Fig. 1

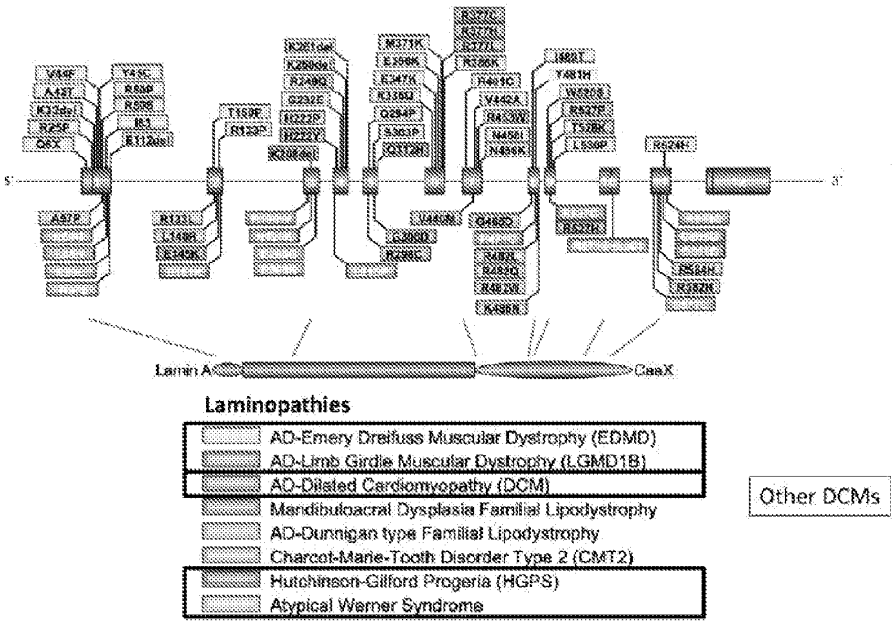

Lamin A
CaaX
Laminopathies
AD-Emery Dreifuss Muscular Dystrophy (EDMD)
AD-Limb Girdle Muscular Dystrophy (LGMD1B)
AD-Dilated Cardiomyopathy (DCM)
Mandibuloacral Dysplasia Familial Lipodystrophy
AD-Dunnigan type Familial Lipodystrophy
Charcot-Marie-Tooth Disorder Type 2 (CMT2)
Hutchinson-Gilford Progeria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

Fig. 2

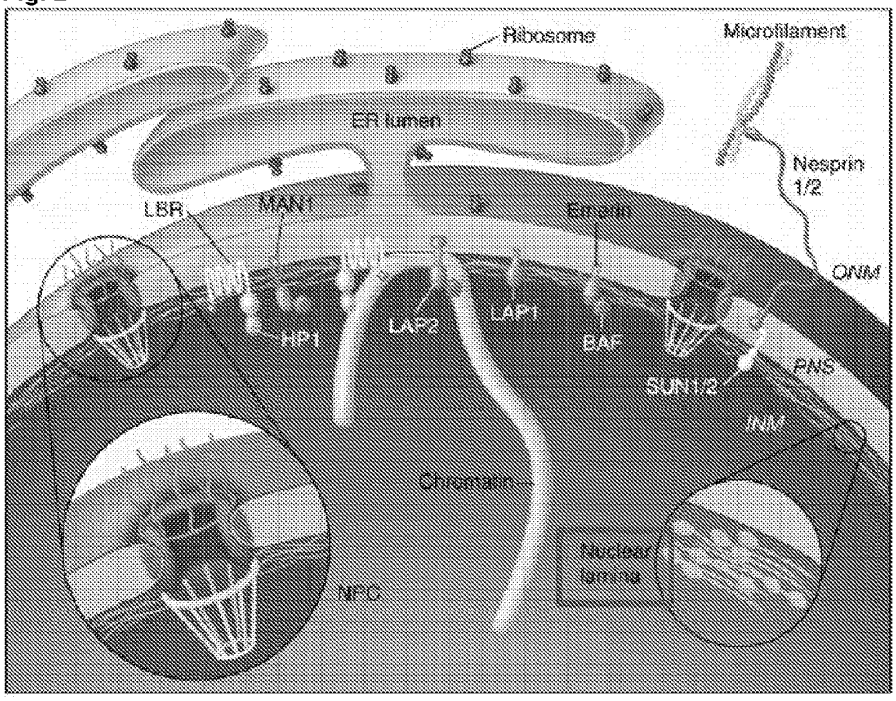

Ribosome
Microfilament
ER lumen
Nesprin 1/2
LBR
MAN1
Emerin
ONM
HP1
LAP2
LAP1
BAF
PNS
SUN1/2
INM
Chromatin
NPC
Nuclear lamina

