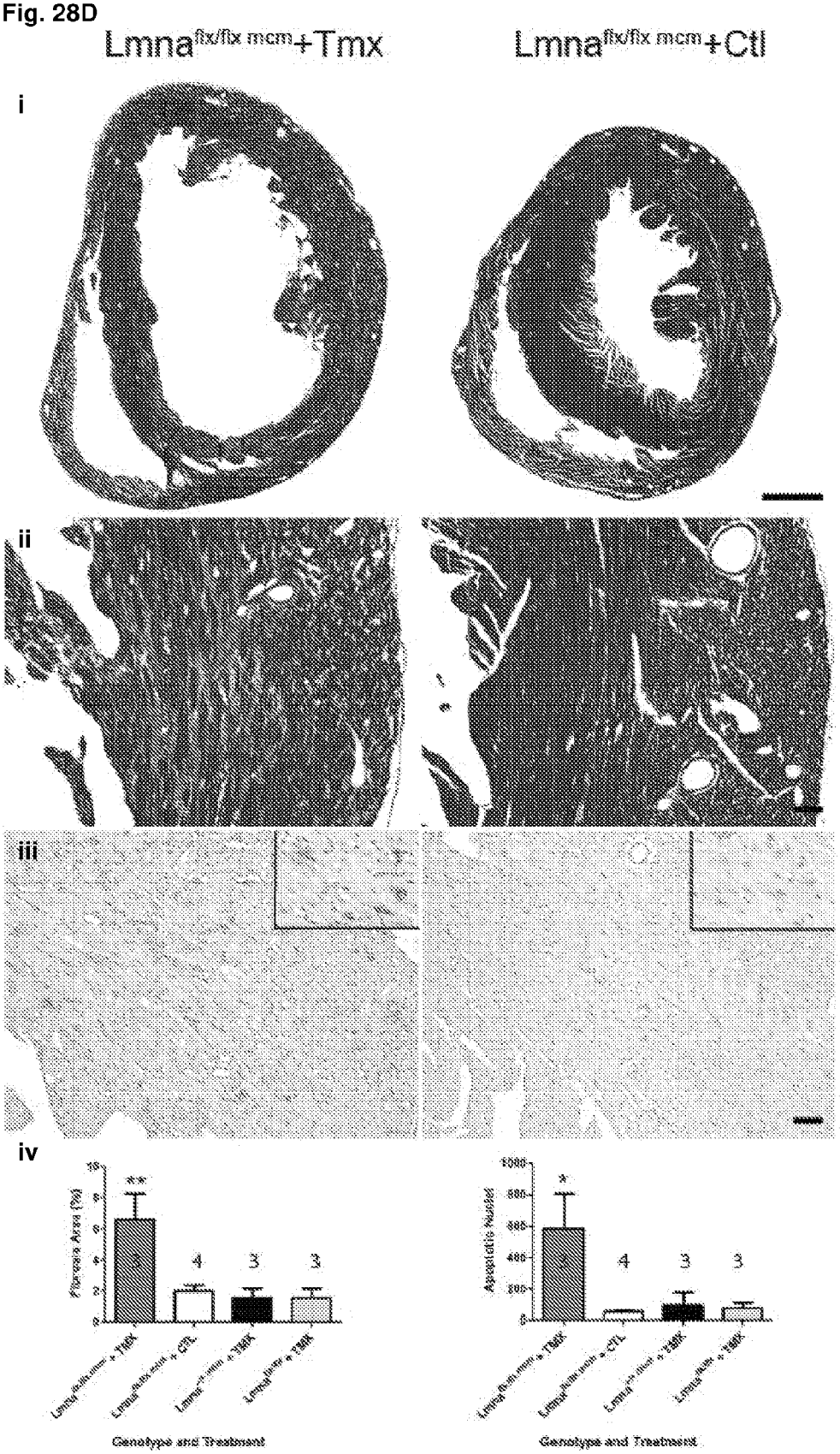

Lmna fix/fix mcm+Tmx
Lmna fix/fix mcm+Ctl
i
ii
iii
iv
Fibrosis Area (%)
**
4
3
3
Genotype and Treatment
Apoptotic Nuclei
*
4
3
3
Genotype and Treatment

Fig. 29A

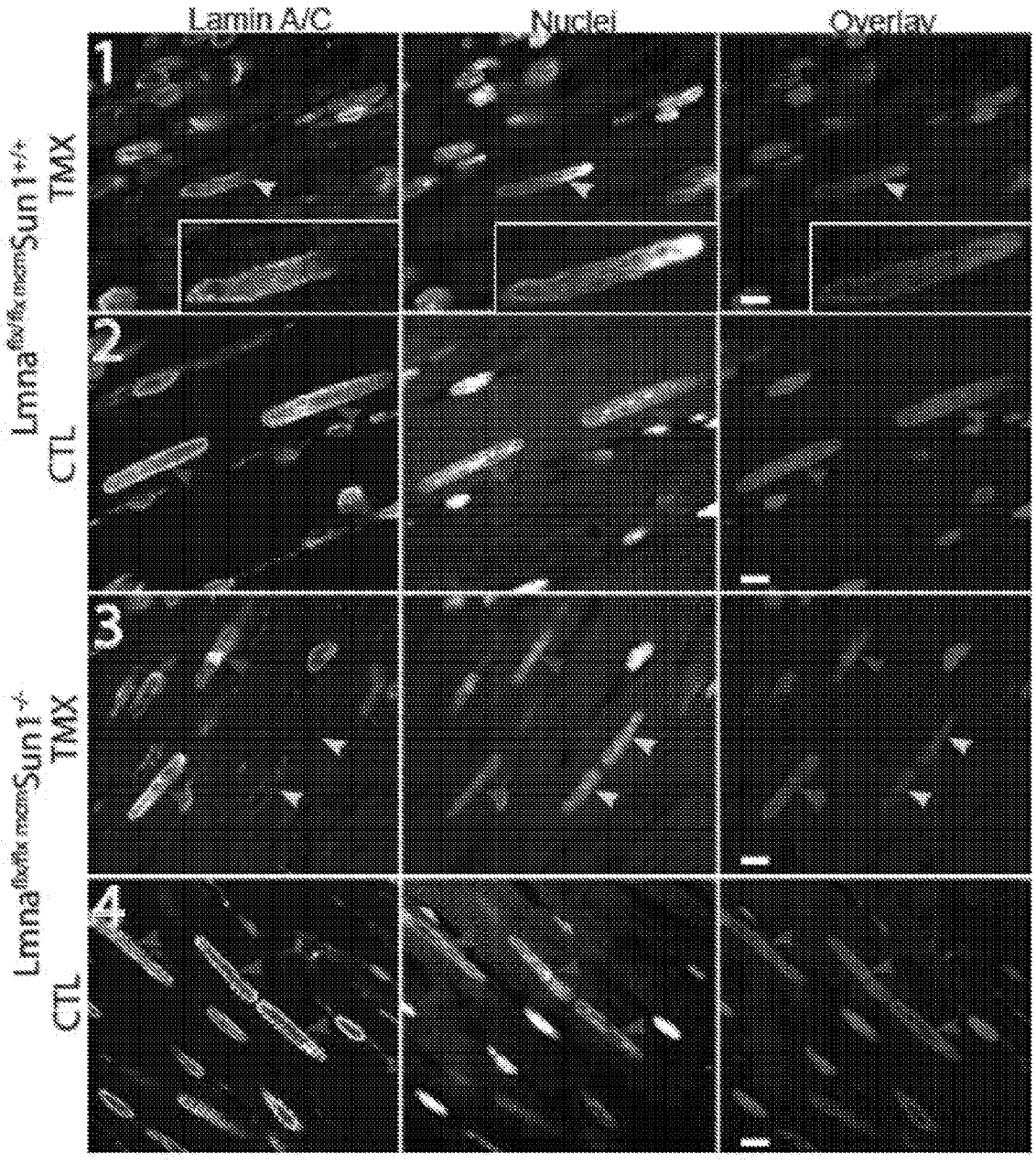
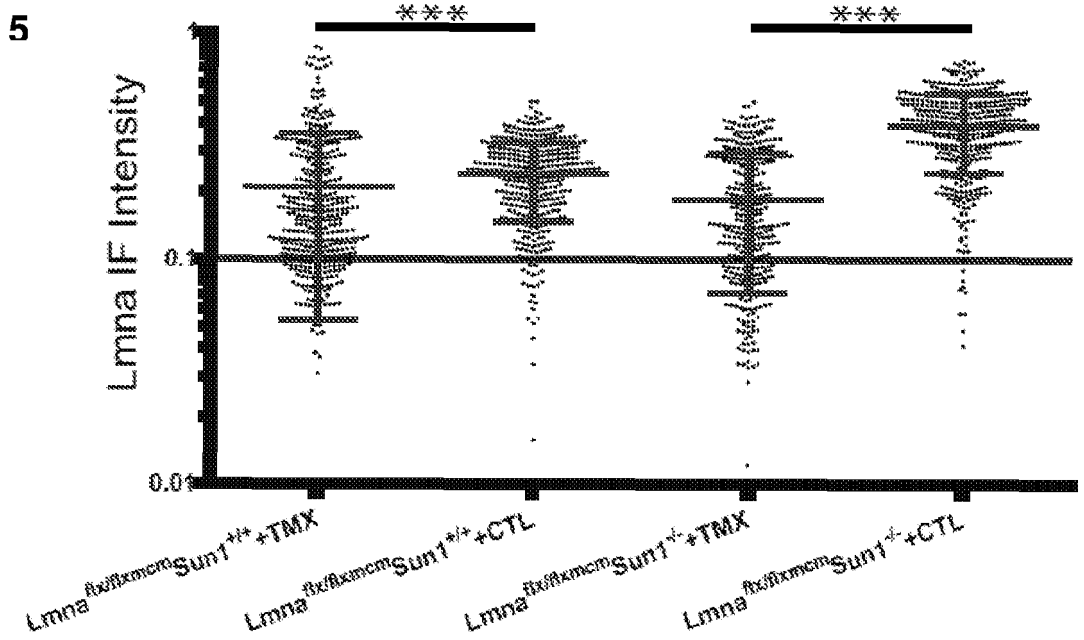

Lamin A/C
Nuclei
Overlay
1
2
3
4
Lmna flox/flox mcm Sun1 +/+
TMX
CTL
Lmna flox/flox mcm Sun1 -/-
TMX
CTL

Fig. 29A (continued)



