SUPPLEMENTAL APPENDIX

Supplemental Materials & Methods

Echocardiography

Echocardiography was measured on mice that were anesthetized initially by 3% isoflurane and maintained asleep at 1%~1.5% isoflurane. Echocardiography was performed with a VINNO6n machine (VINNO Corporation, Suzhou, China). The standard short-axis cardiac videos were acquired using a 23MHz transducer. FS, LVPW and LVID values were measured by averaging results from five consecutive heart beats. The researcher who performed echocardiography was blinded to mouse genotypes.

Tissue sectioning

Paraffin sectioning was performed at Servicebio, China. The harvest tissues were first fixed in 4% paraformaldehyde (PFA) at 4°C overnight and then in 20% sucrose solution overnight. Next, the samples were dehydrated through a serial gradient of ethanol and N-butanol. The samples were waxed in liquid paraffin for 2 h and embedded to make paraffin blocks using a tissue embedder (EG1150, Leica, Germany). Four-micron sections were cut by paraffin slicer (RM2245, Leica, Germany) and adhered to the microscope slides after floating on a water bath (HI1210, Leica, Germany).

For frozen sectioning, the harvest tissues were fixed with 4% PFA and were dehydrated in sucrose solutions (15% followed by 30%) overnight. Then the tissue

samples were embedded in OCT (Sakura) and frozen in -80°C. Tissue sections (7 μm) were cut on a cryostat microtome (CM1950, Leica, Germany).

Histological staining

Histological staining was performed at Servicebio using paraffin sections or cryosections. In brief, for H&E staining, the paraffin sections were dewaxed in xylene, hydrated by passing decreasing concentrations of alcohol baths and then stained in 0.5% hematoxylin (G1004, Servicebio) for 3-5 min. Then the sections were washed in running water and differentiated by dipping in 1% acid ethanol for a few seconds. Next the sections were rinsed in water, and stained in 0.05% eosin Y (G1003, Servicebio) for 10min. Then the sections were dehydrated in increasing concentrations of ethanol, cleared in xylene and mounted for imaging.

For picrosirius red staining, the paraffin sections were dewaxed and dehydrated and then incubated with 0.2% picrosirius red solution dissolved in saturated aqueous picric acid (1.2% picric acid in water) (G1018, Servicebio). Next the sections were rinsed in water, dehydrated in increasing concentrations of ethanol, cleared in xylene and mounted for imaging.

For Oil Red O staining, frozen sections were used. The working solution was made of Oil Red O solution (G1015, Servicebio) diluted with distilled water at the ratio of 6:4. The sections were incubated in Oil Red O solution for 8-10 min in the dark. The

sections were later differentiated by dipping in 60% isopropanol for a few seconds, rinsed in water, and stained in 0.5% hematoxylin (G1004, Servicebio) for 3-5 min. Then the sections were washed in water and mounted for imaging.

All slices were scanned by an all-in-one fluorescence microscope (BZ-X800, Keyence, Japan). ImageJ was used for quantitative measurement. The cross-section areas for biceps femoris, diaphragm and white fat tissue were manually measured.

Western blotting

The tissues were washed once on ice phosphate buffered saline (PBS). Then the tissues were lysed in RIPA Buffer (25mM Tris PH7.0~8.0, 150mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100) containing protease inhibitors (Solarbio, China). Tissue lysates were centrifuged for 15 min at 1,2000 rpm at 4°C. The supernatants were collected and the protein concentration was quantified using BCA Protein assay kit (TransGen Biotech, Beijing, China).

The lysates were diluted into identical protein concentrations, added into 4×SDS sample buffer and heated at 70℃ for 10 min. Then 30 μg proteins of each sample were subjected to SDS-PAGE in a 4%-12% gradient gel (TransGen Biotech, China) using a constant voltage at 120 V for 1 h 30 min. The proteins were transferred to a PVDF membrane and blocked for 1 h in 5% milk/TBST at room temperature. The primary antibodies were incubated with the membranes overnight at 4°C. After the membranes

were washed with TBST, the HRP-conjugated secondary antibodies were used in staining for 1 h at RT. After washing, the ECL Western blotting substrate (PE0010, Solarbio, Beijing, China) was used to detect chemiluminescence signals with a Bio-Rad imager (Bio-Rad, USA). See Table S4 for antibody information.