Fig. 3

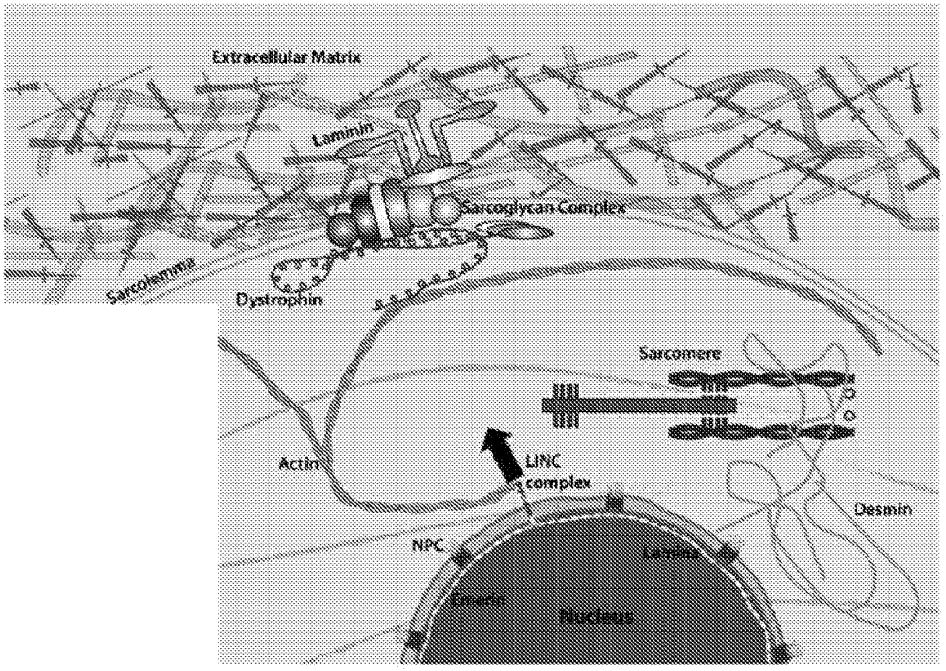

Extracellular Matrix
Laminin
Sarcoglycan Complex
Sarcolemma
Dystrophin
Sarcomere
Actin
LINC complex
Desmin
NPC
Lamin
Nucleus

Fig. 4

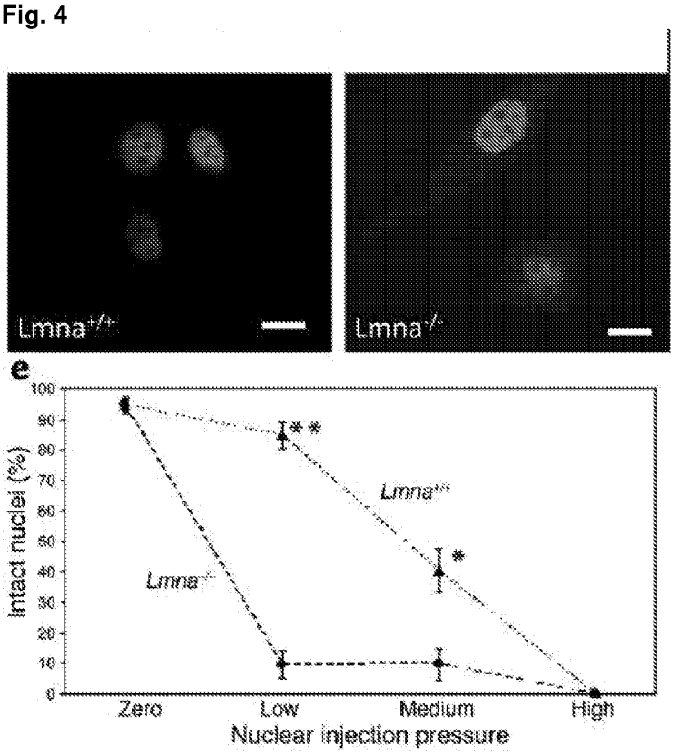

Lmna+/+
Lmna-/-
e
Intact nuclei (%)
Lmna+/+
Lmna-/-
Zero
Low
Medium
High
Nuclear injection pressure

