Disrupting the LINC complex by AAV mediated gene transduction prevents progression of Lamin induced cardiomyopathy

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Mutations in the LaminA gene are a common cause of monogenic dilated cardiomyopathy. Here we show that mice with a cardiomyocyte-specific Lmna deletion develop cardiac failure and die within 3–4 weeks after inducing the mutation. When the same Lmna mutations are induced in mice genetically deficient in the LINC complex protein SUN1, life is extended to more than one year. Disruption of SUN1’s function is also accomplished by transducing and expressing a dominant-negative SUN1 miniprotein in Lmna deficient cardiomyocytes, using the cardiotrophic Adeno Associated Viral Vector 9. The SUN1 miniprotein disrupts binding between the endogenous LINC complex SUN and KASH domains, displacing the cardiomyocyte KASH complexes from the nuclear periphery, resulting in at least a fivefold extension in lifespan. Cardiomyocyte-specific expression of the SUN1 miniprotein prevents cardiomyopathy progression, potentially avoiding the necessity of developing a specific therapeutic tailored to treating each different LMNA cardiomyopathy-inducing mutation of which there are more than 450.
Dilated Cardiomyopathy (DCM) is the most common disease affecting heart muscle, accounting for ~60% of all cardiomyopathies. It is characterized by reduced systolic (contractile) function due to enlargement and thinning of the left ventricular wall. In some cases, both ventricles are affected. DCM often results in sudden heart failure and cardiac death, resulting in high hospital admission rates, the need for heart transplantation, and consequently a high-cost burden. The causes of DCM are varied but include various extrinsic factors (viral, autoimmune infiltration, lifestyle). 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, which encodes the giant sarcomeric protein titin, with truncating mutations having varied but include various extrinsic factors (viral, autoimmune). Mice, therefore, provide a valuable model to determine the underlying molecular pathology of DCM.

LMNA-induced DCM is characterized by cardiac conduction defects, manifested by electrophysiological abnormalities, including atrioventricular block, ventricular arrhythmias, and fibrillation. The risk of sudden cardiac death is more significant in patients with LMNA-cardiomyopathy than patients with other forms of DCM. Some 450 different dominant missense mutations have been identified in the LMNA gene that are linked to DCM. This diversity of mutations complicates genetic approaches to treating LMNA-induced DCM. To a limited extent, LMNA-linked DCM can be treated by fitting a pacemaker. Ultimately, effective treatment is accomplished by heart transplantation.

Mice carrying Lmna mutations die within a few weeks after birth. The cause of their early death is uncertain due to multiple tissues being affected. However, cardiac myopathy is considered to be a significant factor, as many Lmna mutant mice develop DCM with conduction abnormalities with focal myocyte degeneration. Mice, therefore, provide a valuable model to determine the underlying molecular pathology of DCM.

The laminas 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 is composed of the A-type lamins, consisting of 2 predominant forms, lamins A and lamin C, derived by alternate splicing of LMNA. In addition, two B-type lamins (LMNB 1 and 2) are encoded by separate genes, LMNB1 and LMNB2. The lamina provides structural and mechanical integrity to the nucleus, maintains nuclear shape, and contributes to nuclear positioning within the cell. In addition, the lamina is a critical determinant of chromatin organization, through the provision of binding sites at the nuclear periphery for higher-order chromatin domains. The lamins interact with numerous INM proteins, including Emerin, the Lamina-Associated Polypeptides (LAPs), and the SUN domain proteins, many of which are either mutated or present as a variant linked to heart disease. Together, these proteins comprise an integrated protein network, centered on the lamina, where loss or mutation of the lamins can result in either the mislocalization or a change in their expression levels. Among the proteins, whose expression is altered by the loss of mutation of Lmna are SUN and LAP2α, whose levels increase. Genetic reduction of the levels of either protein, particularly SUN1, significantly ameliorates much of the pathology observed in mice with Lmna mutations, so increasing longevity. Whether this is a consequence of SUN1 toxicity due to elevated expression levels versus elimination of a SUN1 function that exacerbates the effects of Lmna deficiency is uncertain.

The SUN (Sad1p, UNC-84) proteins share a conserved C-terminal SUN domain and localize to the INM. In mammals, SUN1 and SUN2 are the two principal SUN proteins widely expressed in virtually all tissues. In the perinuclear space, between the INM and outer nuclear membrane (ONM), the C-termini of either SUN1 or 2 binds to the C-termini (KASH domains) of the different Nesprins/SYNEx/KASH proteins that traverse the ONM. Together, these two families of proteins comprise the LINC complexes, which physically couple interphase nuclei to the cytoskeleton. The N-termini of the SUN domain proteins protrude into the nucleoplasm. With SUN1, this region interacts with pre-lamina and nuclear pore complexes, as well as other nucleoplasmonic/NE proteins such as DROSHA. With the Nesprins/KASH-domain proteins, their large N-terminal domains extend into the cytoplasm adjacent to the ONM. Depending on the particular Nespin/KASH protein, the Nesprins interact directly or indirectly with the three cytoskeletal protein networks (microtubules, actin microfilaments, and intermediate filaments). Consequently, the LINC complex establishes 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 mediates force transmission between the nucleus and cytoskeleton, resulting in changes in gene expression/chromatin organization in response to mechanical/physical stimuli. Although the loss of either SUN1 or SUN2 alone has no overt effect on postnatal growth and longevity, SUN1 null mice are both infertile and deaf. However, simultaneous loss of SUN1 and SUN2 results in perinatal lethality, indicating some degree of redundancy, at least during embryogenesis.

Here we show that in mice that develop Lmna-induced DCM, disruption of the LINC complex, by either genetic ablation or by delivery of a Dominantly Negative acting Sun1 miniprotein (DNSUN1) that destabilizes the LINC complex, significantly ameliorates DCM progression, and leads to at least a fivefold increase in longevity. We demonstrate that disruption of the LINC complex in individuals carrying LMNA mutations, AAV mediated delivery of a DN-SUN1 to CMs may be of therapeutic benefit to patients with LMNA associated DCM.