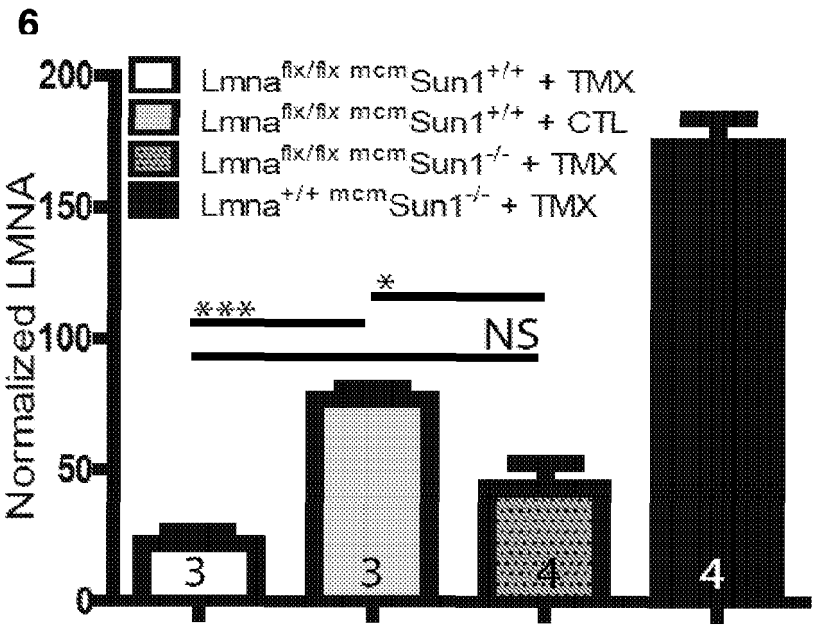


Fig. 29B

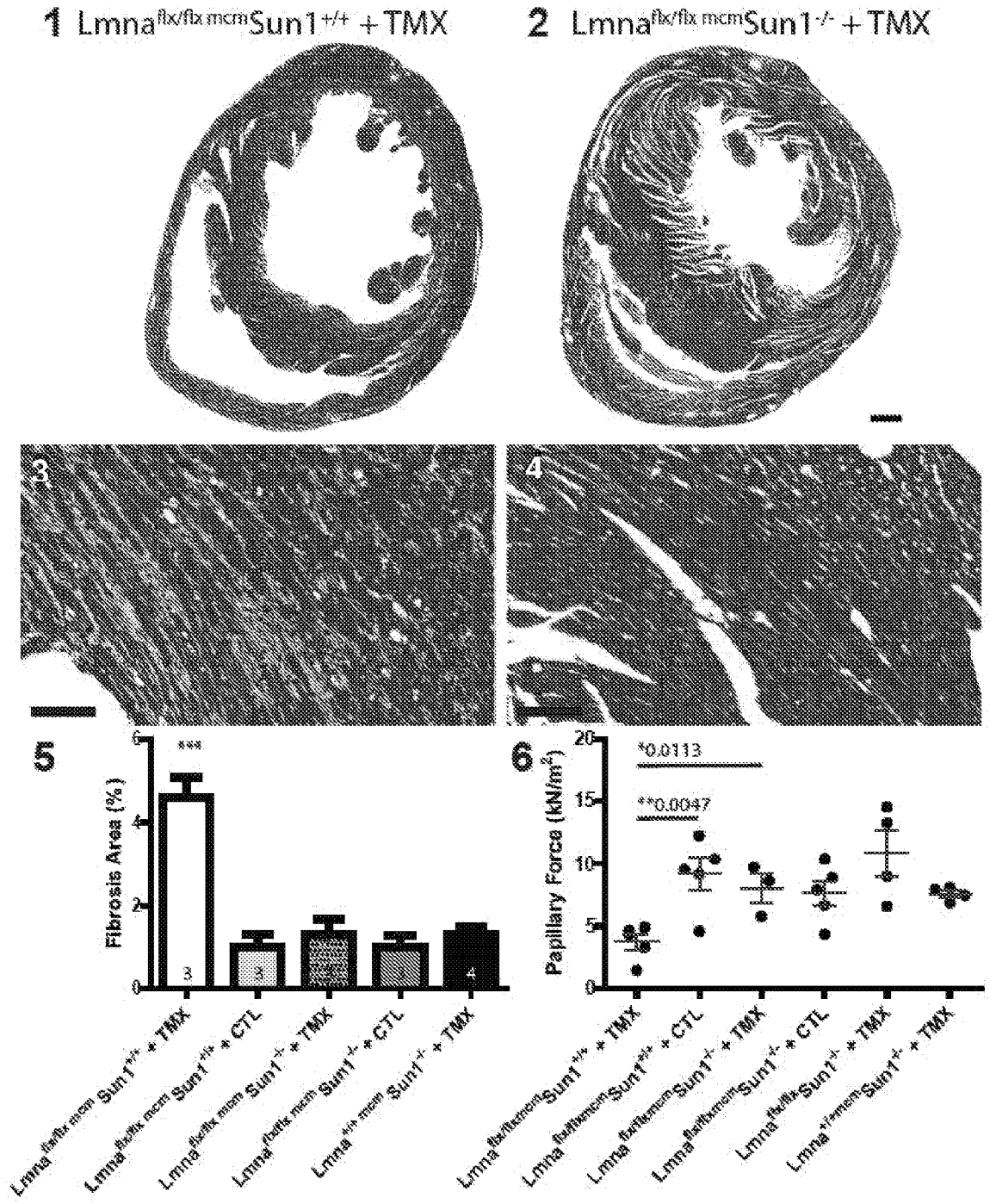

1 Lmna flx/flx mcm Sun1 +/+ + TMX
2 Lmna flx/flx mcm Sun1 -/- + TMX
3
4
5
Fibrosis Area (%)
***
6
Papillary Force (kN/m²)
*0.0113
**0.0047
Lmna flx/flx mcm Sun1 +/+ + TMX
Lmna flx/flx mcm Sun1 +/+ + CTL
Lmna flx/flx mcm Sun1 -/- + TMX
Lmna flx/flx mcm Sun1 -/- + CTL
Lmna +/+ mcm Sun1 -/- + TMX

Fig. 29C

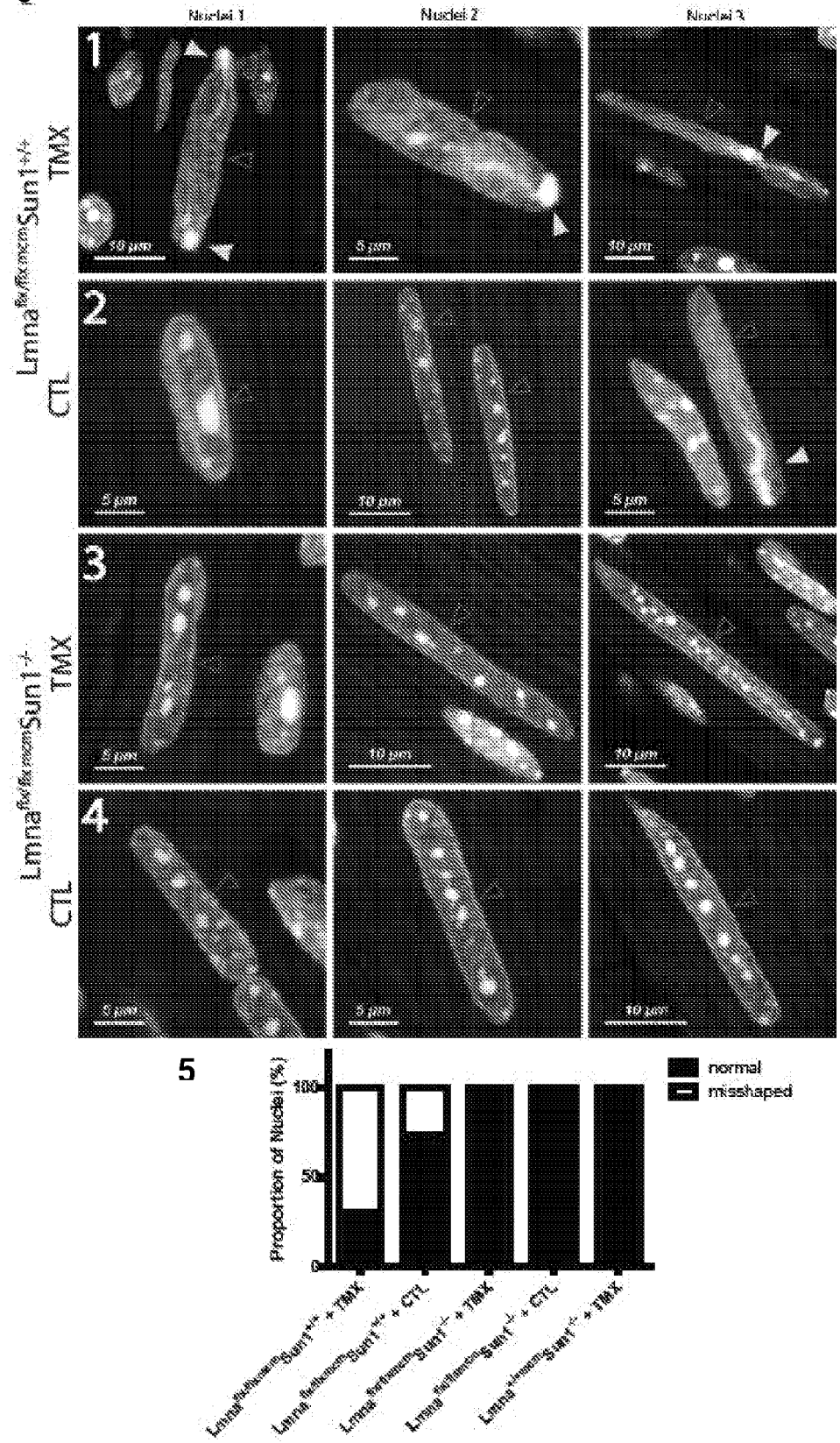

Nuclei 1
Nuclei 2
Nuclei 3
1
2
3
4
5
TMX
CTL
TMX
CTL
5 µm
10 µm
Proportion of Nuclei (%)
100
50
0
normal
misshaped
+ TMX
+ CTL
+ TMX
+ CTL
+ TMX

Fig. 29D

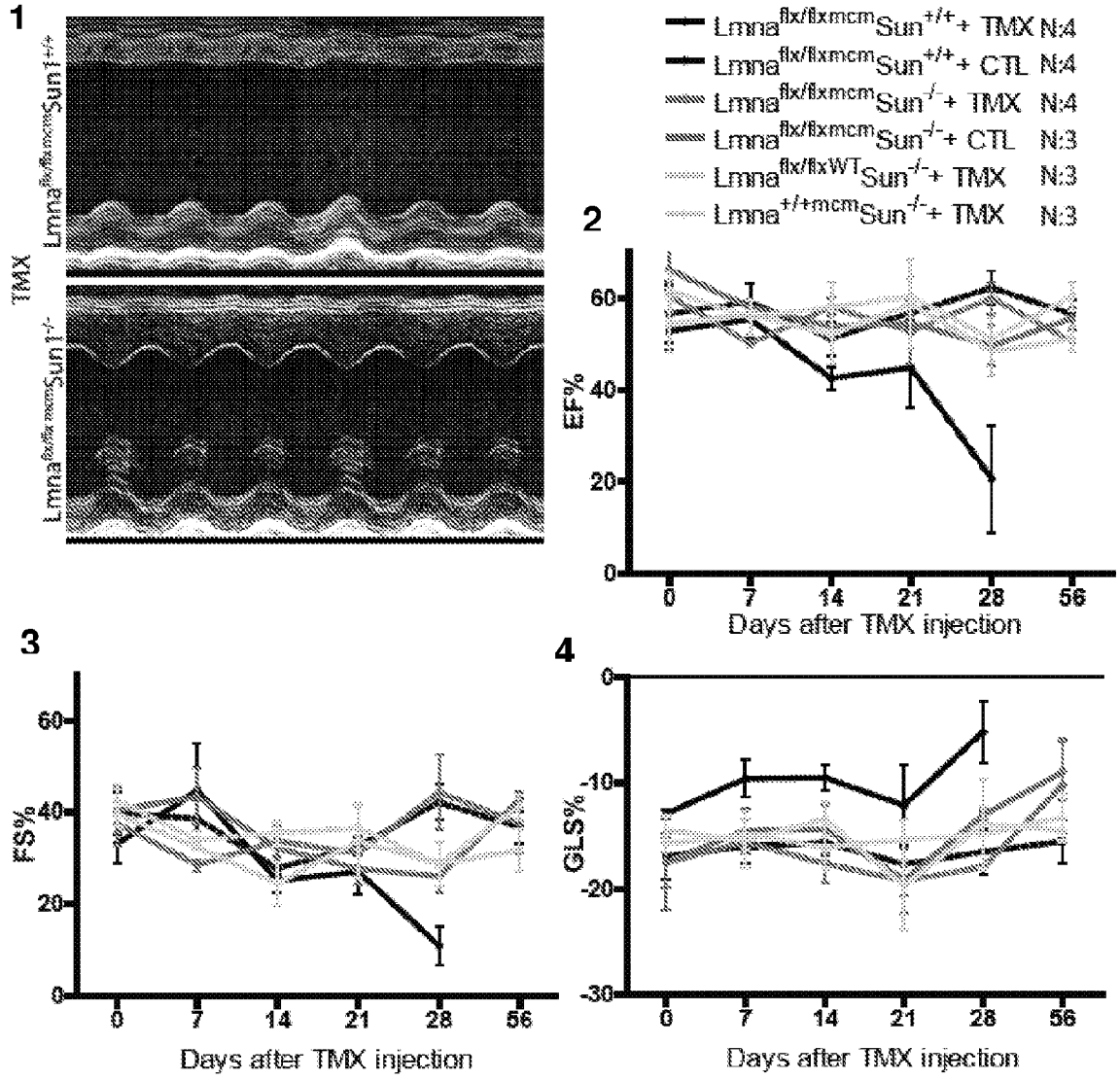

1
TMX
Lmna flx/flxmcm Sun1 +/+
Lmna flx/flxmcm Sun1 -/-
2
Lmna flx/flxmcm Sun +/+ + TMX N:4
Lmna flx/flxmcm Sun +/+ + CTL N:4
Lmna flx/flxmcm Sun -/- + TMX N:4
Lmna flx/flxmcm Sun -/- + CTL N:3
Lmna flx/flxWT Sun -/- + TMX N:3
Lmna +/+mcm Sun -/- + TMX N:3
EF%
Days after TMX injection
3
FS%
Days after TMX injection
4
GLS%
Days after TMX injection