Fig. 5A

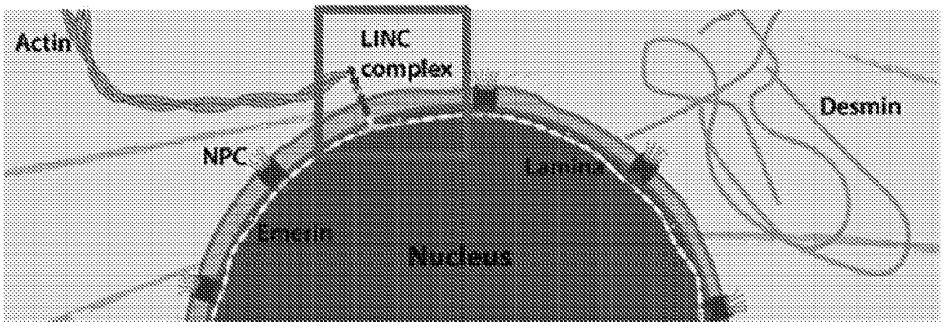

Actin
LINC complex
Desmin
NPC
Lamina
Emerin
Nucleus

Fig. 5B

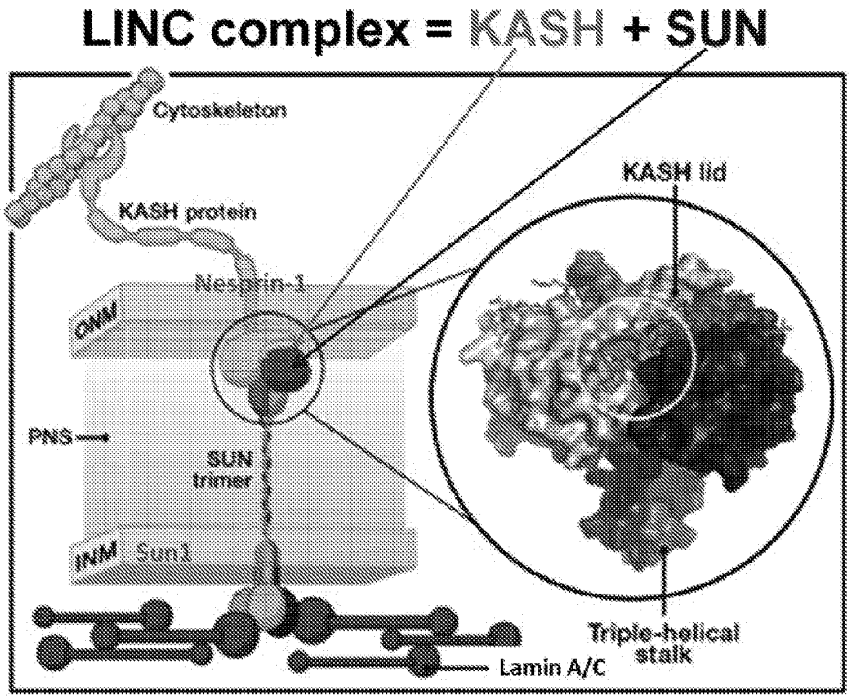

LINC complex = KASH + SUN
Cytoskeleton
KASH protein
Nesprin-1
KASH lid
ONM
PNS
SUN trimer
INM Sun1
Triple-helical stalk
Lamin A/C