RNA extraction and quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from heart apex using the TransZol Up Plus RNA Kit (ER501-01, TransGen, Beijing, China). TransScript II One-Step gDNA Removal and cDNA Synthesis SuperMix (AH311-03, TransGen, Beijing, China) kits were used for genomic DNA removal and reverse transcription. Real-time PCR analysis was performed using Perfect Start Green qPCR Super Mix (+DyeII) (AQ602-24, TransGen, Beijing, China) and the AriaMx Real-Time PCR System (Agilent Technologies). See Table S3 for primer information.

Immunofluorescence

The samples were incubated with primary antibodies for 1h at room temperature. Then the samples were washed with PBS for 3 times, 5 min each. Fluorescent secondary antibodies and dyes were incubated with the samples for 1 h at room temperature. Then the slides were mounted with ProLong™ Diamond antifade mountant (P36961, Invitrogen, Thermo Fisher Scientific, USA). See Table S4 for antibody and dye information.

Epifluorescence images were acquired using an all-in-one fluorescence microscope (BZ-X800, Keyence, Japan). The confocal images were observed by an SP8 (Leica, Germany) confocal microscope equipped with an APO 63X/1.4 oil objective. ImageJ was used for quantitative measurement. Cell morphology, Z-line distance and nuclear morphology parameters were manually measured.

Fluorescence-activated cell sorting (FACS)

The cardiomyocytes were isolated from the $Rosa^{Cas9\text{-}Tom}$ mice that were injected with (CASAAV) or without (control) AAV-Lmna-sgRNA. These cells were re-suspended in perfusion buffer. Using a BD FACSDiscover S8 cell sorter (BD Biosciences, USA), the tdTomato-positive and -negative cardiomyocytes were sorted.

Magnetic resonance imaging (MRI)

A 3.0 T MRI system (Siemens Magnetom Trio, USA) was utilized for MRI imaging. Mice were anesthetized and placed into a mouse-specific coils. Images from the sagittal and coronal planes were obtained with a T2-weighted Turbo Spin Echo (TSE) sequence. Bee DICOM viewer (www.beedicom.com) was used to analyze the MRI images.

Supplemental Table 1. RNA-Seq differential expression analysis of 6-week $Lmna^{\Delta/\Delta}$ versus $Lmna^{+/+}$ ventricles (file separately attached)

Supplemental Table 2. RNA-Seq differential expression analysis of 6-week versus 2-week Lmna$^{+/+}$ ventricles (file separately attached)

Supplemental Table 3. DNA primers in this study

Primers for genotyping		
Genotype	Primer 1	Primer 2
+ (242bp)	CCAGCTTACAGAGCACCGA	TGAGCGCACCAGCTTGCGCA
Δ (237bp)	CCAGCTTACAGAGCACCGA	TGAGCGCACCAGCTTTGGC

Primers for RT-qPCR		
Gene	Primer 1	Primer 2
Lmna	TCCCACCGAAGTTCACCCTA	TGGAGTTGATGAGAGCGGTG
Nppa	TTCCTCGTCTTGGCCTTTTG	CCTCATCTTCTACCGGCATC
Ctgf	CAGGCTGGAGAAGCAGAGTCGT	CTGGTGCAGCCAGAAAGCTCAA
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Gfp RT-(AAV quantification)	AAGCTGACCCTGAAGTTCATCTGC	CTTGTAGTTGCCGTCGTCCTTGAA