Fig. 30A

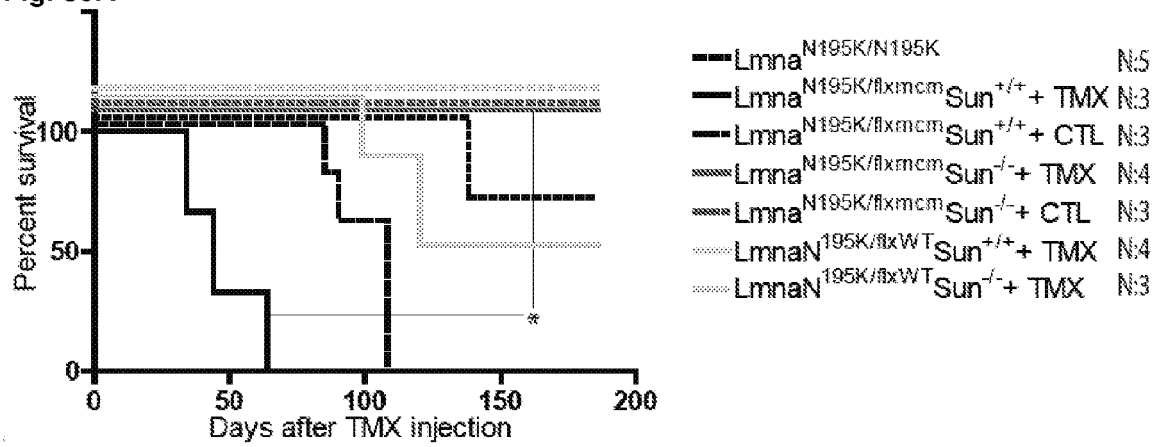


Fig. 30B

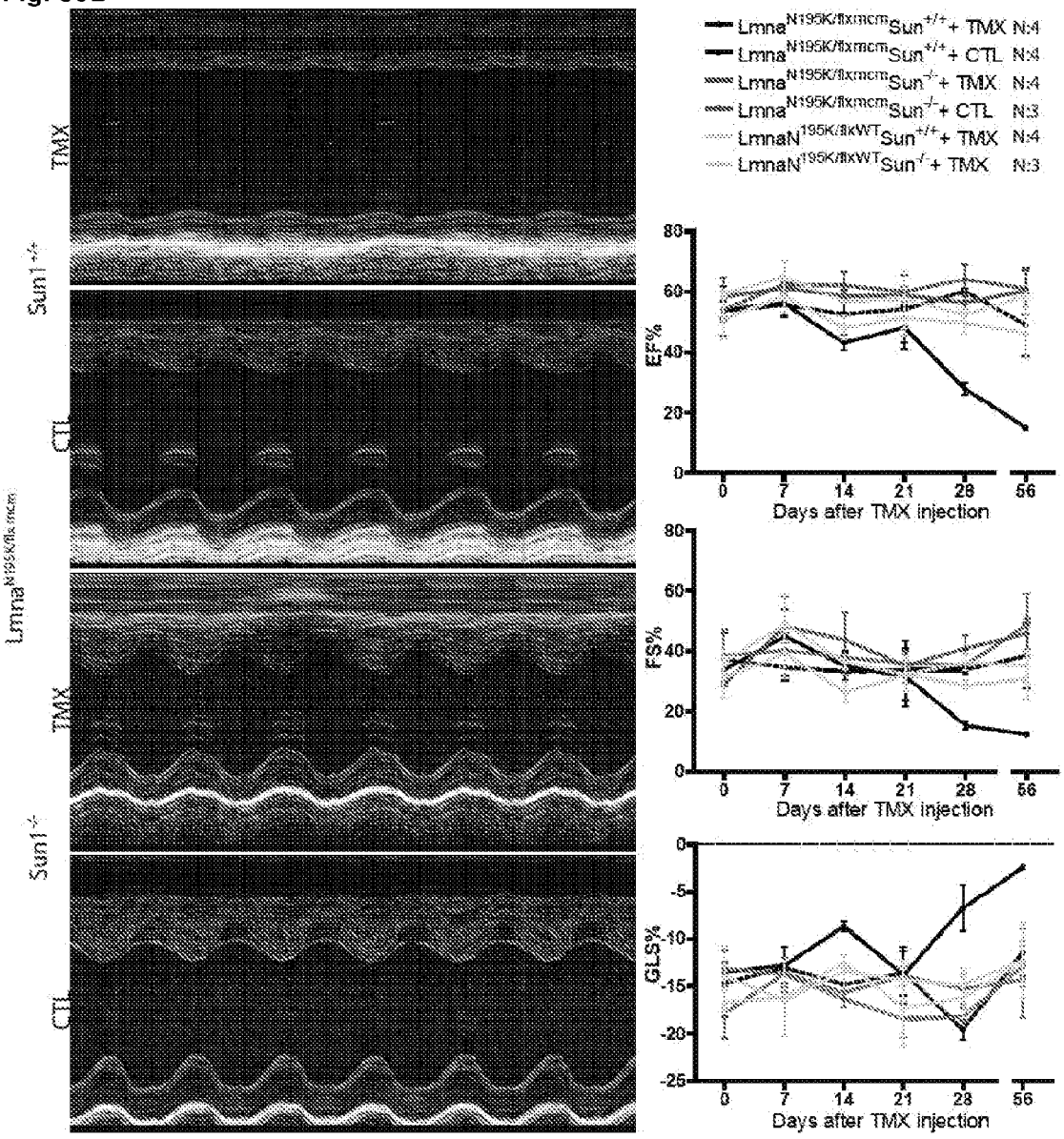


Fig. 31A

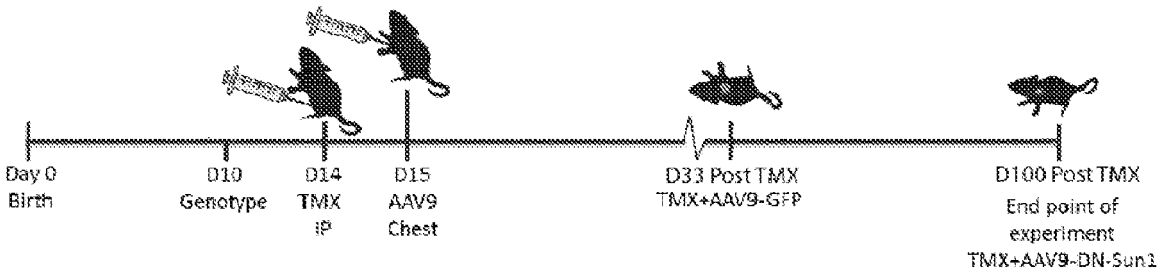


Fig. 31B

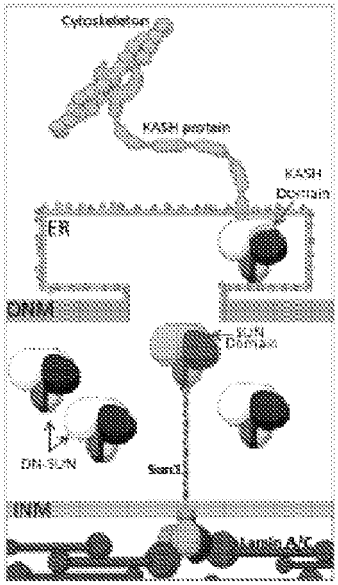


Fig. 31C

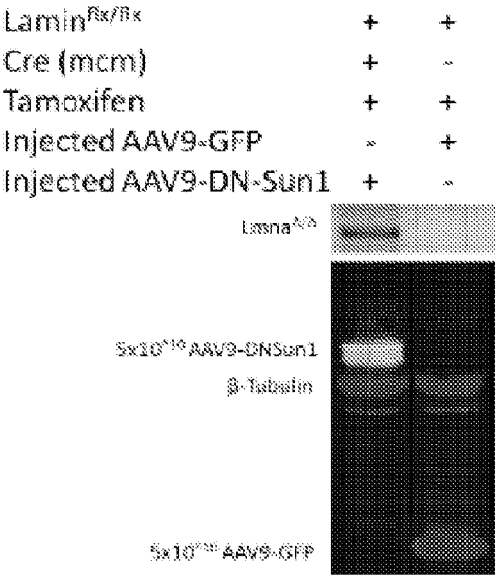


Fig. 31D

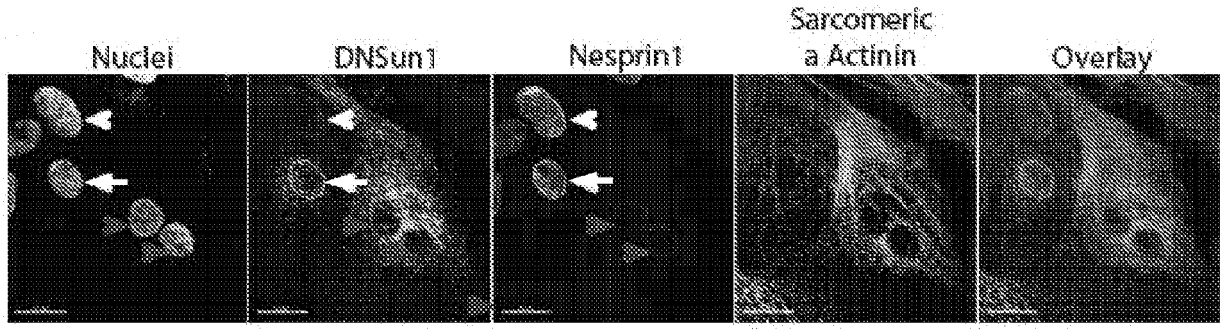


Fig. 31E (i)

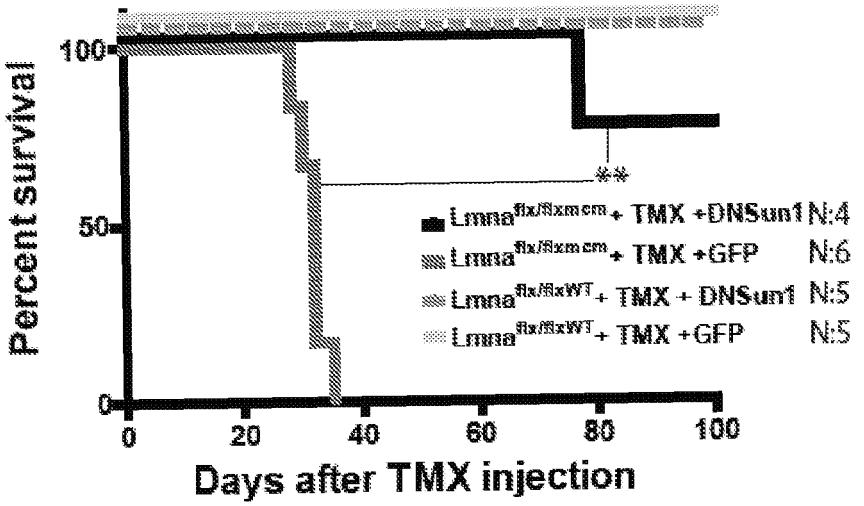


Fig. 31E (ii)