Fig. 6A

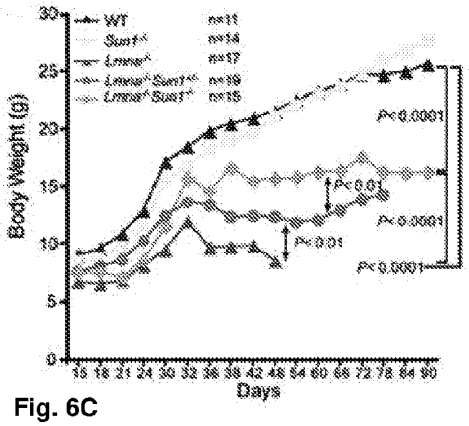


Fig. 6B

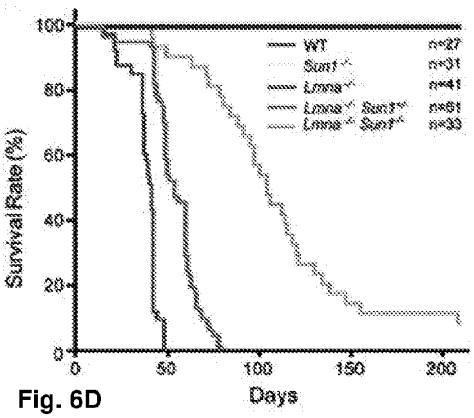


Fig. 6C

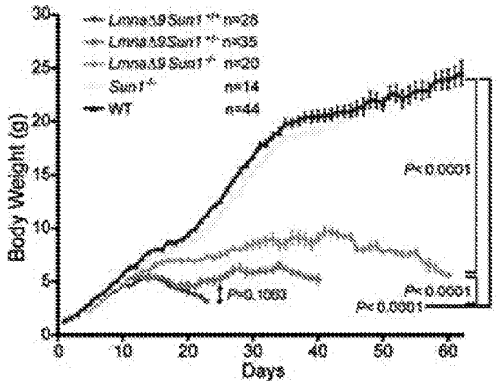


Fig. 6D

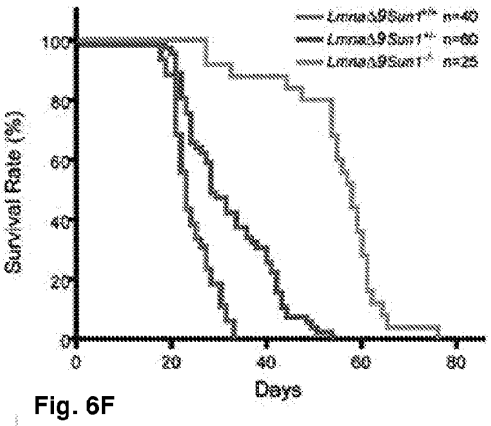


Fig. 6E

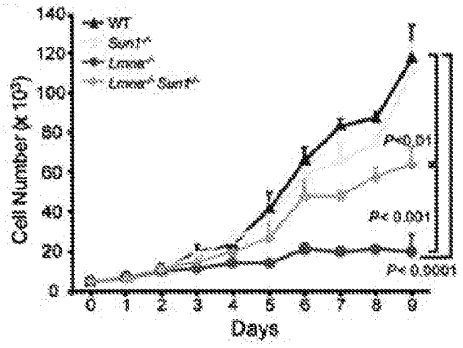


Fig. 6F

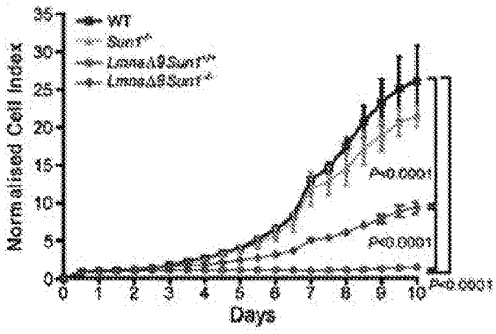


Fig. 7

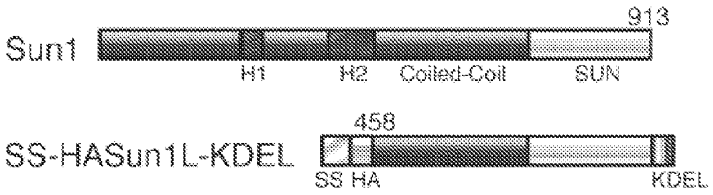

Sun1
913
H1
H2
Coiled-Coil
SUN
SS-HASun1L-KDEL
458
SS
HA
KDEL

Fig. 8

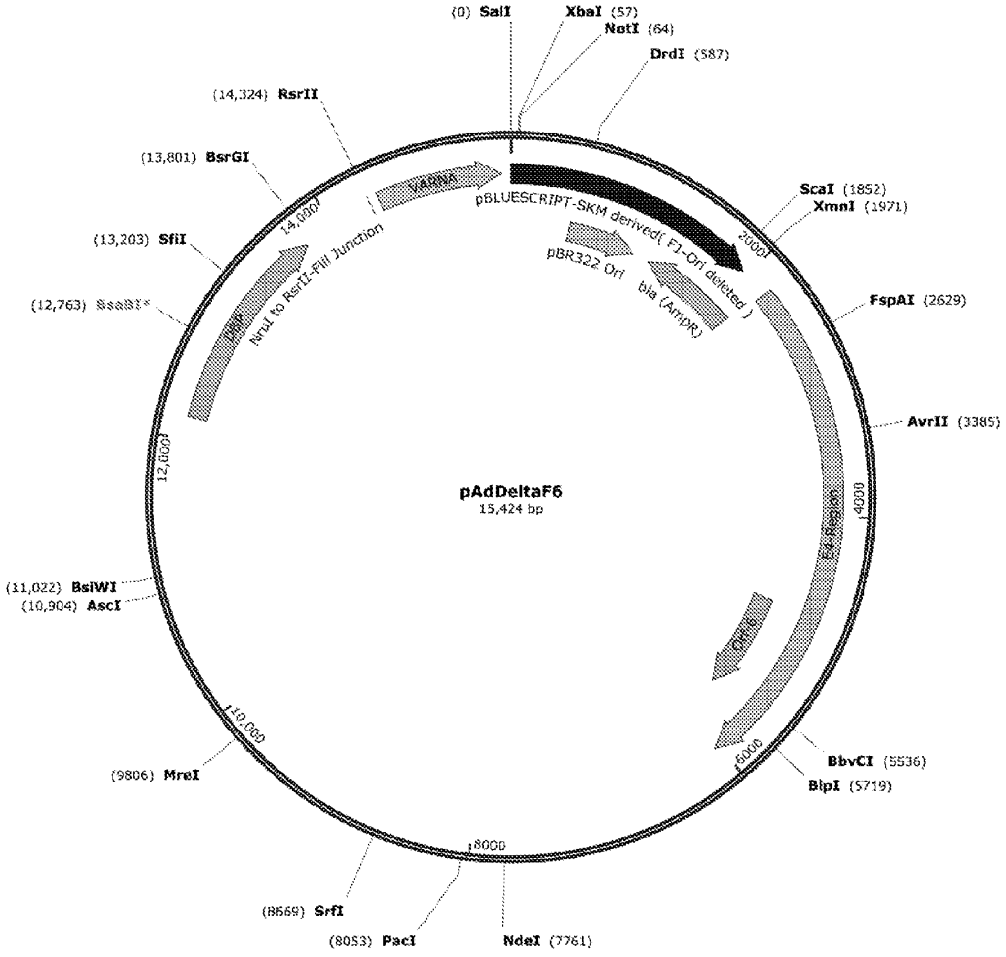

(0) SalI
XbaI (57)
NotI (64)
DrdI (587)
(14,324) RsrII
(13,801) BsrGI
(13,203) SfiI
(12,763) BssSI
SV40 pA
pBLUESCRIPT-SKM derived(F1-Ori deleted)
pBR322 Ori
bla (AmpR)
ScaI (1852)
XmnI (1971)
FspAI (2629)
AvrII (3385)
E4 Region
ORF6
BbvCI (5536)
BipI (5719)
(8669) SrfI
(8053) PacI
NdeI (7761)
(9806) MreI
(11,022) BsiWI
(10,904) AscI
Xmn1 to RsrII-Fill Junction
pAdDeltaF6
15,424 bp