Results
Cardiomyocyte-specific loss of Lmna results in heart failure. To further define the consequences of Lmna loss to postnatal pathology in mice, we specifically ablated the Lmna gene in specific tissues by using a floxed conditional Lmna<sup>fl/fl</sup> line of mice that, when recombined by Cre activation, results in the complete loss of LaminA/C protein as previously described. If the Lmna<sup>fl/fl</sup> is constitutively deleted in all tissues by crossing the Lmna<sup>fl/fl</sup> mice with Zp3-Cre mice, the mean postnatal lifespan is 17.5 days (Fig. 1A). When the same deletion is induced in the absence of Sun1, Lmna<sup>Δ/Δ</sup> Sun1<sup>−/−</sup> mice lived to a mean of 32.5 days, almost a doubling in longevity (Fig. 1A). Performing the same Lmna deletion on a Sun2 null background did not extend the longevity of Lmna<sup>Δ/Δ</sup> mice, revealing the longevity extension is specific to the loss of Sun1 even though Sun2 is expressed in multiple tissues, including cardiomyocytes (Supplementary Fig. 1). As the A-type lamins are widely expressed in almost all adult tissues, we then determined whether Lmna deletion, specifically in cardiomyocytes (CMs), contributes to the early postnatal death of Lmna<sup>Δ/Δ</sup> mice and what consequences the same tissue-specific loss of Lmna would have on a Sun1 null background. We crossed the Lmna<sup>Δ/Δ</sup> to a Myh6-Cre<sup>−/−</sup> mice, in which constitutive Cre expression commences during embryogenesis but is restricted to CMs. These mice survived slightly longer than the Lmna<sup>Δ/Δ</sup> for an average of 26.5 days postnatally (Fig. 1B). The same CM-specific deletion performed on a Sun1<sup>−/−</sup> background resulted in a significant increase in longevity to at least 6 months and beyond after...
birth (Fig. 1B), revealing that loss of SUN1 significantly extends longevity in mice with LmA-induced heart failure. To further define the loss of LmA and its effect in postnatal/adult CMs, we derived mice homozygous for the LmAFF allele carrying the inducible cardiomyocyte-specific Cre-Tg (Myh6-Cre/Esri1) (abbreviated to mcm) in which Cre is induced in CMs by a single injection of tamoxifen (Tmx)\(^{32}\). The average lifespan of 1–5 month-old LmAFF/mcm mice, following Cre induction, was 27 days (Figs. 1C, 2A). LmAFF or WT controls were unaffected by Tmx injection and showed typical longevity. However, on a Sun1 null background, longevity was significantly extended to more than 400 days following Cre induction (see below).

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 LmA mutant mouse line, carrying the N195K missense mutation, in which early death is a consequence of heart failure. LmA mutation has been identified in at least two unrelated patients diagnosed with DCM\(^{33,34}\). Here too, we found that the absence of SUN1 significantly increased the lifespan of this mutant line and improved cardiac function (Fig. 1D). We extended these findings by deriving mice heterozygous for the N195K mutation, where the WT-LmA allele is floxed, i.e., LmA\(^{N195K/F}\) X Sun1\(^{+/+}\). Activating Cre in these mice by Tmx injection (LmA\(^{N195K/F}\)mcm + Tmx) resulted in the cardiomyocyte-specific deletion of the WT floxed LmA allele rendering the CMs hemizygous for the N195K mutation, i.e., LmA\(^{N195K/+}\). These mice had a mean lifespan of fewer than 50 days, a lifespan half that of the original LmA\(^{N195K/N195K}\) homozygotes (Supplementary Fig. 2A), whereas LmA\(^{N195K/+}\) heterozygotes live for more than 1 year. When the LmA\(^{N195K/F}\)mcm mutation was induced on a Sun1 null background, lifespan is significantly extended from <50 days to >200 days (Supplementary Fig. 2A), indicating that loss of Sun1 is also effective at preventing heart failure caused by LmA missense mutations specifically in CMs.

Cardiomyocyte-specific ablation of LmA results in impaired heart function. By 21 days post Cre induction, LmAFF/mcm mice showed labored breathing, a disheveled, ungroomed appearance, increased lethargy, and kyphosis (Fig. 2B). PCR and immunofluorescence analysis confirmed the LmA deletion was specific to the LmAFF/mcm CMs, with no detectable recombination occurring in the brain, diaphragm, lung, liver, and skeletal muscle, or in wild-type control animals (Fig. 2C). LmA A/C protein levels were decreased 3.5 fold in the LmAFF/mcm hearts after Cre induction compared to uninduced LmAFF/mcm littermates (Fig. 2D). Immunofluorescence analysis of isolated CMs and sections through LmAFF/mcm hearts revealed reduced levels of LmA A/C protein with some CM nuclei lacking detectable LmA protein (Fig. 2E, F).

Echocardiograms performed 21 days after Cre induction revealed poor cardiac contractility in LmAFF/mcm mice compared to LmAFF/mcm controls (Fig. 3A). There was a significant reduction in the Ejection Fraction (EF%) and Fractional shortening (FS%) (P < 0.0001) (Fig. 3B). Histological analysis of LmAFF/mcm hearts revealed infiltration of nucleated cells and increased intercellular spaces between CMs compared to LmAFF/mcm control hearts (Fig. 3Ci, Cii). Significantly fewer viable (brick-like) CMs were isolated from the recombined LmAFF/mcm hearts compared to controls (Fig. 3Ciii), with many of the isolated CMs containing large vacuoles (Fig. 3Civ). The left ventricular lumen in Tmx-induced LmAFF/mcm hearts was significantly enlarged (Fig. 3Di). There was an increase in intercellular spaces between LmAFF/mcm + Tmx CMs compared to controls (Fig. 3Dii), indicative of increased fibrosis.
together with significantly increased fibrosis ($P = 0.0098$) (Fig. 3Dii). Increased numbers of apoptotic cells (by tunnel analysis) were identified in LmnaF/F:mcm hearts compared to controls (Fig. 3Diii). However, we did not detect evidence for DNA damage in the CMs, as assessed by Rad51, MRE11, H2AX phosphor-Ser, and 53Bp1 immunostaining.

In the LmnaN195K/F:mcm mice, echocardiograms after Cre induction revealed progressive worsening of cardiac contractility in the LmnaN195K/F:mcm Sun1−/− mice compared to LmnaN195K/F:mcm Sun1+/− mice (Supplementary Fig. 2B). Loss of SUN1 preserved both EF, FS, and Global Longitudinal Strain (GLS) in LmnaN195K+/−:mcmSun1−/− mice compared to LmnaN195K−/−:mcm Sun1−/− mice (Supplementary Fig. 2B).

Deletion of Sun1 ameliorates cardiac pathology induced by Lmna loss. Lmna deletion induced on a Sun1 null background results in the mice living for more than 1 year after Cre-induced deletion of Lmna in CMs (Fig. 1B). Hearts and isolated CMs from LmnaF/F:mcm/Sun1−/− mice, 3 weeks after Tmx induction, were
Fig. 3 Cardiac function and histology in Lmna<sup>F/F;mcm</sup> mice after Cre induction. A Lmna<sup>F/F;mcm</sup> mice show reduced cardiac contractile function at 21 days after Tmx injection. B Lmna<sup>F/F;mcm</sup> hearts have reduced EF% and FS%, with increased LVID. Data were analyzed from the total number of animals (N) per genotype as indicated in the graph. (**** P < 0.0001; ** P = 0.0015; one-way ANOVA, mean ± SD). C Histological analysis revealed increased nucleated cell infiltration and intercellular spaces in Lmna<sup>F/F;mcm</sup> hearts (i and ii). Significantly fewer viable (brick-like) CMs were isolated from Lmna<sup>F/F;mcm</sup> hearts compared to Lmna<sup>F/F;mcm</sup> controls (N = 3 repeats) (iii). The Lmna<sup>F/F;mcm</sup> CMs contain large intracellular vacuoles (red arrowhead, iv). Scale bar 100 μm (i), and 50 μm (ii). D In Lmna<sup>F/F;mcm</sup> hearts the left ventricular lumen was enlarged (i) with increased fibrosis (** P = 0.0007, blue staining) and apoptotic nuclei (* P = 0.0220; one-way ANOVA; mean ± SD) (ii–iv). Scale bar 500 μm (i), 50 μm (ii). All analyses were performed on hearts isolated 21 days after Tmx induction.
compared to those from LmnaF/F:mcm/Sun1+/+ to determine the extent to which SUN1 loss ameliorated the pathological changes induced by Lmna loss in CMs.