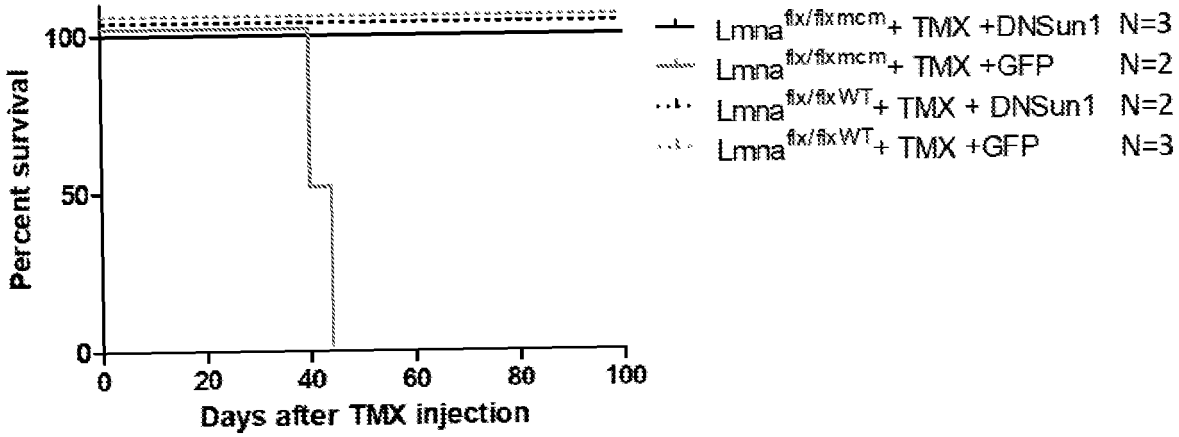


Fig. 31F

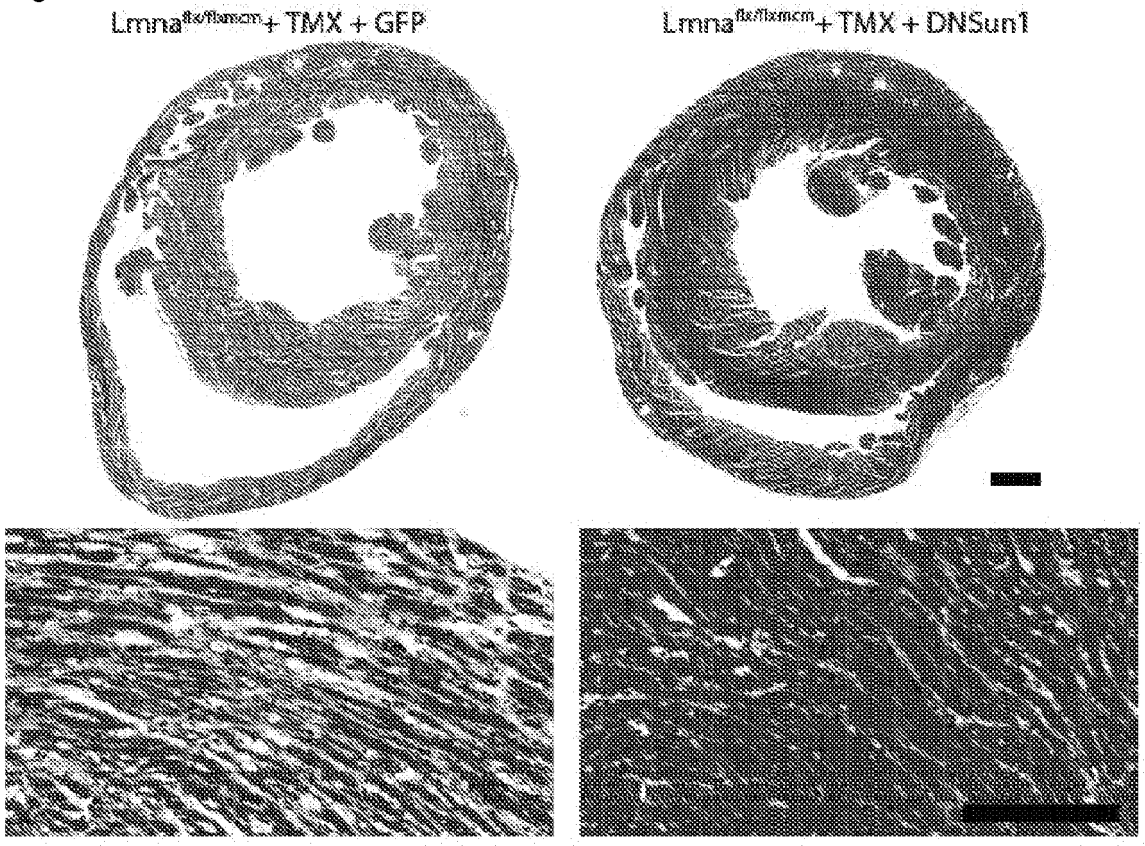


Fig. 31G

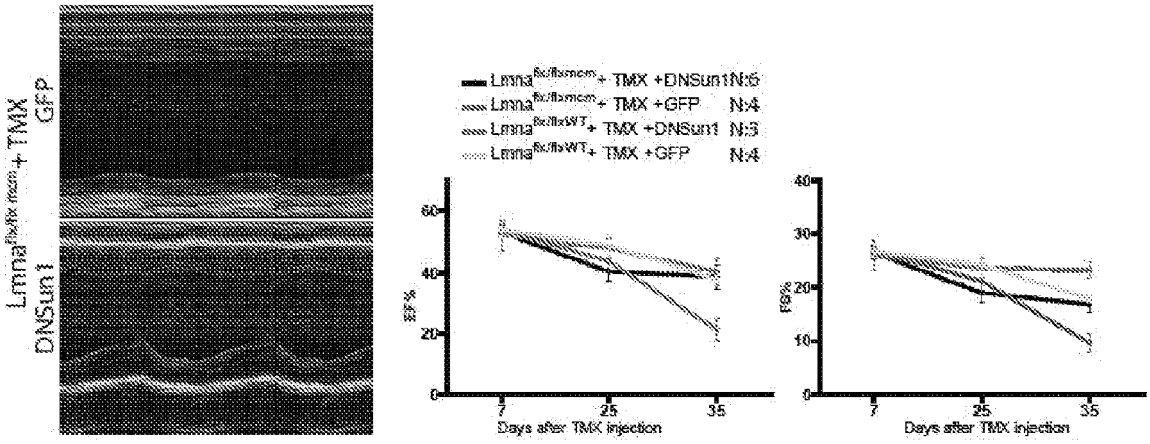


Fig. 32A

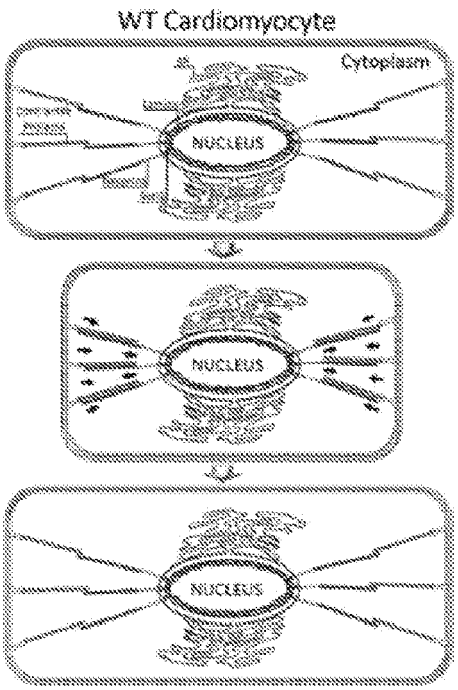


Fig. 32B

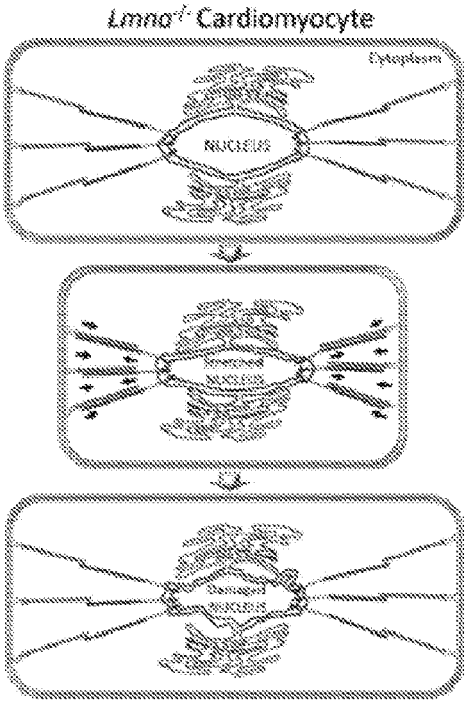


Fig. 32C

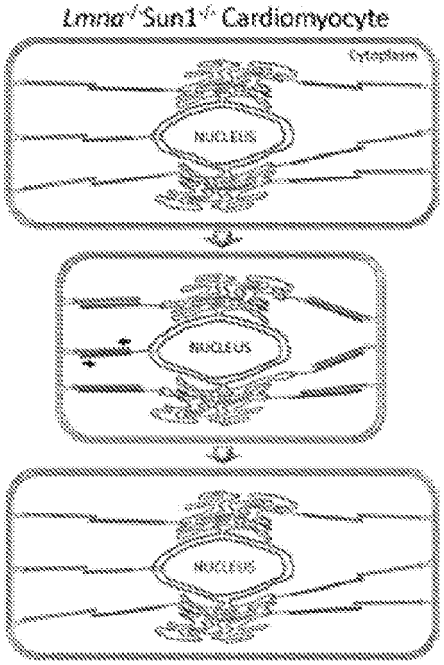


Fig. 32D

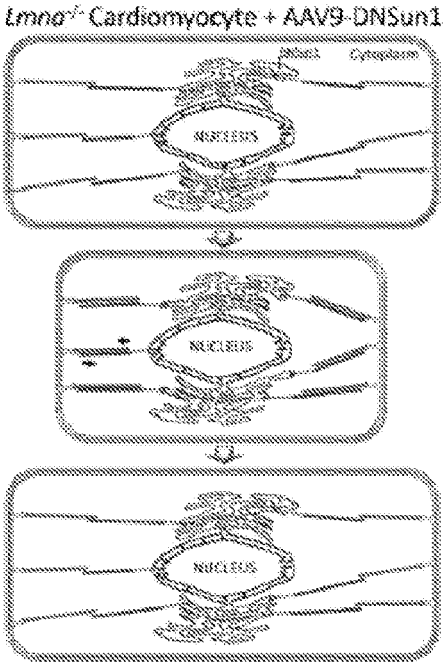


Fig. 33

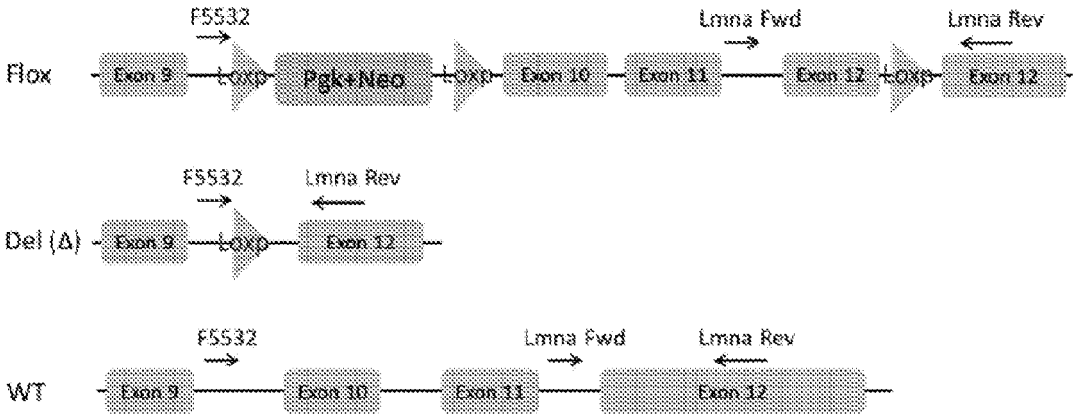


Fig. 34

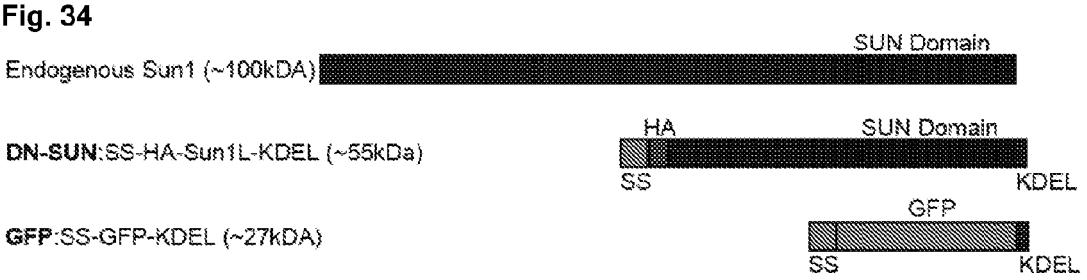


Fig. 35

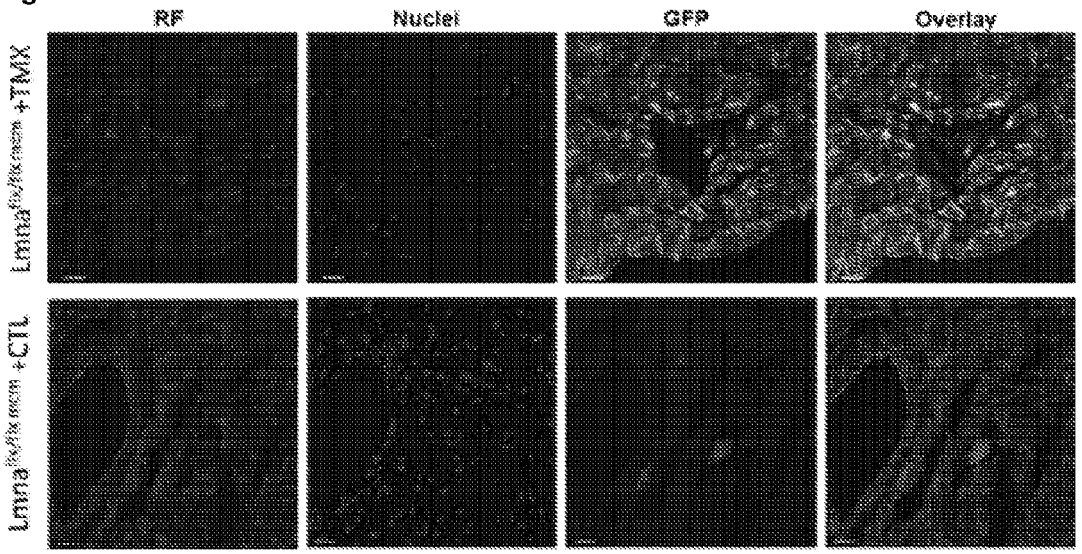
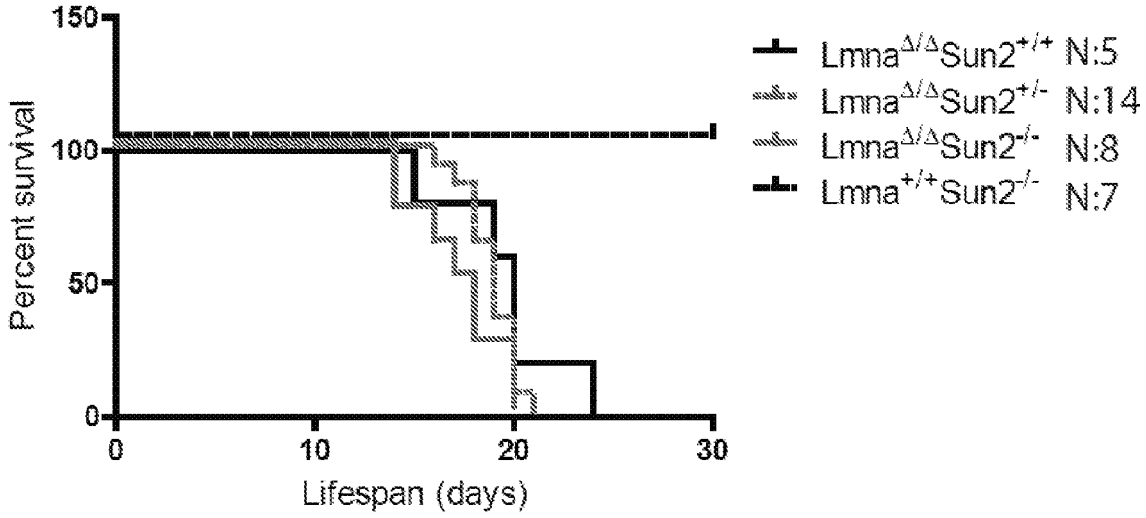