Primers for amplicon sequencing*

Barcoded forward primers	AATGATACGGCGACCACCGAGATCTACAC**tatagcct**ACACTCTTTC CCTACACGACGCTCTTCCGATCT**TGTATAAAGGCCCGGGACAG**
	AATGATACGGCGACCACCGAGATCTACAC**atagaggc**ACACTCTTTC CCTACACGACGCTCTTCCGATCT**TGTATAAAGGCCCGGGACAG**
	AATGATACGGCGACCACCGAGATCTACAC**cctatcct**ACACTCTTTC CCTACACGACGCTCTTCCGATCT**TGTATAAAGGCCCGGGACAG**
	AATGATACGGCGACCACCGAGATCTACAC**ggctctga**ACACTCTTTC CCTACACGACGCTCTTCCGATCT**TGTATAAAGGCCCGGGACAG**
	AATGATACGGCGACCACCGAGATCTACAC**caggacgt**ACACTCTTTC CCTACACGACGCTCTTCCGATCT**TGTATAAAGGCCCGGGACAG**
Barcoded reverse primers	CAAGCAGAAGACGGCATACGAGAT**ttcgcgga**GTGACTGGAGTTCA GACGTGTGCTCTTCCGATCT**AGGAGAGAGAAGAAAGGCCA**

*Barcodes and genome-matching sequences in bold.

Supplemental Table 4. Antibodies and dyes in this study

Antibodies				
Name*	Vendor	Cat. #	Working concentration	Application
Ms-anti-Lamin A/C	Cell Signaling Technology	4777S	1:200	IF
			1:2000	WB
Ms-anti-α-actinin-2	Abcam	ab9465	1:200	IF
Ms-anti-Cav3	Abcam	ab173575	1:200	IF
Ms-anti-GFP	TransGen	HT801	1:2000	WB
Ms-anti-GAPDH	TransGen	HC301	1:5000	WB
HRP-Gt-anti-Ms	TransGen	HS201	1:10000	WB
488-Dk-anti-Ms	TransGen	A21202	1:500	IF
555-Dk-anti-Ms	TransGen	A31570	1:500	IF

*Ms: mouse, Gt: goat, Dk: donkey

Dye				
Name	Vendor	Cat.#	Working concentration	Application
DAPI(1mg/ml)	Thermo Scientific	62248	1:1000	IF
WGA-555(2mg/ml)	AAT Bioquest	25539	1:200	IF