Fig. 9

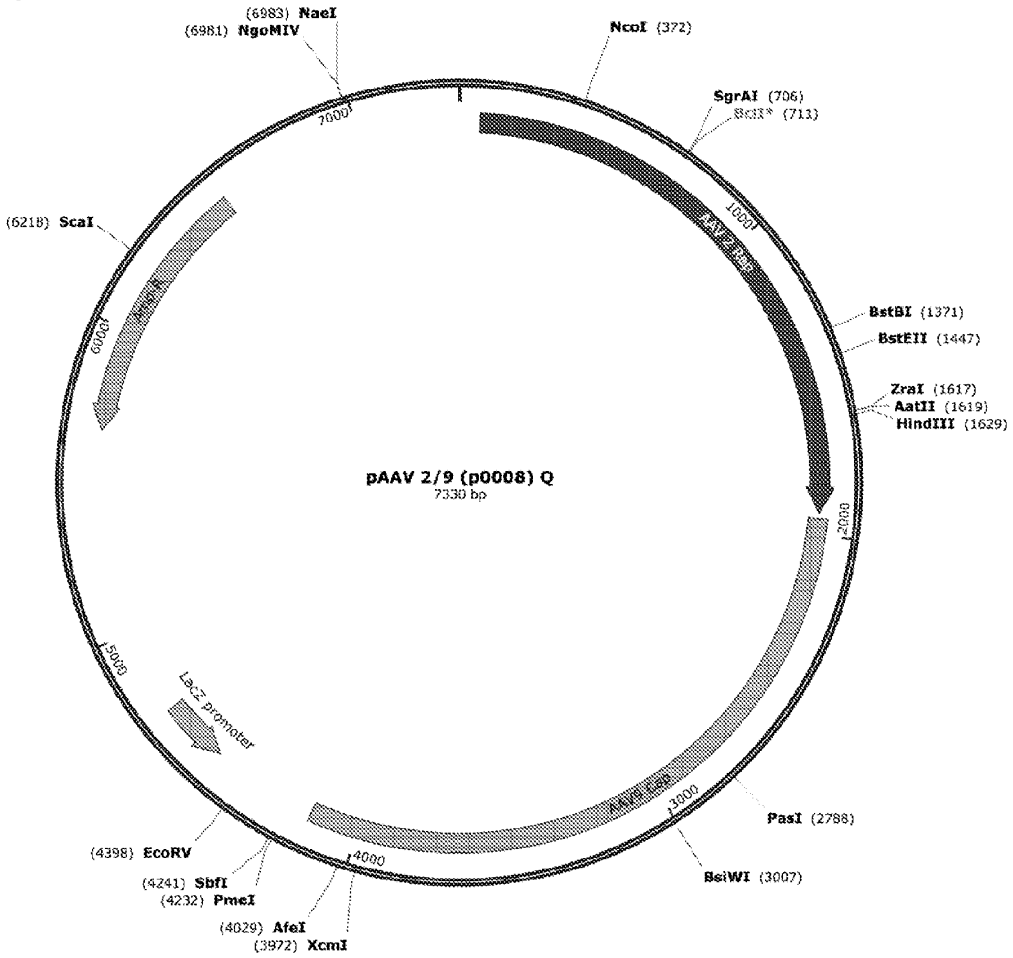

(6983) NaeI
(6981) NgoMIV
NcoI (372)
SgrAI (706)
BclI* (713)
1000
AAV2 ITR
BstBI (1371)
BstEII (1447)
ZraI (1617)
AatII (1619)
HindIII (1629)
2000
pAAV 2/9 (p0008) Q
7330 bp
3000
PasI (2788)
BsiWI (3007)
4000
(4398) EcoRV
(4241) SbfI
(4232) PmeI
(4029) AfeI
(3972) XcmI
5000
LacZ promoter
6000
AmpR
(6218) ScaI
7000