Fig. 36


150
100
50
0
Percent survival
0
10
20
30
Lifespan (days)
Lmna$^{\Delta/\Delta}$Sun2$^{+/+}$ N:5
Lmna$^{\Delta/\Delta}$Sun2$^{+/-}$ N:14
Lmna$^{\Delta/\Delta}$Sun2$^{-/-}$ N:8
Lmna$^{+/+}$Sun2$^{-/-}$ N:7

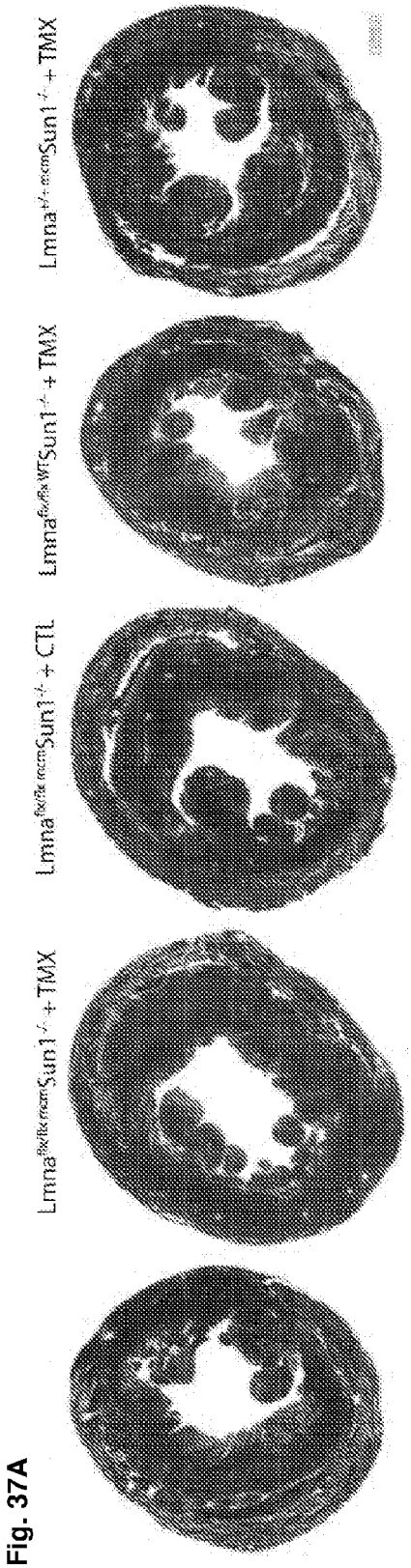

Lmna fl/fl rosa Sun1 -/- + TMX
Lmna fl/fl rosa Sun1 -/- + CTL
Lmna fl/fl WT Sun1 -/- + TMX
Lmna +/+ rosa Sun1 -/- + TMX

Fig. 37A

Fig. 37B

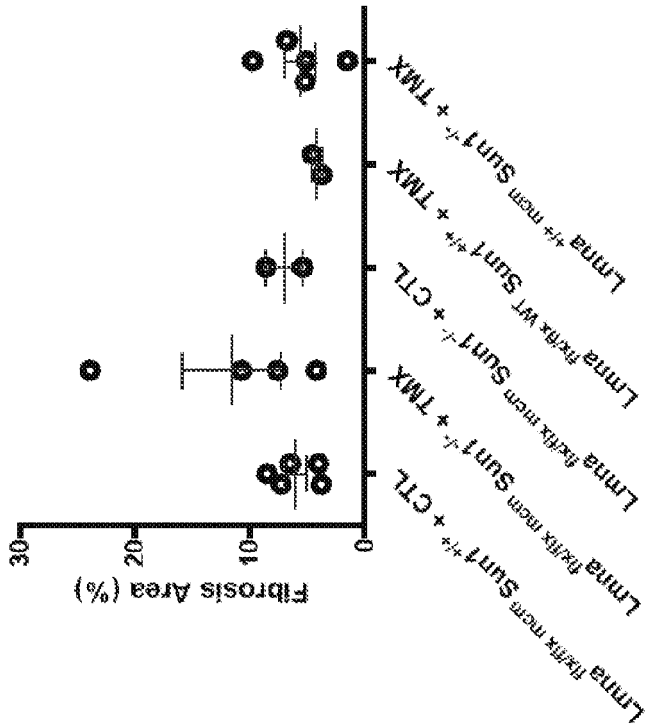


Fig. 37C

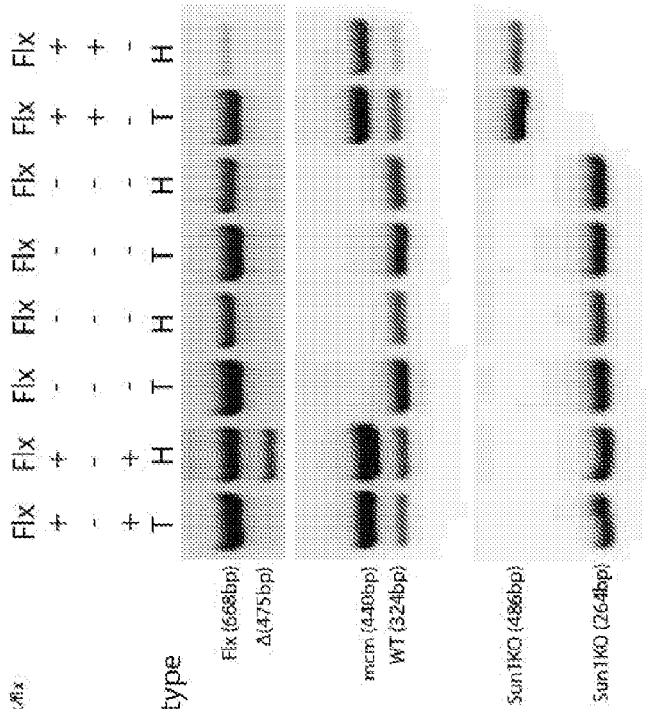


Fig. 37D

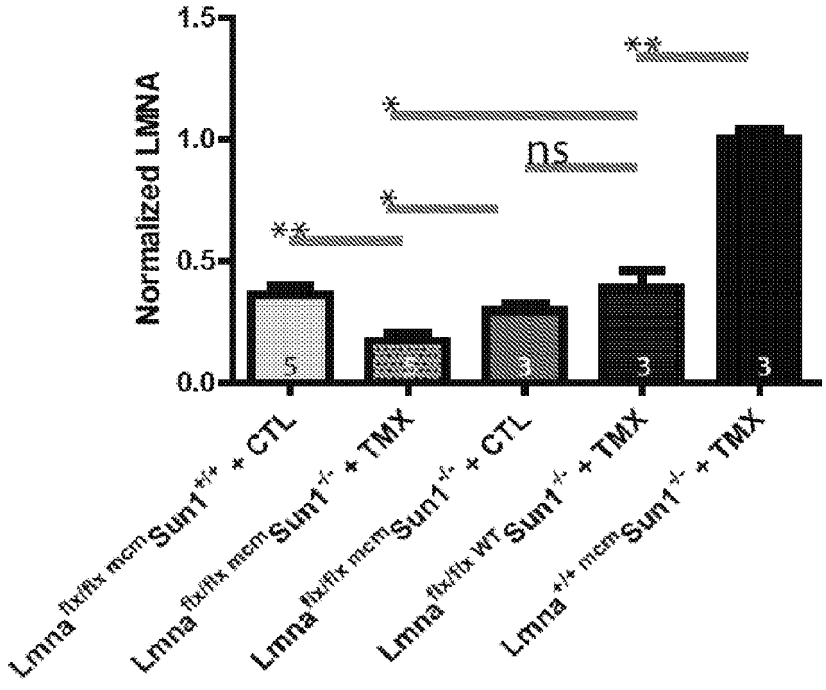

Normalized LMNA
1.5
1.0
0.5
0.0
**
*
*
ns
**
5
3
3
3
Lmna flx/flx mcm Sun1 +/+ + CTL
Lmna flx/flx mcm Sun1 -/- + TMX
Lmna flx/flx mcm Sun1 -/- + CTL
Lmna flx/flx WT Sun1 -/- + TMX
Lmna +/+ mcm Sun1 -/- + TMX

Fig. 37E

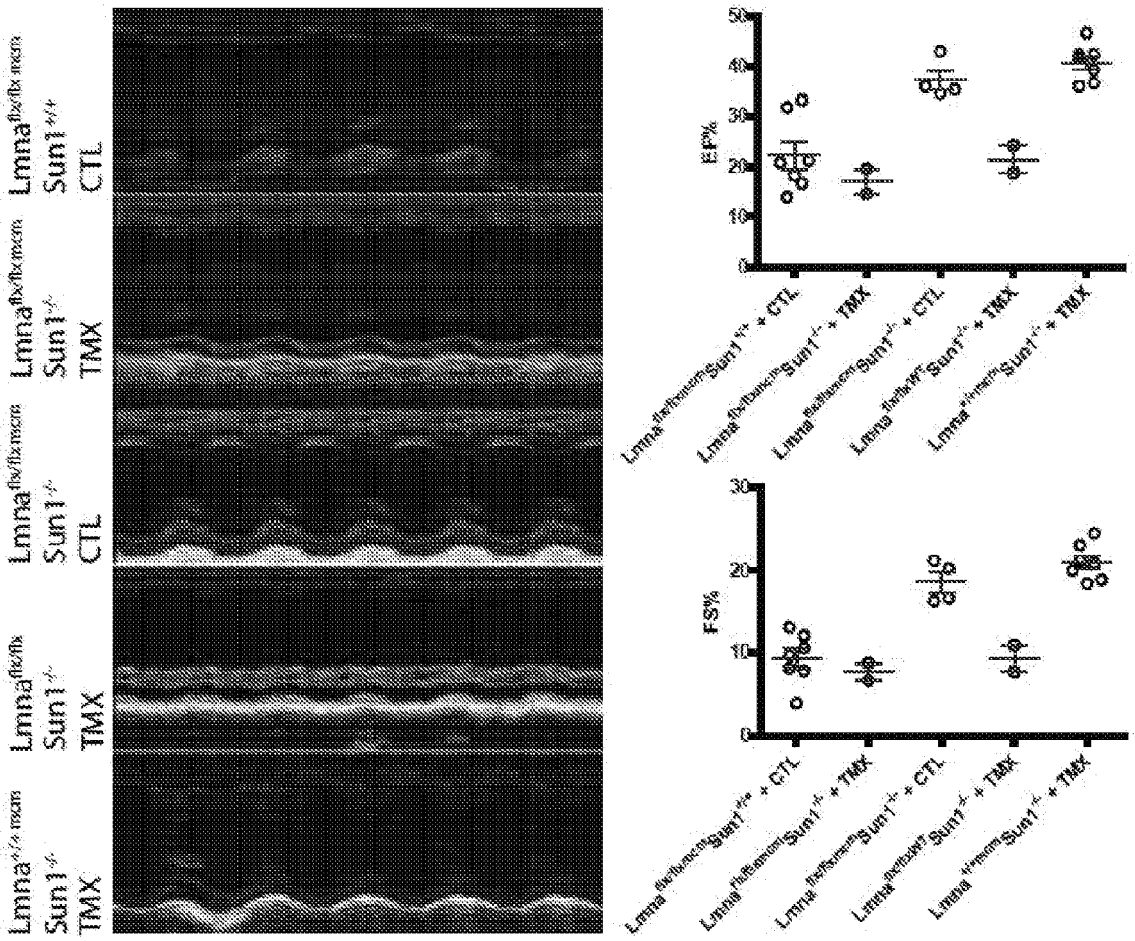

Lmna+/+mcm Sun1-/- TMX
Lmnaflx/flx Sun1-/- TMX
Lmnaflx/flxmcm Sun1-/- CTL
Lmnaflx/flxmcm Sun1-/- TMX
Lmnaflx/flxmcm Sun1+/+ CTL
EF%
FS%
Lmnaflx/flxmcmSun1+/+ + CTL
Lmnaflx/flxmcmSun1-/- + TMX
Lmnaflx/flxmcmSun1-/- + CTL
Lmnaflx/flxmcmSun1-/- + TMX
Lmna+/+mcmSun1-/- + TMX