WGA-488(2mg/ml)	AAT Bioquest	25530	1:200	IF

Supplemental Figures

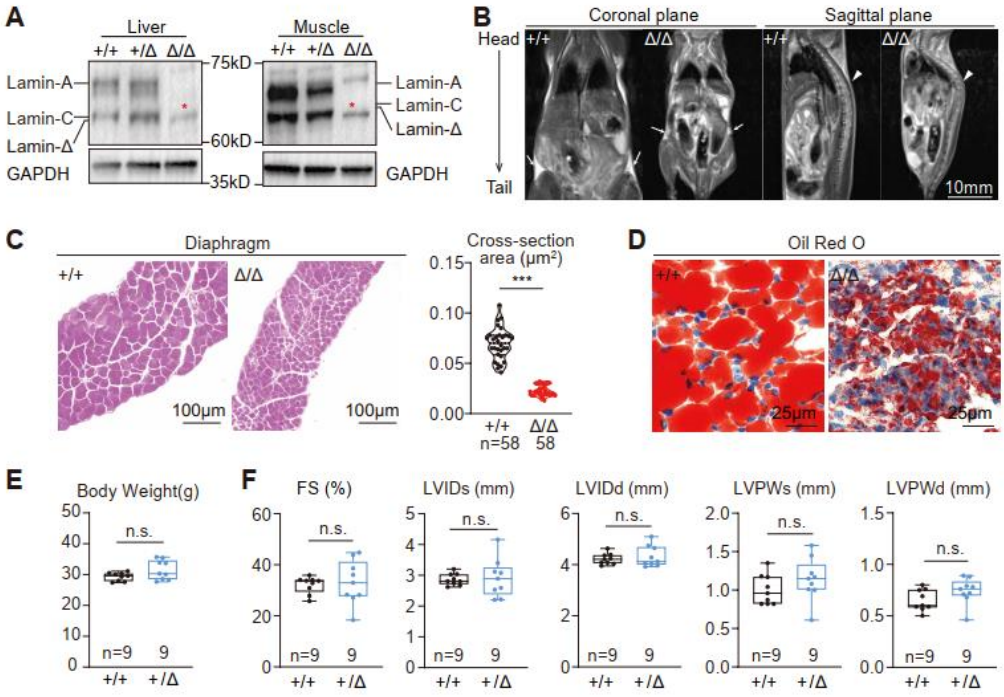

Figure S1. Characterization of the *Lmna*$^{\Delta/\Delta}$ mice. (A) Western blot of liver and skeletal muscle tissues using an antibody recognizing lamin-A/C. Red star, lamin-Δ protein. **(B)** T2-weighted magnetic resonance imaging of 6-week-old mice. White arrow, subcutaneous fat; arrow head, spine curve. N indicates cell number. **(C)** H&E staining of diaphragm sections and quantification of myocyte cross-section area. **(D)** Oil Red O staining on gonadal adipose tissue sections. **(E-F)** 6-month-old mice were assessed by body weight **(E)** and echocardiogram **(F)**. N indicates animal number. Mann-Whitney U test, ***p<0.001; n.s., not significant.