Western analysis and immunostaining of whole heart extracts revealed the predominant isoform of SUN1 was the Δ10 variant and confirmed the absence of SUN1 protein (Fig. 4A). In isolated CMs, SUN1 was absent from the periphery of the CM nuclei (Fig. 4B).

Loss of SUN1 resulted in the KASH-domain protein Nesprin-1 localizing to the poles of the CM nuclei instead of being uniformly distributed around the periphery (Fig. 4B). Furthermore, the two nuclei in each CM were both more elongated and had repositioned to be closer to each other in the Sun1−/− CMs, compared to their broader distribution in Sun1+/+ CMs (Fig. 4B). In Sun1−/− cells, a higher background staining is apparent due to increased unspecific binding of the secondary anti-mouse IgG2b antibody.

Immunofluorescence imaging for Lamin A/C on sections from the left ventricles of whole hearts isolated from mice some 3 weeks after Cre induction revealed many nuclei to be elongated and distorted. In some of these, residual LaminA was displaced to one pole of the nucleus (Fig. 4Ci and insert) in the LmnaF/F:mcm/Sun1−/+ hearts compared to WT hearts (Fig. 4Cii). In contrast, in the LmnaF/F:mcm/Sun1−/− hearts, while there were also many elongated nuclei, these showed few if any malformations/indentations, even when there was no Lamin A/C labeling (Fig. 4Ciii yellow arrowheads compared to Fig. 4Civ).

Quantification of Lamin A/C intensity on those sections revealed a significant reduction in Lamin A/C protein in the LmnaF/F:mcm/Sun1−/− CM nuclei (**P = 0.0008) compared to LmnaF/F:mcm/Sun1+/+ controls (Fig. 4C lower panels), and comparable to the reduction observed in the LmnaF/F:mcm/Sun1−/+ TMX-induced heart nuclei. The LmnaF/F:mcm/Sun1−/+ CM nuclei exhibited increased longitudinal length, together with a segmented appearance, with the segments often being connected by narrow bridges (Fig. 4D panels i -yellow arrows, compare with ii). In the absence of Sun1, LmnaF/F:mcm/Sun1−/− CM nuclei exhibited no chromatin abnormalities or segmentation defects (Fig. 4Dii and iv). In total, 70% of CMs in LmnaF/F:mcm/Sun1+/+ mice had ruptured or misshapen nuclei compared to fewer than 1% of the CMs from the LmnaF/F:mcm/Sun1−/+ animals (Fig. 4D lowest panel).

At the tissue level, enlargement of the left ventricle (LV) was evident in the LmnaF/F:mcm/Sun1−/+ mice but not in the lives of the LmnaF/F:mcm/Sun1−/− hearts (Fig. 5A). The LmnaF/F:mcm/Sun1+/+ hearts exhibited significantly increased fibrosis (**P < 0.0001) compared to controls, whereas slight fibrosis was evident in the LmnaF/F:mcm/Sun1−/+ hearts (Fig. 5A lower panels and graph).

To assess ventricular muscle mechanics, we measured the active force in cardiac papillary muscle. The active force was reduced by 66% in LmnaF/F:mcm/Sun1−/++Tmx papillary muscle (P = 0.0028) when compared with LmnaF/F:mcm/Sun1+/+ controls. In the absence of Sun1, LmnaF/F:mcm/Sun1−/++Tmx cardiac papillary active force was maintained at levels that did not differ significantly from those of controls (Fig. 5B).

Echocardiograms performed before and after Cre induction revealed a progressive worsening of cardiac contractility in the LmnaF/F:mcm/Sun1−/+ hearts compared to LmnaF/F:mcm/Sun1−/− hearts (Fig. 5C). The absence of SUN1 preserved both EF, FS and GLS (GLS is a separate parameter used to assess myocardial contractility, and is a better predictor of heart failure) in the LmnaF/F:mcm/Sun1−/− mice compared to LmnaF/F:mcm/Sun1+/+ mice.

PCR analysis of LmnaF/F:mcm/Sun1−/− hearts 12–14 months after Cre induction confirmed the sustained deletion of the Lmna gene (Supplementary Fig. 3A), and protein quantification revealed a significant reduction of Lamin A/C levels in these hearts (Supplementary Fig. 3B). Histological analysis of the hearts revealed no significant increase in fibrosis than controls (Supplementary Fig. 3C, D). However, echocardiograms showed reduced EF and FS in both the LmnaF/F:mcm/Sun1+/+ (un-induced) and LmnaF/F:mcm/Sun1+/−+Tmx mice (Supplementary Fig. 3E). The average lifespan of Lmna+/− mice is 13–14 months, implying that theloxP sites may compromise lamin expression, resulting in reduced contractile function as the mice aged.

Together these findings demonstrate that loss of Lmna, in 2–3 month adult CMs is sufficient to result in cardiac failure within 3–4 weeks after Cre activation. However, the pathology is significantly reduced by deleting Sun1, resulting in cardiomyopathy progression being retarded for at least a year.

AAV9 mediated transduction of a DNSUN1 ameliorates cardiomyopathy. These results demonstrate that genetic ablation of SUN1 functionality or reduced possibly toxic SUN1 levels could be of therapeutic value in treating DCM. Consequently, our next goal was to determine whether the beneficial effects of SUN1 loss were due to the complete elimination of SUN1’s functions, including interactions involving its nucleoplasmic domain, versus specific disruption of its LINC complex-associated role. In the latter, SUN1 functions as an anchor for KASH-domain proteins in the ONM, and mediate the tethering of the nucleus to components of the cytoskeleton, with the microtubular network, preferentially interacting with SUN1-KASH LINC complexes. To distinguish between these two possibilities, we utilized the Adenovirus Associated Virus (AAV) to transduce and specifically express in CMs, a dominant-negative SUN1 minigene whose protein product would compete with SUN1-KASH binding in the CM perinuclear space. Initially, we used a region corresponding to the entire luminal domain of the mouse Sun1 gene, which was tagged at its N terminus with an HA (HA-SUN1L) epitope. To localize the resulting protein product to both the lumen of 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 secretion of the miniprotein from the ER-PNS, a KDEL tetrapeptide was linked to the C- terminus of SS–HA–SUN1L, forming SS–HA–SUN1L–KDEL. The signal sequence would ensure the HA-SUN1L-KDEL accumulates intracellularly within the contiguous peripheral ER and PNS lumen. The cDNA sequence encoding the minigene was fused to the chicken cardiac troponin promoter (cTnT) to ensure the minigene is only transcribed in CMs. A diagram of how SS–HA–SUN1L (DNSUN1) competes with endogenous SUN1, thereby displacing KASH-domain proteins from NE-associated LINC complexes, is presented in Fig. 6B.