Fig. 38

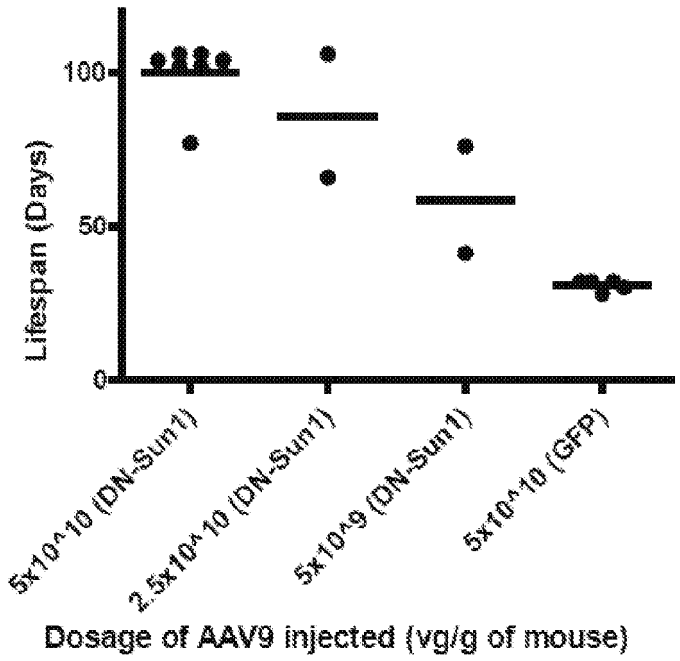

100
50
0
Lifespan (Days)
5x10^10 (DN-Sun1)
2.5x10^10 (DN-Sun1)
5x10^9 (DN-Sun1)
5x10^10 (GFP)
Dosage of AAV9 injected (vg/g of mouse)

Fig. 39A

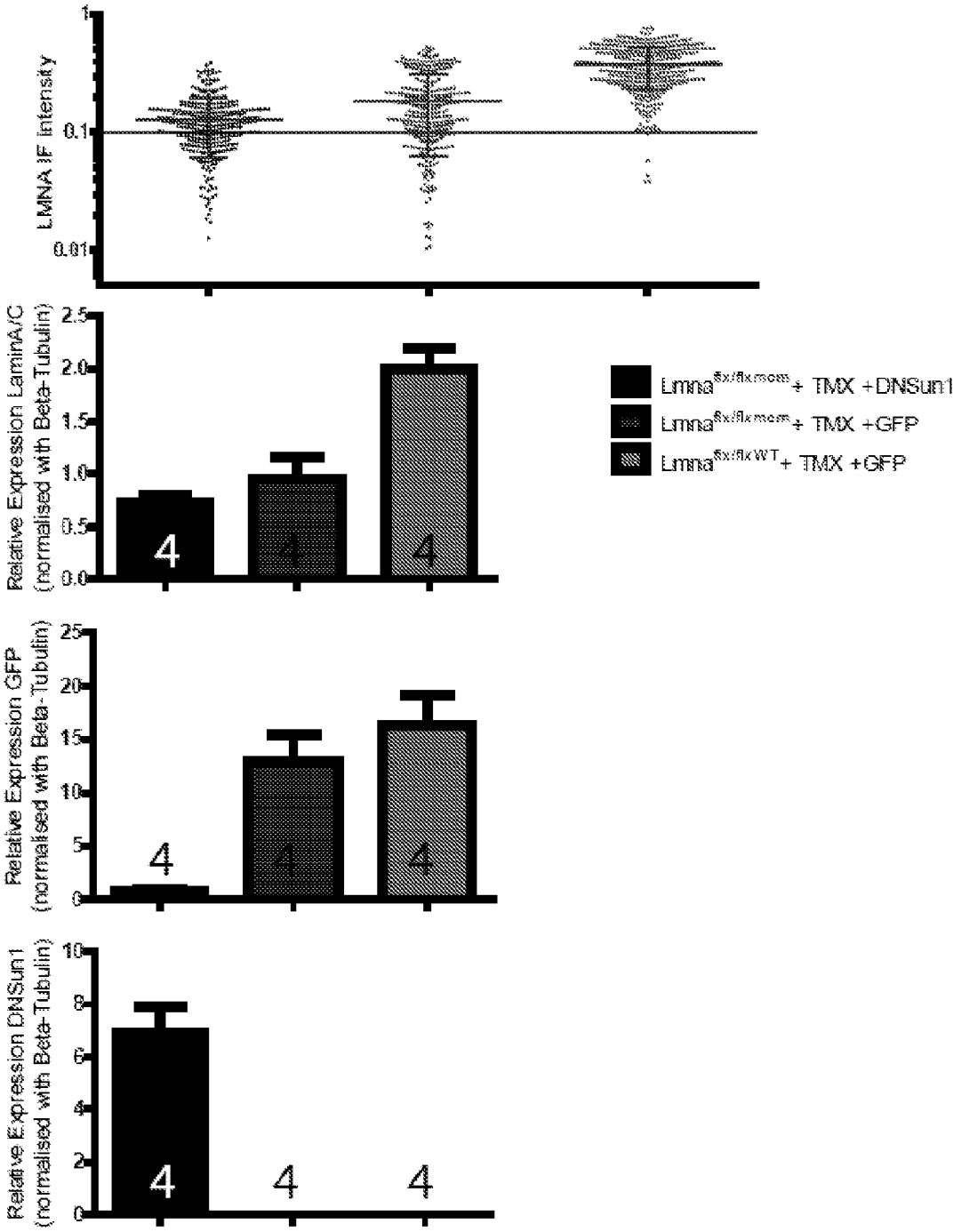

LMNA IF intensity
1
0.1
0.01
Relative Expression LaminA/C (normalised with Beta-Tubulin)
2.5
2.0
1.5
1.0
0.5
0.0
4
4
4
Lmna$^{fx/fxmom}$+ TMX +DNSun1
Lmna$^{fx/fxmom}$+ TMX +GFP
Lmna$^{fx/fxWT}$+ TMX +GFP
Relative Expression GFP (normalised with Beta-Tubulin)
25
20
15
10
5
0
4
4
4
Relative Expression DNSun1 (normalised with Beta-Tubulin)
10
8
6
4
2
0
4
4
4

Fig. 39B

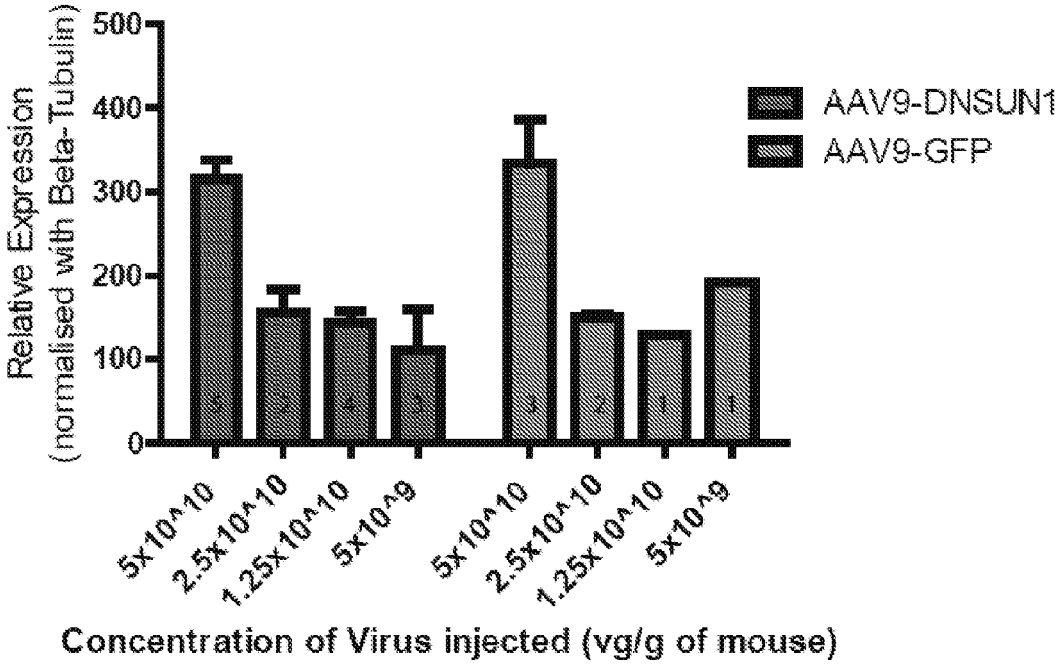


Fig. 39C

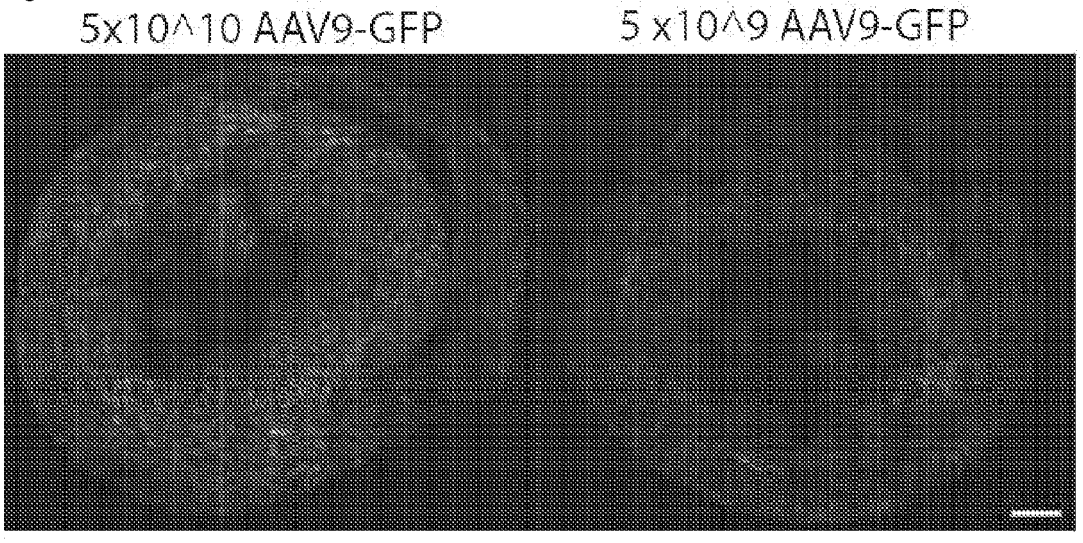


Fig. 40A

```
SEQ ID NO:69   wildtype    CATGACCTTGAGCTGAAACTGCTGCAGAATATCACACACCACATCACCGTGACAGGACAG   2100
SEQ ID NO:70   Sun1_plus4  CATGACCTTGAGCTGAAACTGCTGCAGAAATATCACACACCACATCACCGTGACAGGACAG   2100
SEQ ID NO:71   Sun1_del7   CATGACCTTGAGCTGAAACTGCTGCAGAATATCACACACCACATCACCGTGACAGGACAG   2100
                            ************************************************************

SEQ ID NO:69   wildtype    GCCCCG----ACATCCGAGGCTATTGTGTCTGCCGTGAATCAGGCAGGGATTTCAGGAAT   2156
SEQ ID NO:70   Sun1_plus4  GCCCCGagacACATCCGAGGCTATTGTGTCTGCCGTGAATCAGGCAGGGATTTCAGGAAT   2160
SEQ ID NO:71   Sun1_del7   GCCC----------CGAGGCTATTGTGTCTGCCGTGAATCAGGCAGGGATTTCAGGAAT   2149
                            ****          ************************************************

SEQ ID NO:69   wildtype    CACAGAAGCGcaagcacatatcattgtgaacaatgctctgaagctgtactcccaagacaa   2216
SEQ ID NO:70   Sun1_plus4  CACAGAAGCGcaagcacatatcattgtgaacaatgctctgaagctgtactcccaagacaa   2220
SEQ ID NO:71   Sun1_del7   CACAGAAGCGcaagcacatatcattgtgaacaatgctctgaagctgtactcccaagacaa   2209
                            ************************************************************
```

Fig. 40B

```
SEQ ID NO:72   wildtype       WLLEKLSSRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPTSEAIVSAVNQAGISGIT    720
SEQ ID NO:73   Sun1_plus4     WLLEKLSSRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPRHIRGYCVCRESGRDFRN    720
SEQ ID NO:74   Sun1_del7      WLLEKLSSRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPRLLCLP---*--------    708
                              ****************************************

SEQ ID NO:72   wildtype       EAQAHIIVNNALKLYSQDKTGMVDFALESGGGSILSTRCSETYETKTALLSLFGVPLWYF    780
SEQ ID NO:73   Sun1_plus4     --------HRSASTYHCEQCSEAVLPRQDGDGGL----C---------------SGVWRW    753
SEQ ID NO:74   Sun1_del7      ------------------------------------------------------------    708

SEQ ID NO:72   wildtype       SQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMKIYPTTFTMEHIPKTLSPTGNISSAPK    840
SEQ ID NO:73   Sun1_plus4     QHPKHSVL*-----------------------------------------------------    761
SEQ ID NO:74   Sun1_del7      ------------------------------------------------------------    708

SEQ ID NO:72   wildtype       DFAVYGLETEYQEEGQPLGRFTYDQEGDSLQMFHTLERPDQAFQIVELRVLSNWGHPEYT    900
SEQ ID NO:73   Sun1_plus4     ------------------------------------------------------------    761
SEQ ID NO:74   Sun1_del7      ------------------------------------------------------------    708

SEQ ID NO:72   wildtype       CLYRFRVHGEPIQ*    913
SEQ ID NO:73   Sun1_plus4     --------------    761
SEQ ID NO:74   Sun1_del7      --------------    708
```