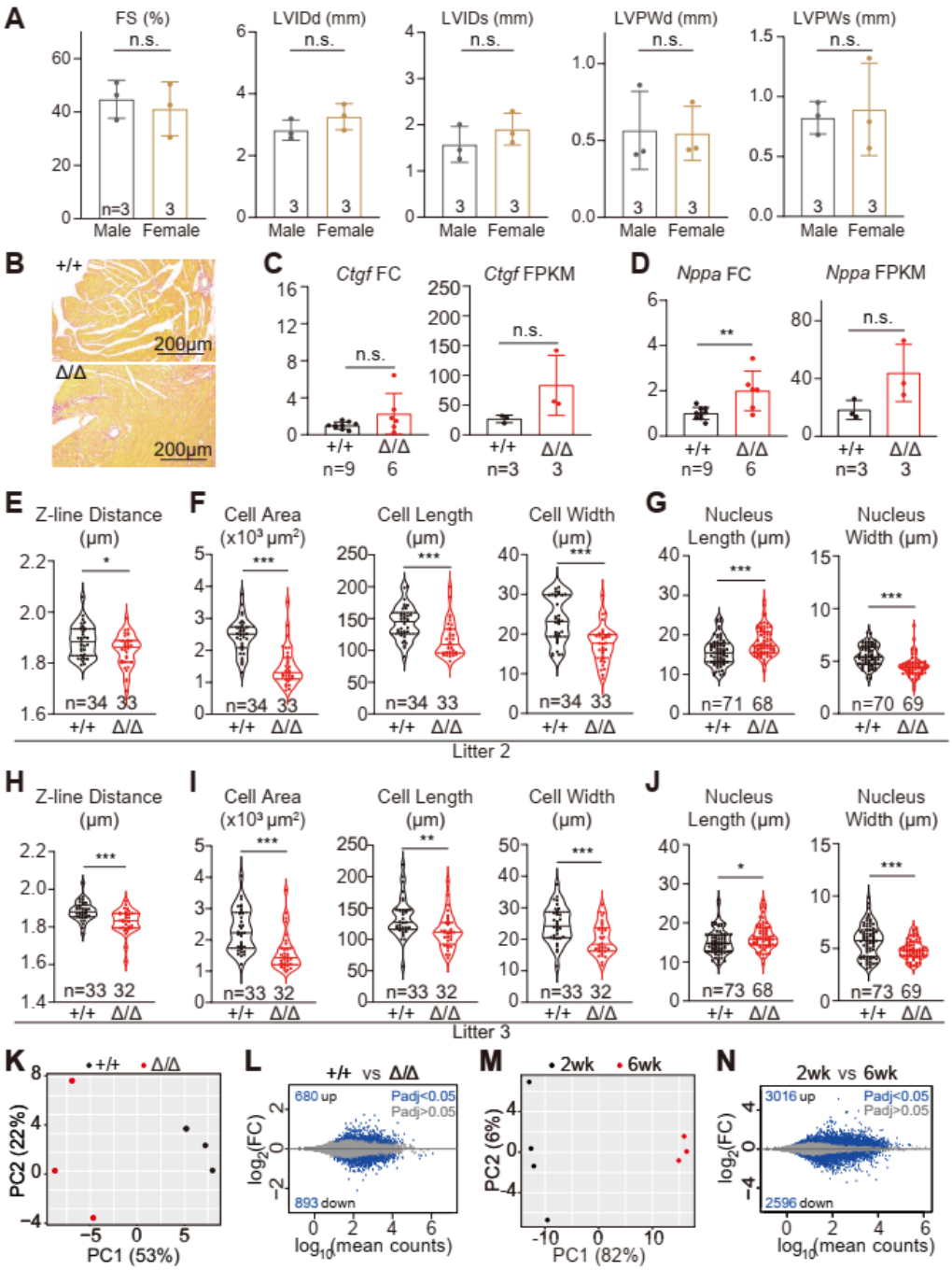

Figure S2. Characterization of the heart phenotypes. (A) Echocardiogram analysis of 6-week-old *Lmna*$^{\Delta/\Delta}$ mice of distinct genders. N indicates animal number. **(B)** Picrosirius red staining on paraffin sections of the heart. **(C-D)** RT-qPCR and RNA-seq quantification of key cardiac pathological markers. FC, fold change. RPKM, reads per kilobase per million mapped reads. **(E-J)** Quantitative analysis of sarcomere Z-line distances **(E, H)**, cell morphology **(F, I)**, and nuclear morphology **(G, J)** in cardiomyocytes isolated from two pairs of littermates. N indicates cell number. Mann-Whitney U test, *p<0.05; **p<0.01; ***p<0.001; n.s., not significant in **(A, C-J)**. **(K-N)** PCA **(K, M)** and MA **(L, N)** plots of 6-week *Lmna*$^{\Delta/\Delta}$ versus *Lmna*$^{+/+}$ ventricles **(K-L)** or 6-week versus 2-week *Lmna*$^{+/+}$ ventricles **(M-N)**. The adjust P values were calculated by DESeq2 with Benjamini–Hochberg correction.