To verify that the DNmSUN1 functioned in CMs, we initially transduced human CMs derived from iPSC stem cells using the AVV-DJ system to provide a higher infectivity rate in cultured cells than the AAV9 serotype. DNsUN1, under transcriptional control of the cTnT promoter, effectively displaced Nesprin1 from nuclear envelopes in the CMs expressing the DNsUN1 (Fig. 6Cii and iii) confirming that the DNsUN1 could disrupt LINC complexes in CMs.

We then used AAV (serotype 9) to transduce and express the DNsUN1 minigene in the hearts of postnatal mice, initially by intrathoracic injection at a dose of 5 × 1010 vg/g per mouse. The injected mice were sacrificed for analysis at 99 days after Tmx-induced deletion of Lmna. Detection by PCR of the Lmna deletion in the hearts confirmed Cre induction following Tmx injection as expected (Fig. 6D). The localization and expression...
Fig. 4 Sun1 loss in Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> hearts inhibits changes in nuclear morphology and organization caused by Lmna loss. A Heart extracts from Lmna<sup>ff</sup>/Mcm<sup>−/+</sup> and Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> confirmed the absence of Sun1 in the Sun<sup>−/−</sup> hearts (red). GAPDH was used as a loading control (green). A representative blot is shown for the number of animals (N) per genotype is indicated below the image. Markers are in kDa (B) Immunofluorescence imaging of CMs isolated from 2-month-old Lmna<sup>ff</sup>/Mcm<sup>−/+</sup> and Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> mice. Absence of Sun1 results in the redistribution of Nesprin1 to the poles of the nuclei indicated by the yellow arrows. Note that the two nuclei in each cardiomyocyte are positioned closer to each other and are elongated. Images are representative for N = 3 per genotype shown. Hoechst staining is shown in blue, sarcomeric α-actinin is magenta, (scale bar 10 μm). C Immunostaining of CM nuclei in sections through the left heart ventricle 25 days after Cre induction (Tmx). CM nuclei with redistributed or absent Lamin A/C levels in nuclei of cardiomyocytes shows a reduction after Cre-induction (***P = 0.0008; ****P < 0.0001; unpaired two-tailed T-test, mean ± SD) (lower panel). Data are analysed of nuclei from Lmna<sup>ff</sup>/Mcm<sup>−/+</sup> Tmx (n = 400), Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> CTL (n = 384), Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> Tmx (n = 328) and Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> CTL (n = 399). Scale bar 10 μm. D Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> CM nuclear morphologies are distorted with indentations (red arrowheads) at the nuclear periphery and DAPI intense foci localizing to the tips of the nuclei (yellow arrowheads). In the absence of Sun1, Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> CMs show no nuclear indentations or chromatin redistribution. 70% of CM nuclei in Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> hearts had NE ruptures/distortions or were misshapen compared to less than 1% of CM nuclei in Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> Cre-induced mice (lower panel). Data are analysed of nuclei from 2 animals per genotype Lmna<sup>ff</sup>/Mcm<sup>−/+</sup> Tmx (n = 206), Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> CTL (n = 152), Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> Tmx (n = 131) and Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> CTL (n = 150), Lmna<sup>ff</sup>/Mcm<sup>−/−</sup> Tmx (n = 144) (No statistical analysis was performed). Source data are provided as a Source Data file.
levels of the DNmSUN1 minigene was determined by total protein extraction from half the heart. Western analysis revealed robust expression of both AAV9-DNmSUN1 and AAV9-GFP control protein 99 days after AAV injection (Fig. 6D lower panel), with the expression levels of both proteins being dependent on the dose of viral particles injected (Supplementary Fig. 4B, C).

To further verify that the DNmSUN1 was disrupting the LINC complex, displacing Nesprin1 in CMs, CMs were isolated from mice transduced with AAV9 delivering DNmSUN1 or controls injected with PBS only. The CMs were immunostained with anti-Nesprin1 and anti-SUN1. The intensity or levels of Nesprin1 at the NE were reduced, revealing displacement of Nesprin1 protein. (Fig. 6E).

Fig. 5 Cardiac pathology of LmnaWt/Wt:mcm/Sun1+/+ hearts after Cre induction. A Left ventricular enlargement (LV) was apparent in the Cre-induced (TMX) LmnaFF:mcm/Sun1+/+ (n = 3) hearts but not in the LV of the Cre-induced (TMX) LmnaFF:mcm/Sun1−/− (n = 3) hearts. B Cardiac papillary muscle active force measurements were significantly reduced from the Cre-induced (TMX) LmnaFF:mcm/Sun1+/+ (n = 5) compared to LmnaFF:mcm/Sun1+/+ controls (CTL) (n = 5) (unpaired two-tailed T-test) and Cre-induced (TMX) LmnaFF:mcm/Sun1−/− (n = 5) hearts but not in the LV of the Cre-induced (TMX) LmnaFF:mcm/Sun1−/− (n = 5).
revealed a drop in the median of Nesprin1 intensity at the nuclear rim to 41.13% in CMs isolated from AAV9-DNHSUN1 transduced mice (*P < 0.0001, unpaired two-tailed T-test, mean ± SD). n = 35 over three independent experiments. Source data are provided as a Source Data file.

In these experiments, LmnaF/F:mcm mice injected with the AAV9-GFP control lived an average of 34.5 days after Cre induction (Supplementary Fig. 4A). LmnaF/F:mcm mice injected with AAV9-DNHSUN1 (5 × 10^10 vg/g) lived significantly longer, with the majority surviving at least 99 days after Tmx induction, before being sacrificed for analysis (P = 0.0002) (Supplementary Fig. 5A). At 35 days post-Tmx injection, fibrosis was detected in both the LmnaF/F:mcm + AAV9-DNHSUN1 and LmnaF/F:mcm + AAV9-GFP hearts (Supplementary Fig. 5B), although fibrosis levels in the LmnaF/F:mcm + AAV9-DNHSUN1 hearts was significantly lower than the levels in the LmnaF/F:mcm + AAV9-GFP hearts (Supplementary Fig. 5B lower panels). ECG analysis confirmed LmnaF/F:mcm + AAV9-DNHSUN1 hearts were functioning better than LmnaF/F:mcm + AAV9-GFP hearts at 35 days post-Tmx (Supplementary Fig. 5C). Although the LmnaF/F:mcm + AAV9-DNHSUN1 mice were alive at 100 days after induction, both EF% and FS% were lower compared to control LmnaF/F:mcm mice (Supplementary Fig. 5C).