Fig. 40C

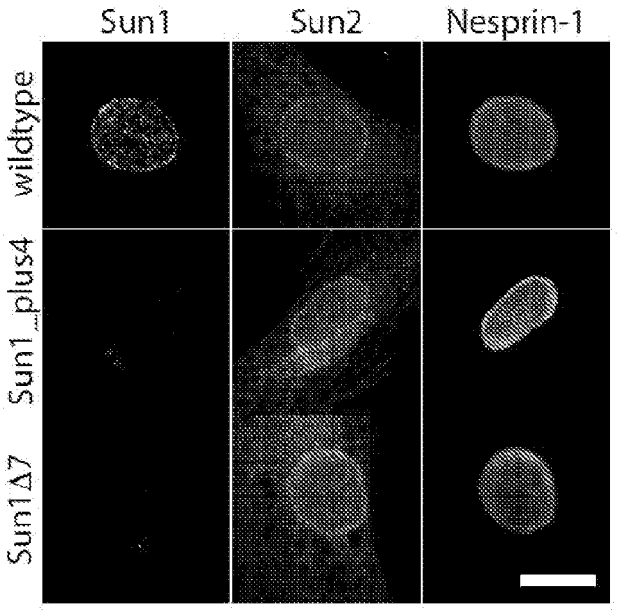


Fig. 41

A

```
SEQ ID NO:75   wildtype        TATTGGACTCACCTGCCTTGTACCCATGTCAGAGAAAGACTACAGCTGTGCCCTCTCCAA     180
SEQ ID NO:76   Syne1_CTdel8    TATTGGACTCACCTGCCTTGTACCCATGTCAGAGAAAGACTACAGCTGTGCCCTCTCCAA     180
                               ************************************************************

SEQ ID NO:75   wildtype        CAACTTTGCCCGATCCTTCCATCCGATGCTCAGATATACCAACGGTCCTCCTCCACTCTG     240
SEQ ID NO:76   Syne1_CTdel8    CAACTTTGCCCGATCCTTCCATCCG--------ATATACCAACGGTCCTCCTCCACTCTG     232
                               *************************        *************************

SEQ ID NO:75   wildtype        AAGCAAGCAGACATCCCCACACAAGTGcaggcagtaagaggagaaggaatatcaaatggc     300
SEQ ID NO:76   Syne1_CTdel8    AAGCAAGCAGACATCCCCACACAAGTGcaggcagtaagaggagaaggaatatcaaatggc     292
                               ************************************************************
```

B

```
SEQ ID NO:77   wildtype         STRDGSDSSRSDPRPERVGRAFLFRILRAALPFQLLLLLLIGLTCLVPMSEKDYSCALSN    60
SEQ ID NO:78   Nesprin1_CTdel8  STRDGSDSSRSDPRPERVGRAFLFRILRAALPFQLLLLLLIGLTCLVPMSEKDYSCALSN    60
                                ************************************************************

SEQ ID NO:77   wildtype         NFARSFHPMLRYTNGPPPL*----------------------------------------    79
SEQ ID NO:78   Nesprin1_CTdel8  NFARSFHPIYQRSSSTLKQADIPTQVQAVRGEGISNGREAPKEKFNILNHQGNANQNNPE    120
                                ********: : :..

SEQ ID NO:77   wildtype         ----------      79
SEQ ID NO:78   Nesprin1_CTdel8  METNAPPCS*      129
```

Fig. 41 (continued)

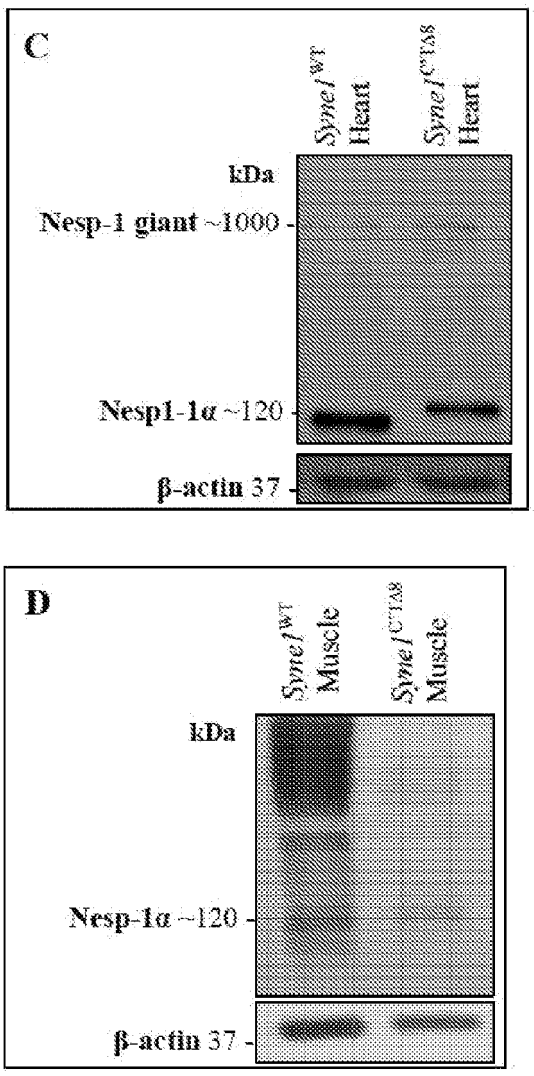


Fig. 42

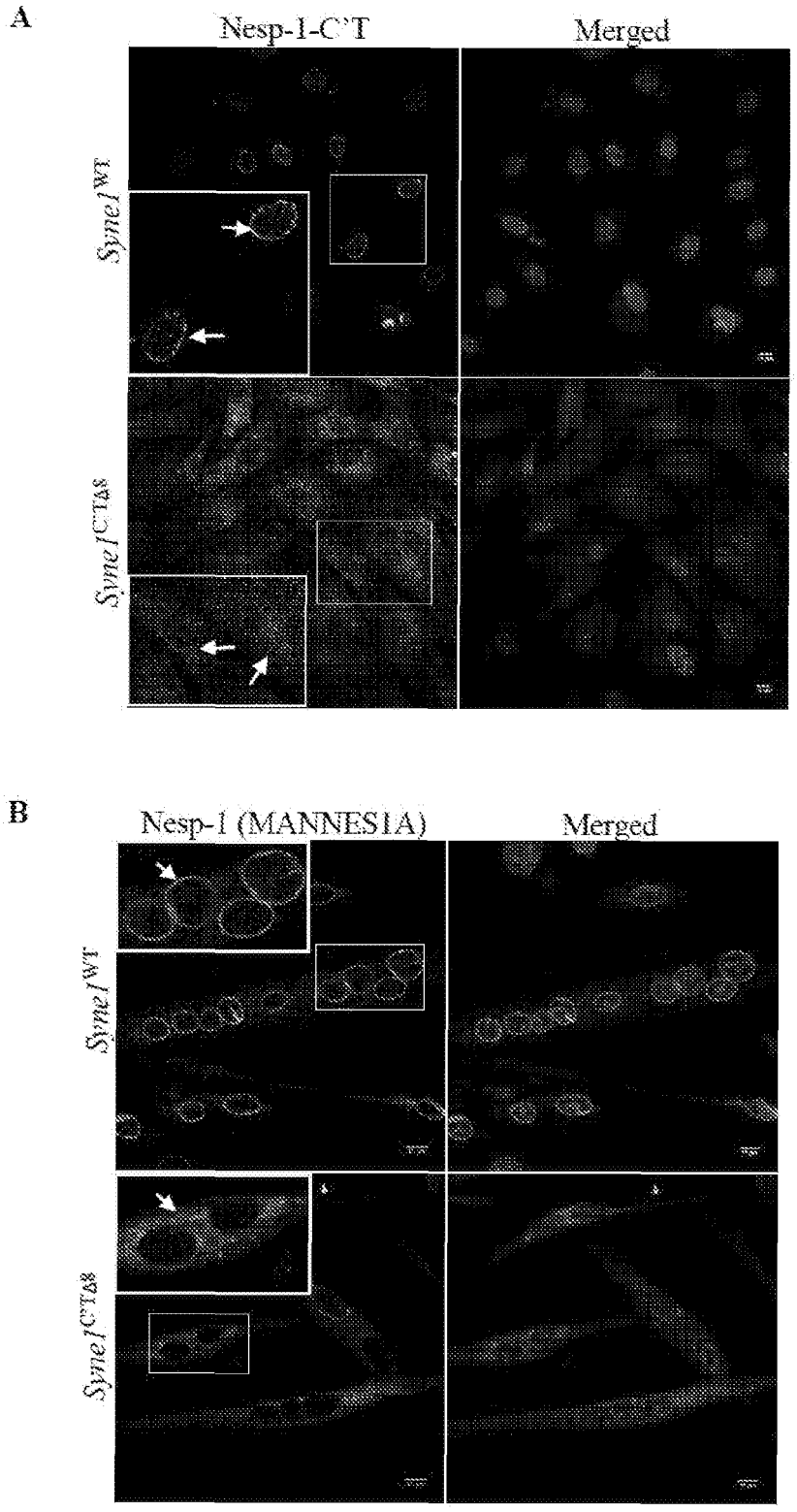

A
Nesp-1-CT
Merged
Syne1WT
Syne1CTΔ8
B
Nesp-1 (MANNES1A)
Merged
Syne1WT
Syne1CTΔ8

Fig. 43

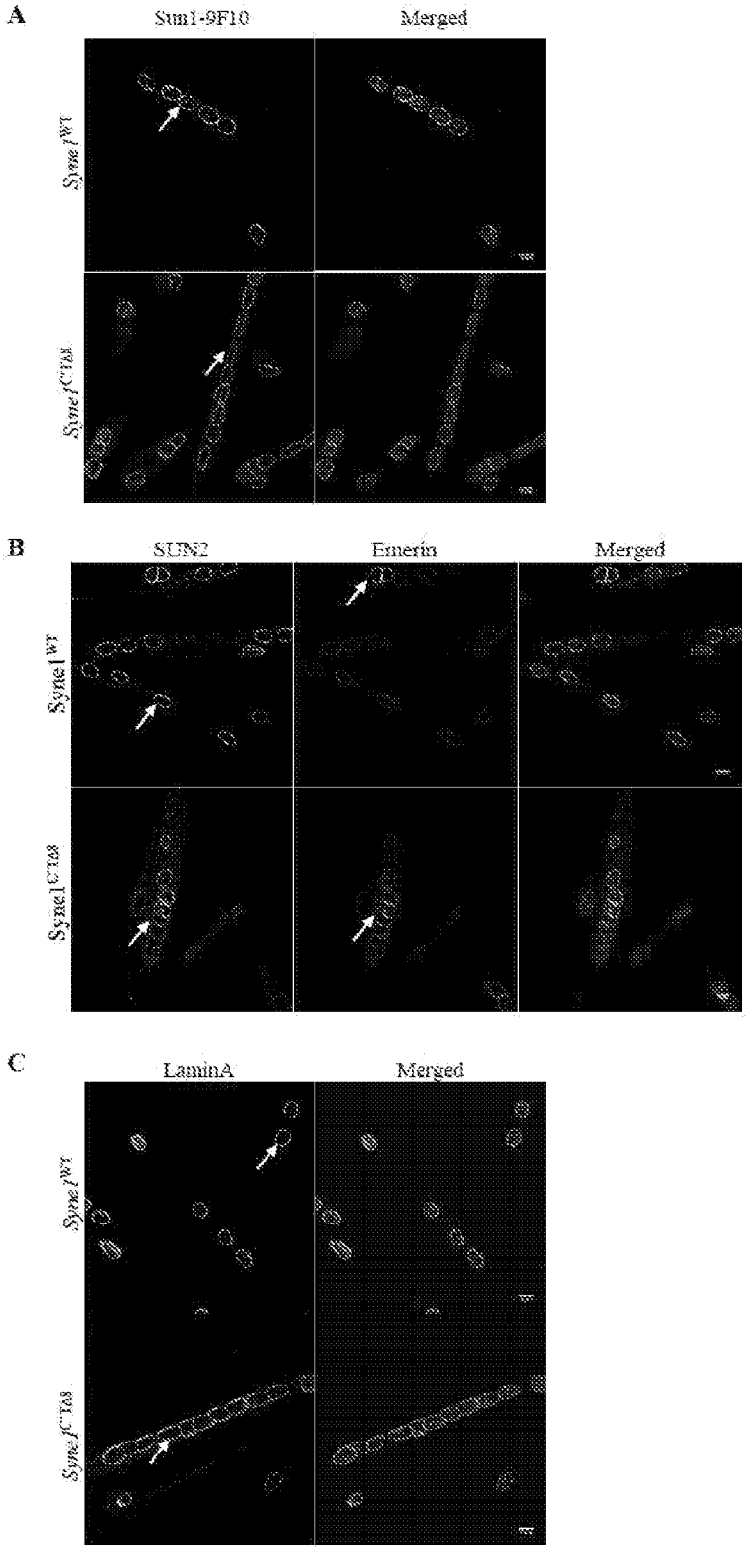

A
Sun1-9F10
Merged
Syne1WT
Syne1CTΔ8
B
SUN2
Emerin
Merged
Syne1WT
Syne1CTΔ8
C
LaminA
Merged
Syne1WT
Syne1CTΔ8