Fig. 10


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

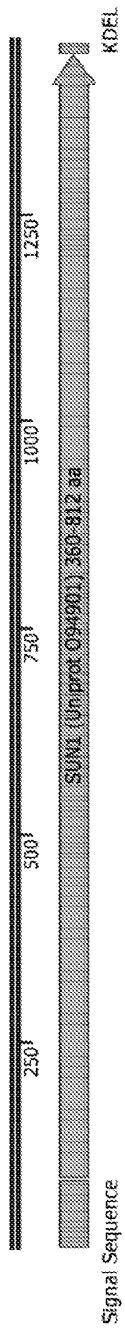

250
500
750
1000
1250
SUN1 (Uniprot Q9H9Q1) 360-812 aa
KDEL
Signal Sequence

Fig. 11

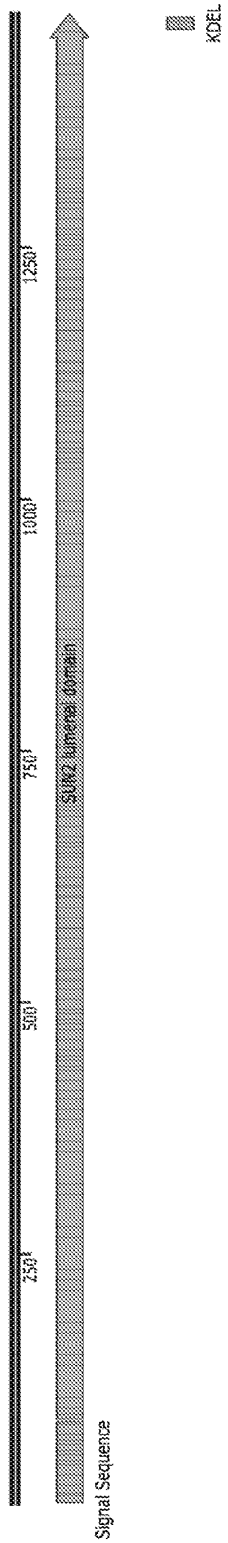

250
500
750
1000
1250
SUN2 luminal domain
KDEL
Signal Sequence

Fig. 12

Fig. 13

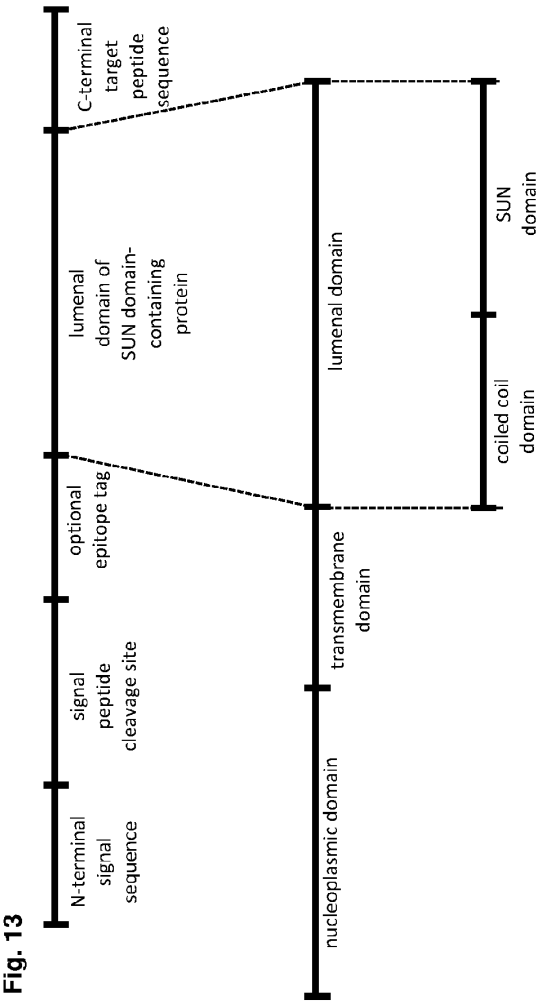


Fig. 14

```
SEQ ID NO:7  KASH1_aminoacid      RGFLFRVLRA--ALP----LQLLLLLLIGLACLVFMSEEDYSCALSNNFARSFHPMLRYTNG
SEQ ID NO:9  KASH2_aminoacid      RSFLSRVVRA--ALP----LQLLLLLLLLACLLFSSEEDYSCTQANNFARSFYPMLRYTNG
SEQ ID NO:11 KASH3_aminoacid      GSLFRRACCV--ALP----LQLLLLLFLLLLFLLPIREEDRSCTLANNFARSFTLMLRYWGP