The expression of either the AAV9-DNHSUN1 or AAV9-GFP proteins did not affect Lamin A/C protein levels (Supplementary Fig. 4A, Supplementary Fig. 4B). The AAV9 human/murine LmnaF/F:mcm miniprotein competes with endogenous SUN1 for binding to the KASH-domain of the Nesprins (which in CMs is Nesprin1). It displaces Nesprin1 from the nuclear envelope, disrupting LINC complex-mediated attachment of the nucleus to the cytoskeleton. Nesprin1 is critical for cardiac function and remodeling. Although Nesprin1 levels were suppressed by AAV9-DNHSUN1, EF% and FS% were not significantly changed compared to control LmnaF/F:mcm mice (Supplementary Fig. 4A, Supplementary Fig. 4B). The expression of Nesprin1 was confirmed by western blot analysis of whole heart lysates at 35 days after induction (Supplementary Fig. 5A). Western blot was repeated twice with N = 4 per AAV9 construct. Markers in kDa. LmnaF/F:mcm hearts were functioning better than LmnaF/F:mcm and LmnaF/F:mcm hearts at 100 days after induction with 5 × 10^10 vg/g of AAV9-GFP than expression levels from a 10-fold lower dose of (5 × 10^9 AAV9-GFP) (Supplementary Fig. 4C). autumn.
A subsequent, more extensive series of AAV transductions were performed using a human DNhSUN1 construct. Instead of using the intrathoracic route for introducing the AAV suspension, we used retro-orbital injection37. LmnaF/F:mcm mice injected with the AAV9-GFP control lived an average of 34.5 days after Cre induction (Fig. 7A). In contrast, LmnaF/F:mcm mice injected with AAV9-DNhSUN1 (2 × 10¹⁰ VG/g) lived significantly longer, with the majority surviving at least 66 days after Tmx induction before they sacrifice for analysis (P = 0.0002) (Fig. 7A). In Fig. 7B, the results of both low (2 × 10¹⁰ vg/g) and high (4 × 10¹⁰ VG/g) doses of DNhSUN1 are presented and show that doubling the concentration of the injected DNhSUN1 extends longevity even further to at least 300 days after Tmx injection. The results further suggested that females may survive longer than males; a divergence also noted in mice where Lmna deletion in the liver38. Injection of the DNhSUN1 into wild-type mice revealed the DNhSUN1 had no overt detrimental effects on the longevity of normal mice with the mice living for over 1 year with DNhSUN1 protein expression still being detectable in heart extracts after 1 year (Fig. 7D). Histological examination of the hearts revealed
Fig. 7 Lmna<sup>+/Ficm</sup> mice transduced with AAV9-DNhSUN1 show improved cardiac function and extended longevity. **A** Transduction of AAV9-DNhSUN1 extends the lifespan of the Lmna<sup>+/Ficm</sup> mice after tamoxifen induction. Lmna<sup>+/Ficm</sup> + AAV9-DNhSUN1 transduced mice with the standard dose (2 × 10<sup>10</sup> vg g<sup>−1</sup>) live for a median of 66 days post-Tmx induction, whereas the Lmna<sup>+/Ficm</sup> + AAV9-GFP transduced mice have a shorter lifespan (36.5 days) (P < 0.0001; Log-rank (Mantel-Cox) test). All animals were induced with Tmx. **B** Transduction of a double dose (4 × 10<sup>10</sup> VG/g) of AAV9-DNhSUN1 increases the lifespan of Lmna<sup>+/Ficm</sup> animals. Male Lmna<sup>+/Ficm</sup> injected with a double dose of DHNSUN1 live for an average of 205 days and females to 309 days post-Tmx induction, whereas the Lmna<sup>+/Ficm</sup> injected with the standard dose DHNSUN1 have a shorter median lifespan (males 66 days, females 104 days). **C** Experimental procedure for AAV transduction. Tmx induction by ip injection at postnatal day 14, followed on day 15 in initial experiments by intrathoracic injection of AAV9-DNmSUN1 or AAV9-GFP as control (AAV). In subsequent experiments with the DNhSUN1 delivery was by retro-orbital injection. The expected lifespan of Lmna<sup>+/Ficm</sup> mice injected with AAV9-GFP was 33 days after Tmx induction. The endpoint of this study is the date of death of Lmna<sup>+/Ficm</sup> animals injected with AAV9-DNhSUN1 (DOD). **D** The DNhSUN1 protein is detected by western blot analysis at 3 weeks after injection and after 1 year following injection at similar levels in whole heart lysates with an antibody specific to the C-terminus of human SUN1. A representative blot is shown for the number of animals (N = 2) per condition. Western blots were performed in triplicate. Markers in kDa (E–F). At 21 days after Tmx induction, extensive fibrosis (blue staining) was detected in Lmna<sup>+/Ficm</sup> mice injected with AAV9-GFP (N = 5) in comparison to Lmna<sup>+/Ficm</sup> treated with AAV9-DNhSUN1 (N = 5). The following genotypes served as controls: Lmna<sup>+/Ficm</sup> + AAV9-DNhSUN1 (N = 5), Lmna<sup>+/Ficm</sup> + AAV9-GFP (N = 4). Data are presented as mean ± SD. (scale bars 500 μm, 40 μm). **E** ECG analysis of mice injected with a standard AAV-DHNsUN1 dose shows improved cardiac function at day 28 after Tmx induction. **F** Cardiac function after transduction with AAV9-DNhSUN1 is improved in Lmna<sup>+/Ficm</sup> animals. ECG analysis revealed an significant improvement of Fractional Shortening (FS), Global Longitudinal Strain (GLS) and Ejection Fraction in Lmna<sup>+/Ficm</sup> + AAV9-DNhSUN1 animals compared to Lmna<sup>+/Ficm</sup> + AAV9-GFP control animals at day 28 (F5 *** p = 0.0009; GLS *** p = 0.0003; EF *** p < 0.0001; One-way ANOVA with Tukey correction). Data were analyzed from the total number of animals (N) per genotype as indicated in the graph. Data are presented as mean ± SD. Source data are provided as a Source Data file.

significantly reduced fibrosis in the DNHsUN1 expressing hearts (Fig. 7E, F) with conserved ventricular dilatation in the DNHsUN1 transduced hearts (Supplementary Fig. 6A, D). ECG analysis confirmed Lmna<sup>+/Ficm</sup> + AAV9-DNhSUN1 hearts were functioning better than Lmna<sup>+/Ficm</sup> + AAV9-GFP hearts at 28 days post-Tmx (Fig. 7G, H), with heart function, also improved by doubling the dose of AAA (Supplementary Fig. 6C, E). Although the Lmna<sup>+/Ficm</sup> + AAV9-DNhSUN1 mice were alive at 100 days after induction both EF% and FS% were still lower compared to control Lmna<sup>+/Ficm</sup> (Fig. 7H). Importantly, expression of the DNHsUN1 in Wt mice had no detrimental effect on cardiac function at 160 days after Tmx.