Fig. 44

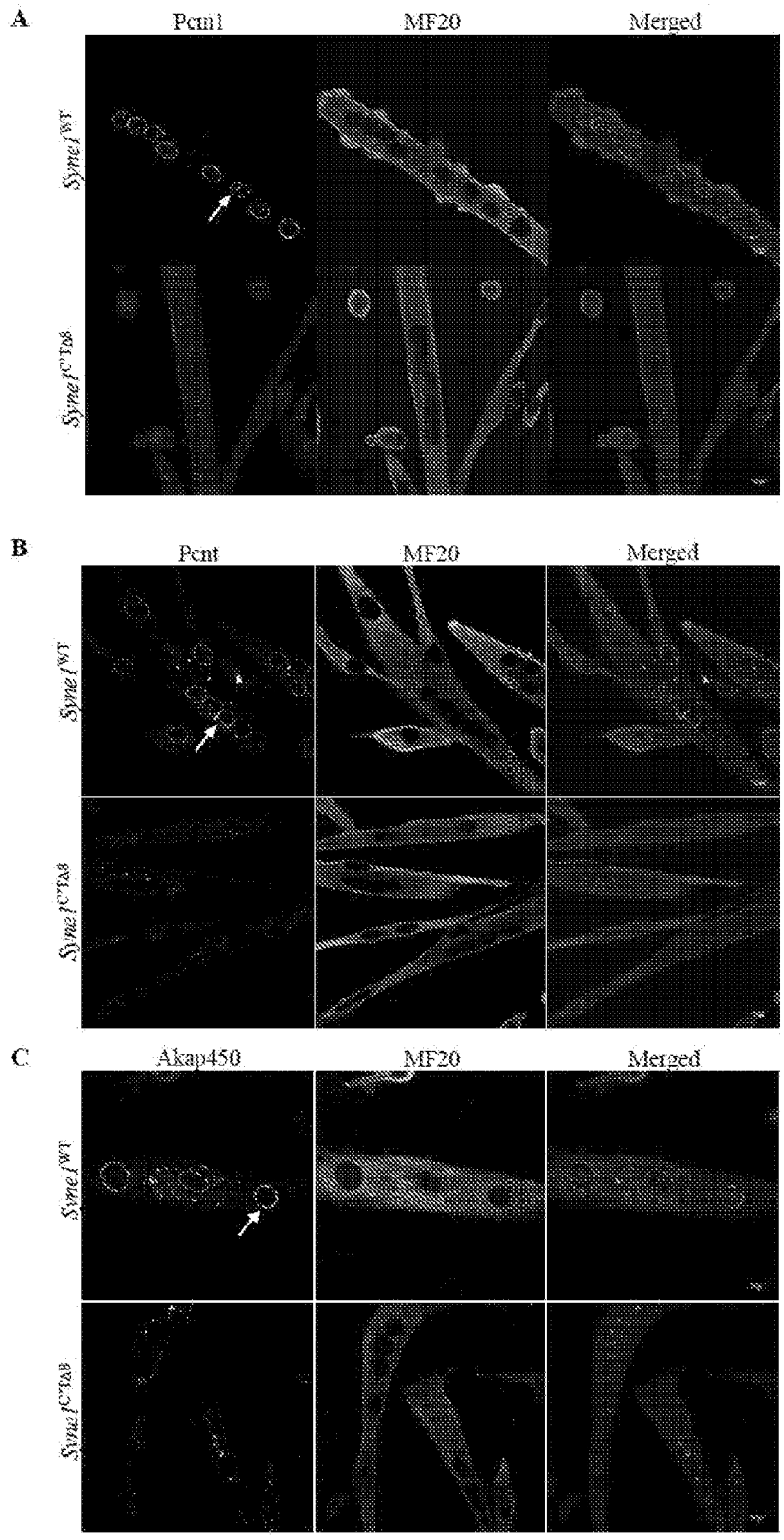
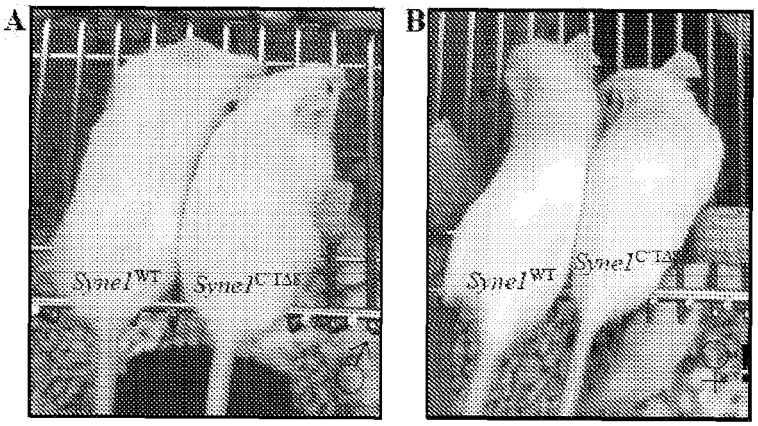

A
Pcm1
MF20
Merged
Syne1WT
Syne1CTa8
B
Pcnt
MF20
Merged
Syne1WT
Syne1CTa8
C
Akap450
MF20
Merged
Syne1WT
Syne1CTa8

Fig. 45


A
B
Syne1WT
Syne1C-TΔ8
Syne1WT
Syne1C-TΔ8

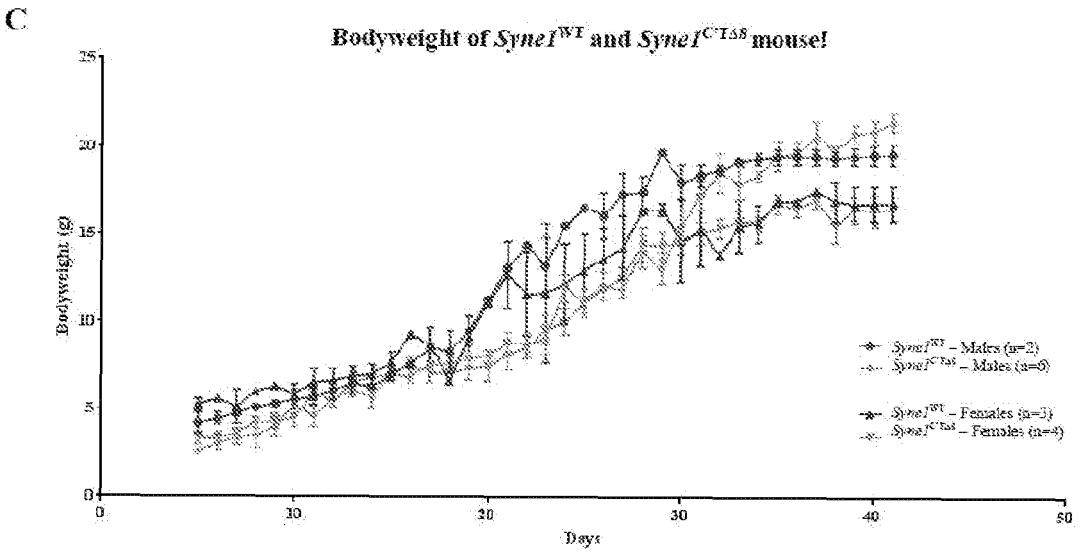

C
Bodyweight of Syne1WT and Syne1C-TΔ8 mouse!
Bodyweight (g)
Days
0
5
10
15
20
25
0
10
20
30
40
50
Syne1WT – Males (n=2)
Syne1C-TΔ8 – Males (n=6)
Syne1WT – Females (n=3)
Syne1C-TΔ8 – Females (n=4)

Fig. 46A

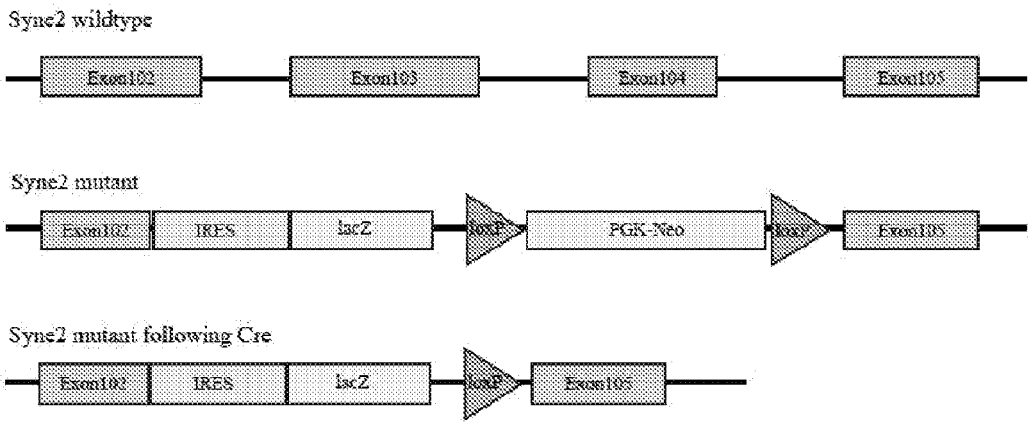


Fig. 46B

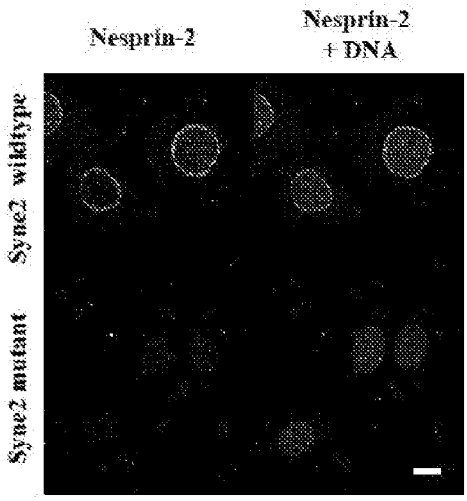


Fig. 46C

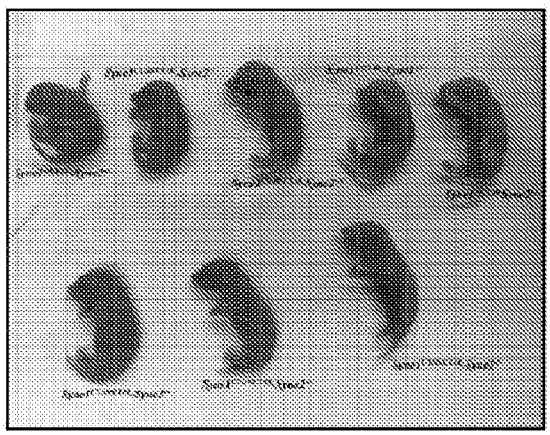
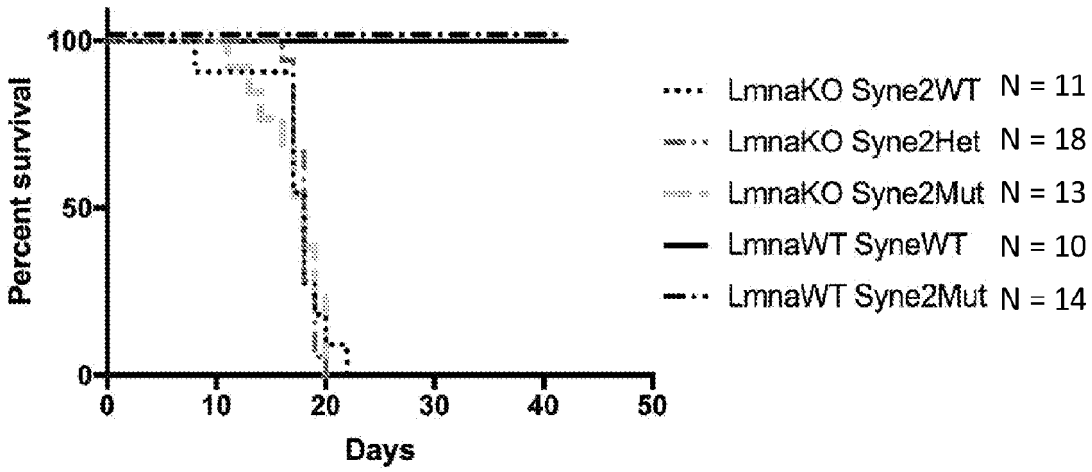


Fig. 47


Percent survival
100
50
0
0
10
20
30
40
50
Days
LmnaKO Syne2WT N = 11
LmnaKO Syne2Het N = 18
LmnaKO Syne2Mut N = 13
LmnaWT SyneWT N = 10
LmnaWT Syne2Mut N = 14

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