SEQ ID NO:13 KASH4_aminoacid      ----DFASRQPLTFLLILFLLFLLLVGAMFLLP--ASGGPCCSHARIPRTPYLVLSYVNG
SEQ ID NO:15 KASH5_aminoacid      -----LRVTRHPLIPAPVLGLLLLLLLSVLLLGPSPPPTWP----------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

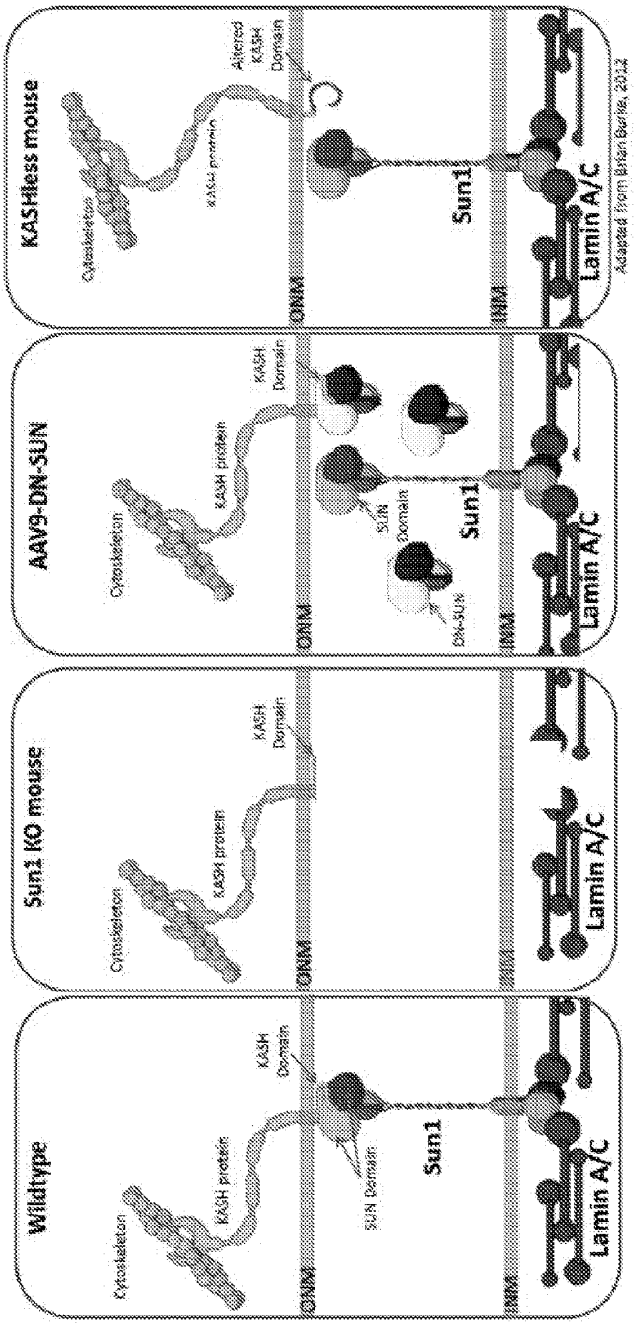

Wildtype
Cytoskeleton
KASH protein
KASH Domain
SUN Domain
Sun1
Lamin A/C
ONM
INM
Sun1 KO mouse
AAV9-DN-SUN
DN-SUN
KASHless mouse
Altered KASH Domain
Adapted from Ishan Barika, 2018

Fig. 16

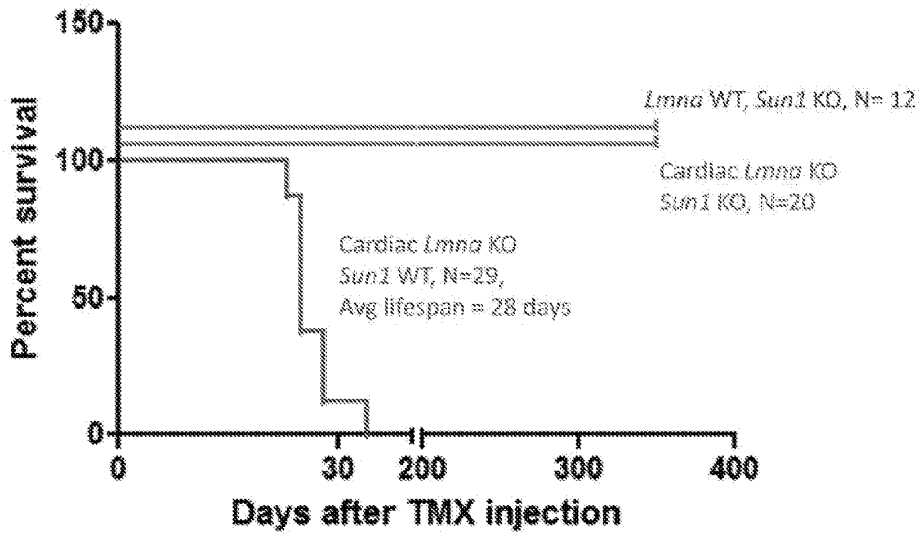

150
100
50
0
Percent survival
Lmna WT, Sun1 KO, N= 12
Cardiac Lmna KO