Discussion

Here we show that disrupting the LINC complex protein, SUN1, suppresses DCM progression caused by LMNA mutations. LMNA associated DCM is regarded as a particularly aggressive form of heart failure, frequently leading to premature death or cardiac transplantation. By 60 years, 55% of LMNA mutation patients die of cardiovascular failure or receive a heart transplant, compared with 11% with idiopathic cardiomyopathy. Attempts to ameliorate DCM by fitting a pacemaker have been, at best, of transient benefit. Consequently, it is necessary to develop new therapeutic avenues to treat DCM caused by LMNA mutations.

The majority of LMNA mutations causing DCM are dominant missense, primarily due to a single base change. Treatment by gene therapy to repair each mutation would be a daunting task. Moreover, simply eliminating the mutated allele, leaving the patient haplo-insufficient for the remaining WT allele would almost certainly be ineffective at preventing heart failure as a patient, who was effectively heterozygous for LMNA, developed DCM. Various other routes downstream of the LMNA gene have been explored as potential therapeutic pathways. These have included inhibiting mTOR with rapamycin/rapalogues, MEK1/2 kinase pathway inhibitors, upregulation of YY1, and most recently inhibiting the transcription factor bromodomain-containing protein 4 (BRD4). Many of these procedures necessitated repeated injections (often daily) of the compounds, some of which were associated with significant side effects.

In contrast, the AAV delivery system requires a single injection. Our data revealed that following a single injection of the vector expression of the DNSUN1 constructs is still detectable 1 year after injection. In all studies, as here, the primary endpoint was lifespan extension, with fibrosis reduction and cardiac function being the secondary endpoints. All approaches resulted in increased longevity, improved ventricular function, and reduced fibrosis (10–40%), but the lifespan extension and long-term efficacy were less than that observed by depletion and genetic disruption of SUN1. However, longevity is extended to close to 1 year after administering and persistent expression of the DNHSUN1 protein for at least 1 year.

The molecular mechanisms underlying the varied phenotypes of the laminopathies are still not 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, altering gene expression. Evidence supporting this hypothesis comes from studies reporting changes in signaling pathways including the AKT-mTOR, WNT/β-catenin, TGF-β/Smad5, MAP Kinase and the ERK1/2–CTGF/CCN2 pathways. While all these changes have been documented, it is not established whether these changes are merely a secondary compensatory effect in diseased tissue.

The second hypothesis centers on LMNA loss or mutation that 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. This hypothesis is similar to that proposed for Duchenne muscular dystrophy (DMD), where the loss of dystrophin increases the fragility of the muscle cell membrane, making them susceptible to tension-stress forces during muscle contraction and results in muscle cell rupture and death. LMNA mutant fibroblasts show nuclear deformation, defective mechanotransduction, reduced viability when subjected to mechanical strain, and increased nuclear rupture at low and moderate pressures compared to WT nuclei. Within the context of contracting murine CMs, mechanical stress and tension forces caused by 500–600 contractions per minute are exerted on the NE via the LINC complex, resulting in nuclear distortion, damage, and eventual death/loss as presented in Figs. 3, 4. Such forces may cause significant damage to the fragile NE of Lmna-null CMs, resulting in CM death. Whether such forces also induce DNA damage in the CMs, as reported for myoblasts derived from Lmna mutant mice, is still unclear as we could not detect any convincing evidence for DNA damage in the LaminA depleted CMs. If this tension-stress...
hypothesis is correct and that the cytoskeleton itself promotes damage to the NE, then unlinking the LINC complex by interfering with SUN1 function should reduce the stress on the CM nuclei. Such uncoupling would predict the prevention of CM cell death in the mutant CMs. To test this tension-stress hypothesis, a DNhSUN1 construct was used to compete with endogenous SUN1 for KASH-domain-binding to decouple CM LINC complexes (Fig. 6B, E). The AAV9 vector, which has a high affinity for CMs, was used to deliver the DNSUN1 hybrid gene under the control of the CtnT promoter to CMs. Our results showed the successful delivery and expression of the GFP tagged miniprotein to CMs (Supplementary Fig. 4C), with robust expression of both the control GFP and DNSU1 proteins (Figs. 6, 7D) with expression persisting 1 year after injection, indicating significant perdurance of the DNSU1 protein (Fig. 7D). Minimal off-target expression, i.e., liver, was detected only after injection of very high vector concentrations (Supplementary Fig. 4D). Significantly, expression of DNSU1 was associated with reduced levels of expression of both the GFP tagged miniprotein to CMs with an otherwise compromised lamina (Figs. 6C, E, D). Intriguingly, the loss of SUN2, the other widely expressed SUN domain protein, did not rescue the mice carrying mutations in Lmna or SUN1. These results indicate that loss or mutation of Lmna results in instability of the CM lamina and nuclei due to incorrect assembly of the lamins. Incorrect lamin assembly, therefore, makes the nuclei susceptible to the tension/stress forces exerted via the LINC complex from the contractile sarcomeres of the CMs or other force-generating/transmitting cytoskeletal components (Fig. 8A, B). In the absence of functional SUN1, due to Sun1 gene ablation or DNSU1 expression disrupting the SUN1 trimer complex, tethering of the KASH proteins to the ONM is decreased. The KASH domain or at least Nesprin-1 association with the NE is then diminished, resulting in reduced tensional force transmission to the CM nuclei, thereby enabling the survival of CMs with an otherwise compromised lamina (Figs. 8C, 7D). Intriguingly, the loss of SUN2, the other widely expressed SUN domain protein, did not rescue the mice carrying Lmna mutations. In mice, SUN2 loss induces mild cardiac hypertrophy, which reduces the necessity for repeat AAV injections, resulting in immune responses to the AAV, potentially compromising the efficacy of the vector. As a delivery route for patients, the AAV system is approved and stable to treat an increasing number of diseases. It is becoming more widely used with multiple ongoing clinical trials, including its 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 so effective in preventing Lmna mutation-induced cell death in murine skeletal muscle, as LmnaΔ/Δ,Sun1 Δ/Δ mice 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 earlier murine lethality remain to be identified.