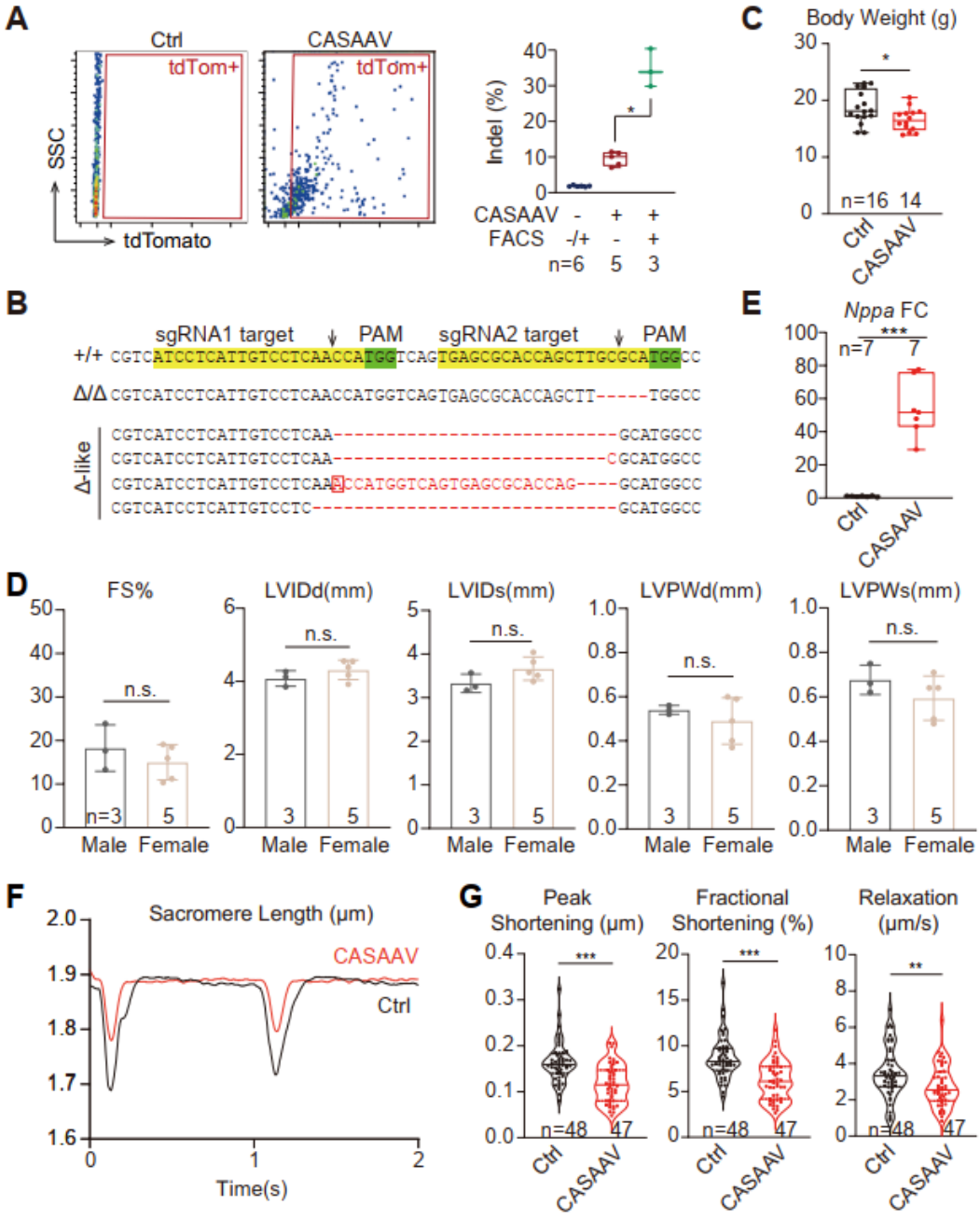

Figure S3. Validation and characterization of CASAAV-mediated *Lmna* mutagenesis. (A) Representative FACS plots to sort CASAAV-treated cardiomyocytes and amplicon-sequencing quantification of indels. SSC, side scatter. **(B)** Representative reads showing CASAAV-induced *Lmna*Δ-like mutations. Arrow, Cas9 cut sites. **(C)** Body weight analysis of 6-week-old high-dose-treated mice. **(D)** Echocardiogram analysis of CASAAV-treated animals separated by gender. **(E)** RT-qPCR analysis of the hearts. N indicates animal number in **(A, C-E)**. **(F-G)** Representative traces **(F)** and quantification **(G)** of cardiomyocyte contraction that was paced by 1 Hz. N indicates cell number in **(G)**. Mann-Whitney U test: *p<0.05; **p<0.01; ***p<0.001; n.s., not significant.