Methods

Mouse lines. Mouse (C57Bl/6J and 129SvJ) strains were maintained at the A*STAR Biological Resource Center facility and the NUS Animal Facility on a 12 h light/dark cycle in ventilated animal barrier facilities with the temperature set to

Fig. 8 Breaking the LINC by disrupting Sun1 protects cardiomyocytes from contraction-induced stress. A CM nuclei expressing normal Lmna, withstand mechanical stress and tension forces transmitted via the LINC complex from the sarcoplasmic cytoskeleton, predominantly the MT system to the NE. B The loss of or introduction of a mutation within the Lmna gene results in loss/or incorrect assembly of the nuclear lamina, resulting in a compromised and weakened Lamina/NE. The weakened nuclei are damaged due to the tension/stress forces exerted via the MT network and LINC complex from the contractile sarcomeres of the cardiomyocytes. C, D In the absence of SUN1 or by disrupting its binding to the KASH domains by expression of DNSU1, the now untreated LINC complexes exert less tensional force on the CM nuclei, enabling the survival of the Lmna mutant CMs.
21 ± 1 °C, humidity at 55–70% and with food and water provided ad libitum. Ethical oversight and approval were granted by the Institutional Animal Care and Use Committee at the ASTAR Biologicals (ABT, RGC) and the NUS AICUC and the animal facility/committee (Comparative Medicine protocol R16-213) and is governed by the association of AAALAC (USA) providing guidelines to both AALAS (USA) and AVS (Singapore) to which NUS adheres.

The Lmna−/− mice were generated and characterized as previously described4,6,8. To derive a cell line with a global deletion Lmna (Lmna−/−), we crossed the floxed allele (Lmnafl/fl) to mice in which Cre recombinase is driven by the regulatory sequences of the mouse zellweger 3 gene (Zp3;Tg(Zp3-cre)93Krw, JAX stock 003651)8. Cardiomyocyte (CM)-specific deletion of Lmna (Lmnafl/fms) was performed using 2 lines where Cre recombinase is expressed specifically in CMs. The first Lmnafl/fms line where Cre expression is driven by the CM-specific murine alpha myostin-alpha heavy chain promoter (Myh6, myosin, heavy polypeptide 6, cardiac muscle, alpha) promoter (MyHcGc(Myhca-cre)2182Mds, JAX stock 011038). In addition, we derived a tamoxifen-inducible CM-specific deletion of Lmna (Lmnafl/fmcn) by crossing the Lmnafl/fms line where Cre expression was driven by the mouse CM-specific alpha-myosin heavy chain promoter (aMHC or alpha-MHC;Myh6 abbreviated to mcm) that expresses a tamoxifen-inducible Cre recombinase (MerCreMer) specifically in juvenile (14d) and adult (3–36 month) cardiac myocytes (mcm;Tg(Myh6-cre/Esr1)1Hmk, JAX stock 005675). The specificity of mcm Cre expression to CMs was confirmed by crossing Cre lines to the mEmerald reporter mice8. Generation of the SUN1−/− mice was previously described6, as was the LmnaN195K/N195K line that carries a Lmna N195K missense mutation that also results in death associated with heart failure. The Lmnafl/Suni−/+ and Lmnafl/Suni−/− mice were obtained by crossing the respective Lamin-Cre mice strains with Sun1+/− mice as Sun1−/− mice are infertile.

To test for the insertion of loxp sites and the conditional deletion allele, genotyping was performed with a duplex PCR protocol. The primer sequences are in Supplementary Table 1.

Tamoxifen injection and tissue collection. Mice (14 days old) and adults (3–5 months old) were injected once with 40 mg/kg of Tamoxifen (Sigma) dissolved in corn oil (Sigma). Mice were sacrificed by CO2 euthanasia or anesthetized with a mixture of 1% Isoflurane (Biovac) and 1.5 L O2 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 flushed with 4% paraformaldehyde (PFA), left in 4% paraformaldehyde (PFA) overnight, dehydrated in 70% ethanol for at least 24 h and embedded in paraffin. For immunofluorescence and enzymatic digestion were performed on rehydrated sections with trypsin and collagenase gummy gum (Aldrich) in isopentane in liquid N2, cut at 9 μm sections using a 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. Cardiomyocyte isolation was performed as per standard protocol8. Briefly, mice were anesthetized with isoflurane (100% O2 at 0.5 L/min, isoflurane 1.5%). Hearts were perfused with 15% KCl, the first 1 mm-sized aorta was cut, and hearts flushed with 7 mL of EDTA buffer through the right ventricle. The ascending aorta was clamped using Reynolds forceps, and the entire heart removed and placed in a 60 mm dish containing fresh EDTA buffer. Hearts were digested by sequential injection of 10 mL EDTA buffer, 3 mL perfusion buffer, and 30–50 mL collagenase buffer into the left ventricle. Forceps were used to gently pull the digested heart into smaller pieces ~1 mm and gentle trituration. Enzymatic digestion was performed on rehydrated sections with trypsin and collagenase gummy gum (Aldrich) in isopentane in liquid N2, cut at 9 μm sections using a 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.

Histological and immunofluorescence microscopy. For histological studies, sections (9 μm) were stained with standard Haematoxylin and Eosin for cell morphology, Masson’s trichrome stain to detect collagen, and TUNEL assay (Abbcam) to detect apoptotic nuclei. Images were obtained with a Zeiss Axio Imager [morphology, Masson

Western analysis for LMNA, SUN1, Ha-tag, and GFP. Whole hearts were homogenized in RIPA lysis buffer and the extract spun at 13200 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 or overnight at 4 °C, washed with TBST washing solution, and incubated in Odyssey IRDye secondary antibodies (1:5000) for 1 h before visualization with the Odyssey Infrared Imaging System (Li-Cor Biosciences). The primary antibodies used: for detection of LaminA/C (Rabbit, 1:500, Cell Signaling) that is specific to an epitope in the first 50 amino acids in LMNA, mSuni (mouse mAB, clone 12.11, neat, from B. Burke), GFP (rabbit mAB, clone 1500, Abcam), anti-HA epitope (rabbit mAB, 3F10, 1:1000, Roche), GAPDH (rabbit, 1:500, Abcam), and anti-β-tubulin (mouse, Tub 2.1, 1:1000, Sigma). For detection of the AAV9-DNSuni transgene, a mouse mAB specific to the C-terminus of human Sun1 (Suni1, clone 9.1, neat, from B. Burke) was used in combination with protein A conjugated to HRP (1:1500, Cell Signaling). GAPDH (rabbit, 1:1500, Abcam) and anti-β-tubulin (mouse, Tub 2.1, 1:1000, Sigma). The level of signal displacement for Western blotting was performed within 24 h with the confocal microscope Olympus FV3000. Quantification was performed after normalization of the signal intensity with LabVIEW 2013 (National instrument). At least 5 mice were tested for each experimental group.

Echocardiography. Cardiac function was measured by echocardiography using the Vehlo2100 and Vehlo3100 (VisualSonics). Mice were shaved 1 day before ultrasound examination. The animals were anesthetized with 1.5% isoflurane mixed with oxygen. Readings of B-mode and M-mode were taken at heart rates between 450 bpm and 350 bpm. FS, EF, and GLS were calculated from the parasternal long axis using the Vehlostrain feature of the VehloLab software (VisualSonics). Cardiac measurements of the left ventricular interior diameter, interventricular septum, and left ventricle posterior wall were taken from the parasternal short-axis for the diastolic and systolic state.