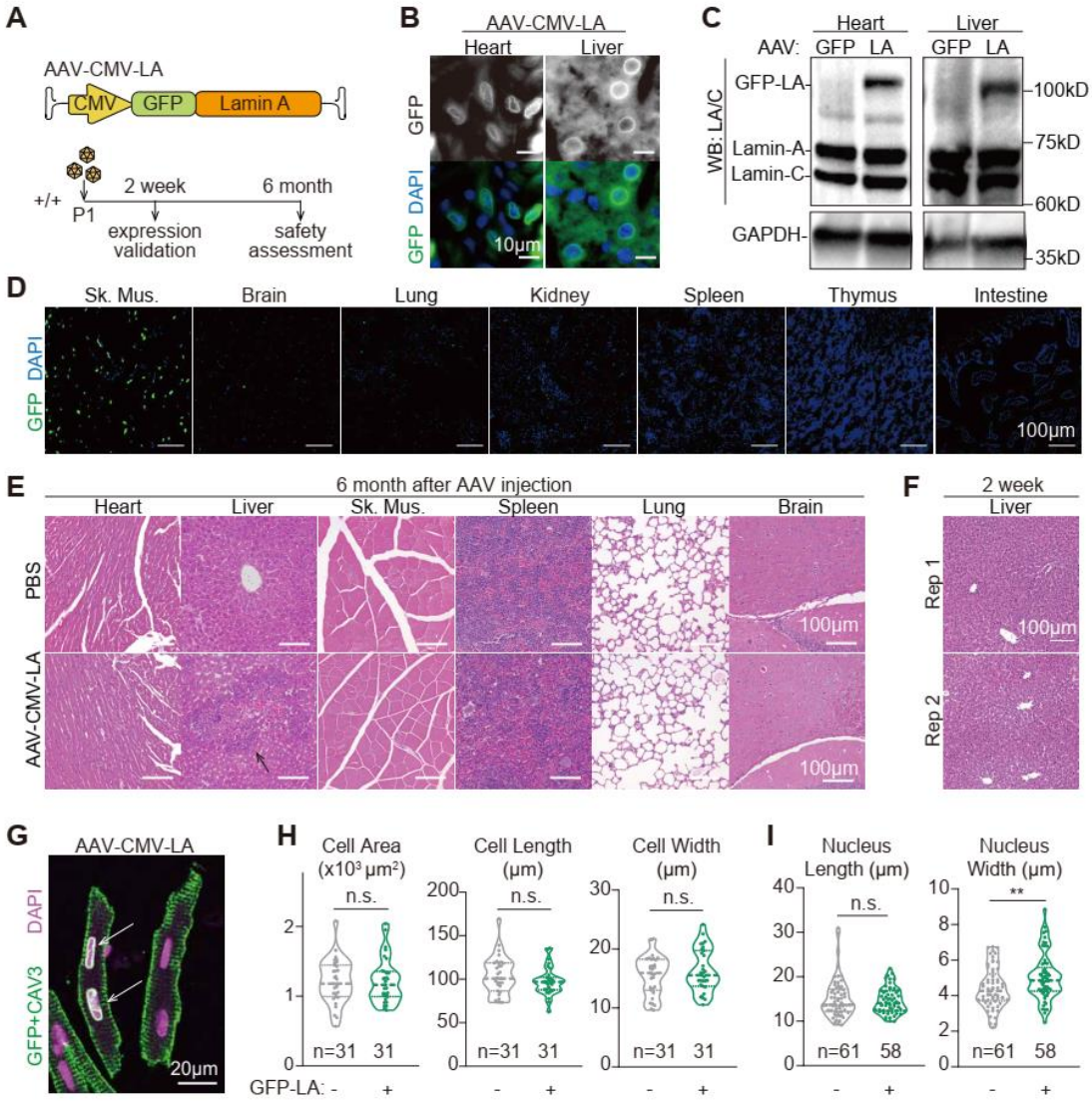

Figure S4. Characterization of the AAV-CMV-LA vector. (A) A diagram showing the AAV assessment experiments in wildtype mice. **(B)** Fluorescence images of AAV-CMV-LA-treated tissues at P14. **(C)** Western blot analysis of lamin-A/C in heart and liver at P14. **(D)** Fluorescence images of multiple tissues that were collected from mice treated with AAV-CMV-LA. Sk. Mus., skeletal muscle. **(E)** H&E staining of various tissues that were collected from mice treated with AAV-CMV-LA for 6 months. Arrow indicates infiltrated cells in the liver. **(F)** Two replicates of liver samples from *Lmna*$^{\Delta/\Delta}$ mice that were treated with AAV-CMV-LA for 2 weeks. **(G)** Immunofluorescence of isolated *Lmna*$^{\Delta/\Delta}$ cardiomyocytes. Arrows indicate the GFP-lamin-A-positive nuclei. A GFP-lamin-A -negative cell is to the right. CAV3, caveolin-3, cell membrane marker. **(H-I)** Quantification of cellular **(H)** and nuclear **(I)** morphology. Mann-Whitney U test: **p<0.01; n.s., not significant. N indicates cell number.

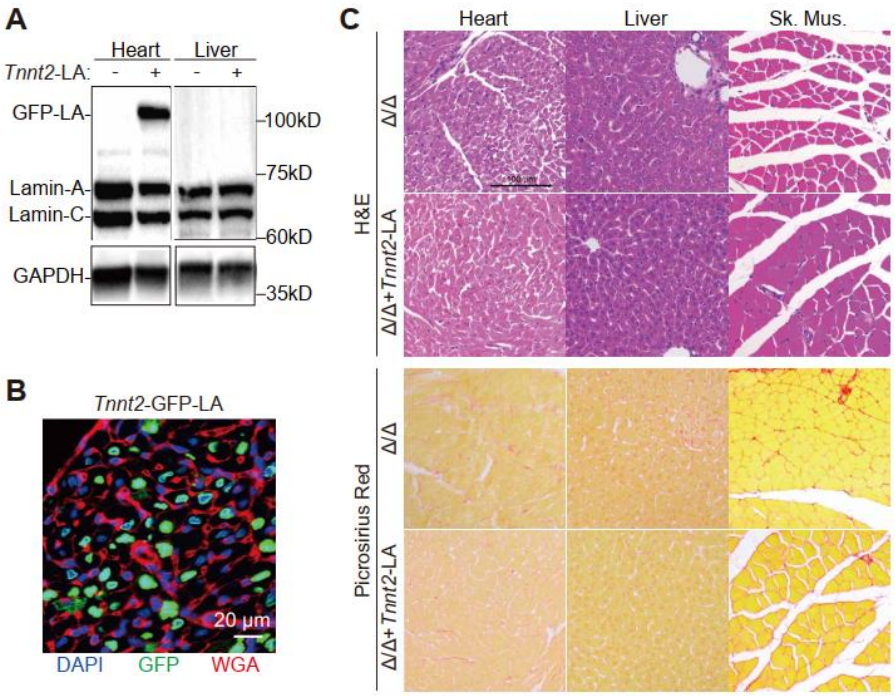

Figure S5. Characterization of AAV-*Tnnt2*-LA-treated hearts. (A) Western blot analysis of high-dose AAV-*Tnnt2*-LA-treated wildtype heart and liver at P14. **(B)** Fluorescence images of a heart section treated with high-dose AAV-*Tnnt2*-LA. **(C)** Histological analysis of P14 $Lmna^{\Delta/\Delta}$ samples under AAV-*Tnnt2*-LA treatment.

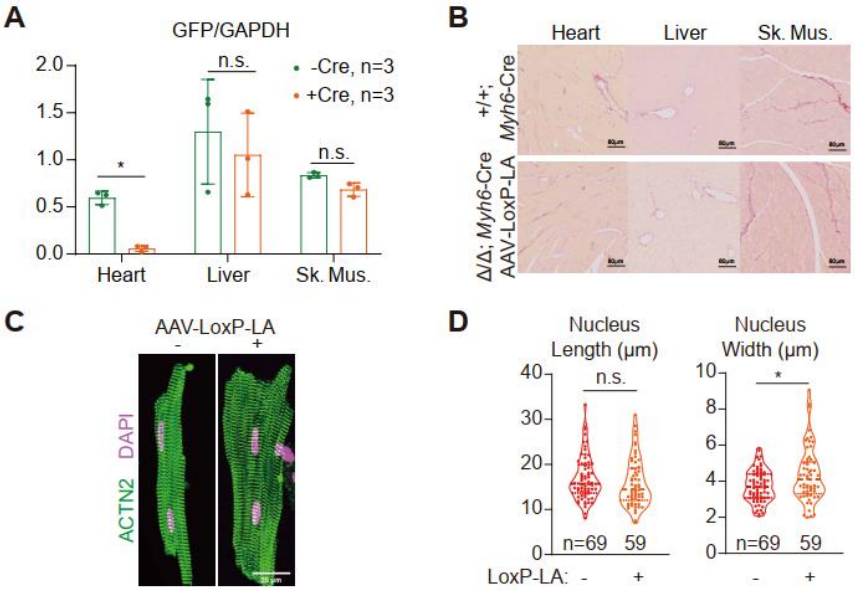

Figure S6. Characterization of AAV-LoxP-LA-treated hearts. (A) Western blot quantification of high-dose AAV-LoxP-LA-treated samples. N indicates animal number. **(B)** Picrosirius red staining of AAV-LoxP-LA-treated samples. **(C)** Representative images of cardiomyocytes in the AAV-LoxP-LA experiments. **(D)** Quantification of nuclear shape. N indicates cell number. Mann-Whitney U test: *p<0.05; n.s., not significant.