Active force measurement of the cardiac papillary muscle. Mouse papillary muscle from the left ventricle was prepared according to the methods described before57. Briefly, the explanted mouse heart was immediately rinsed with oxygenated ice-cold Krebs–Henseleit solution with 12 U/mL heparin sodium (EDQM) and 30 mM 2,3-Butanedione monoamine BDM (Sigma) and excess blood removed. Hearts were then transferred to ice-cold Krebs–Henseleit solution in a glass petri-dish under a dissection microscope with a cooling stage. Cylindrical papillary muscle (200–300 μm in diameter and 1.5–2 mm in length) were excised from the left ventricle. T-shaped aluminum clips with a hole were clamped onto the ends of the papillary preparation and the papillary preparation clamps 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. Force measurements were performed as previously described68. The T-shaped aluminum clips at the ends of the papillary preparations were attached to the hooks of a force transducer (AEBO1, HJK Sensors+Systems) and servo-motor in the experimental rig was glued with shellac in ethanol (Sigma) to minimize the movement during the measurements. The papillary contractile force was measured at 20 °C. The maximum contractile force was measured inactivating solution (100 mM TES, 6.5 Mm MgCl2, 25 mM Ca-EGTA, 5.7 mM Na2ATP, 20 mM glutathione, pH = 7.1, Ionic strength 150 mmol/L) with 32 μmol/L free Ca2+. Data were collected and processed from the force transducer and DAQ data acquisition device (National Instrument) using a customized software program developed by LabVIEW 2013 (National instrument). At least 5 fibers were tested from each mouse, and at least 3 mice were tested for each experimental group.

Derivation of human-induced pluripotent stem cells (iPSCs). Human iPSCs were generated from a healthy male patient19 using the episomal reprogramming method27. Informed consent was obtained for this procedure with UCSF Committee on Human Research which approved the study protocol. The human iPSC cell lines used in this study were generated from a healthy male patient, WTC10 and WTC11. Pluripotent stem cells were maintained on Matrigel (BD

Morris), Myosin Heavy Chain (mouse mAB, MF20, 1:50, Developmental Studies Hybridoma Bank), PC1- (rabbit, 1:200, Sigma) and sarcomere–α-actinin (mouse Evans, EAB, EAC3, 1:200, rabbit mAB, 1:500, Invitrogen) (DAPI, 1:500, Invitrogen). For isolated cardiomyocyte immunofluorescence, myocytes were stained on coverslips. Permeabilization was performed with 0.1% TritonX in PBS for 20 min, followed by gentle washing with PBS, blocking with 3% BSA in 0.1% TritonX in PBS for 1 h, incubated overnight at 8 °C with primary antibody prepared in blocking solution, followed by gentle washing with PBS, then incubated with the secondary antibody for 1 h at room temperature, followed by gentle washing with PBS, and mounted with Prolong Gold Antifade (Invitrogen). Imaging was performed within 24 h with the confocal microscope Olympus FV3000.
Biosciences) coated polystyrene culture plates in StemFlex medium (Thermo Fisher Scientific). Cells were supplemented with Y-27632 (10 μM) (StemCell Technolo-
gies), a Rho-associated kinase (ROCK) inhibitor after passaging to promote cell
survival.

Directed cardiomyocyte differentiation from human pluripotent stem cells.
Differentiation of human iPSCs to cardiomyocytes (iPSC-CMs) was performed by
modulating WNT/β-catenin signaling as described (the GiWi protocol)\(^1\). Human
pluripotent stem cells were seeded at 5 × 10⁴ cells/cm² onto 12-well plates coated
with Matrigel (BD Biosciences) in StemFlex medium for 3 days. On the day of
differentiation, the medium was switched to RPMI medium supplemented with
B27 without insulin (RPMI/B27-) (Life Technologies) and CHIR99021 (12 μM)
(Tocris) for exactly 24 hr before replacing with fresh RPMI/B27- medium. After
48 h, cells were treated with IWP2 (5 μM) (Tocris) in RPMI/B27- for 2 days before
replacing with fresh RPMI/B27- medium. After 2 days, the medium was then
switched to RPMI/B27+ medium for 4–8 days before using a metabolic selection
protocol to purify iPSC-CMs\(^2\). Cells were repl-
ated and maintained on RPMI/B27+ medium for 4 days and then replaced with
lactate medium (glucose-free DMEM containing sodium pyruvate and buffered
lactate (4 mM) supplemented with Glutamax and nonessential amino acids). Cells
were treated with lactate medium twice, with each treatment lasting for 2 days.
The purified iPSC-CMs were then maintained in RPMI/B27+ medium for 1 week
before harvesting for further analyses.

AAV9-DNNSUN1 and AAV9-GFP virus. The DNNSUN1 (SS-HA-Sun1-βcat)- and GFP
(SS-GFP-KDEL) vectors are described as described\(^2\). Briefly, almost the entire luminal
domain of Sun1 was tagged at its NH₂-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 peptide cleavage sites of human serum albumin was
fused to the NH₂-terminus of HA-Sun1L to yield SS-HA-Sun1L-ΔKDEL. 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 a
GGP sequence to generate the SS-GFP-KDEL. The DN-Sun1 and GFP fragments
were amplified with the primers listed below (saving 1.5% Isolurase was used with
oxygen). The AAV9 working solution was prepared freshly before administration. Depending on
the concentration of viral genomes, the respective AAV9 stock solutions were diluted
with PBS containing 0.001% Pluronic F-68.

Statistical analysis. All statistical analyses were performed using Excel 2016 and
Graphpad Prism 9.1.0. Results are shown as mean with ±SD. Data were analyzed
using One-way ANOVA or unpaired T-test as indicated. For lifespan analysis,
significance was tested with Log-rank test. To calculate the significance of cardiac
data Tukey’s post hoc test was used for multiple groups.

Reporting summary. Further information on research design is available in the Nature
Research Reporting Summary linked to this article.

Data availability.
The data supporting the conclusions of this paper are provided in the article and the
Supplementary Information. Any remaining raw data will be available from the
corresponding author upon reasonable request. Source Data are provided with this paper.

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Production of AAV for SUN1 dominant-negative treatments. AAV Virus was produced
as per standard protocol\(^3\). 9C3T cells were expanded in 15 cm dishes or
T-flasks before being seeded into a 10-chamber CellSTACK (Corning Inc., Corn-
ing, NY, USA). AAPlV-2 (gift of J. M. Wilson, UPenn Vector Core, Philadelphia,
PA), pFlpE (Part No. 340202, Cellbiolabs, Inc., San Diego, CA, USA), and
plasmid-containing the same inserts were transfected with PEI Max (Polysciences,
Warrington, PA, USA). 4 days after transfection, the cell pellet and supernatant were harvested.

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