Sun1 KO, N=20
Cardiac Lmna KO
Sun1 WT, N=29,
Avg lifespan = 28 days
0
30
200
300
400
Days after TMX injection

Fig. 17

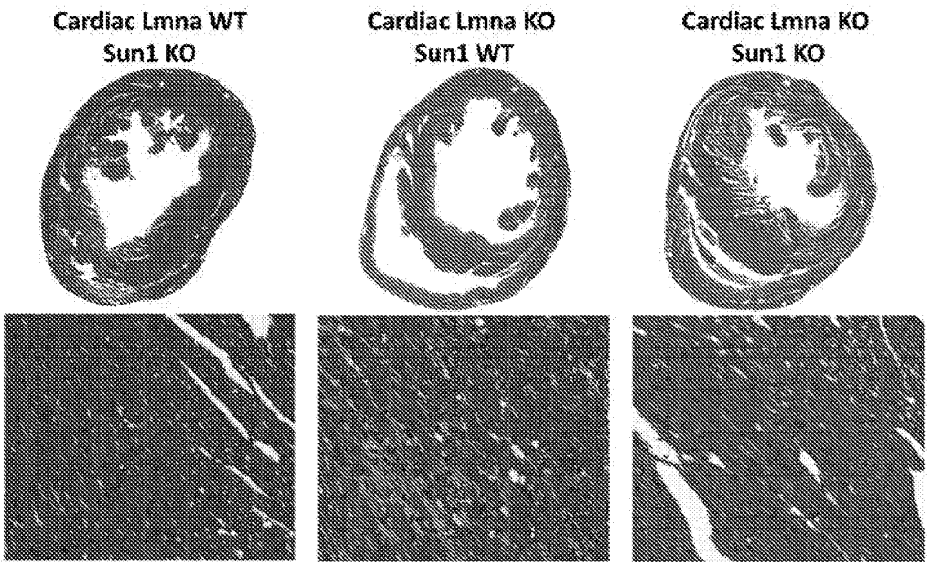

Cardiac Lmna WT
Sun1 KO
Cardiac Lmna KO
Sun1 WT
Cardiac Lmna KO
Sun1 KO

Fig. 18

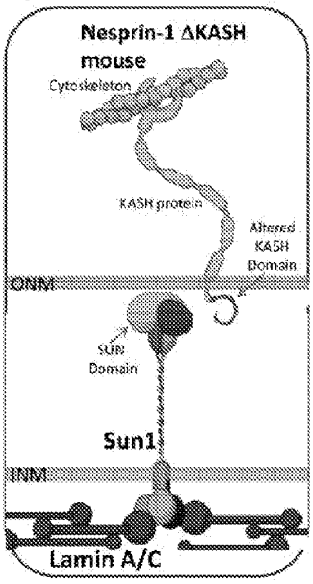


Fig. 19A

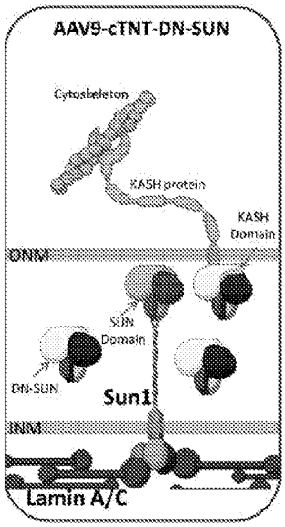


Fig. 19B

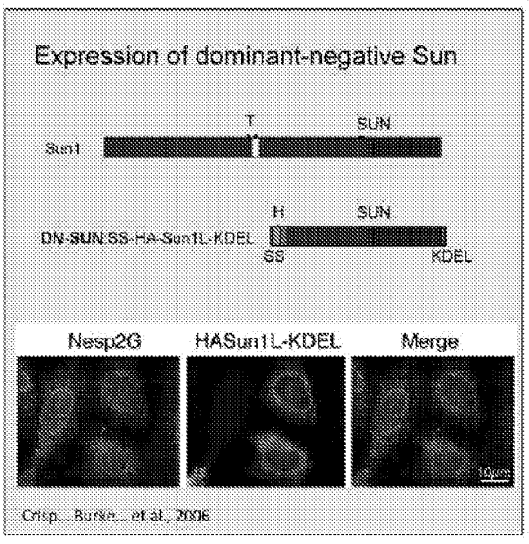


Fig. 20

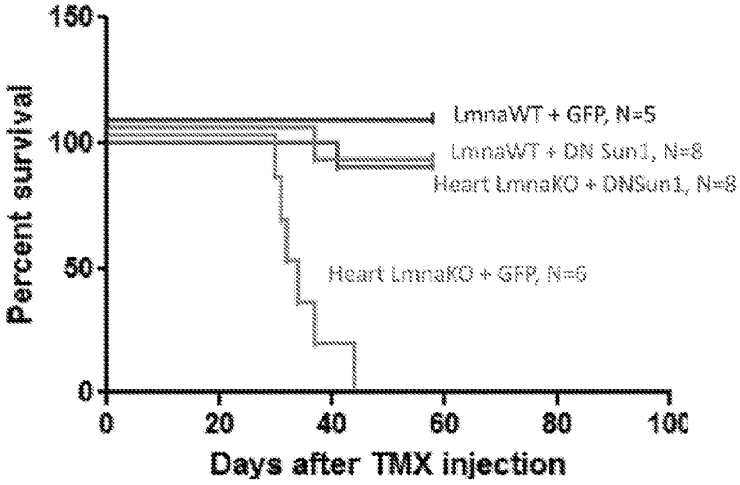

150
100
50
0
Percent survival
LmnaWT + GFP, N=5
LmnaWT + DN Sun1, N=8
Heart LmnaKO + DNSun1, N=8
Heart LmnaKO + GFP, N=6
0
20
40
60
80
100
Days after TMX injection

Fig. 21

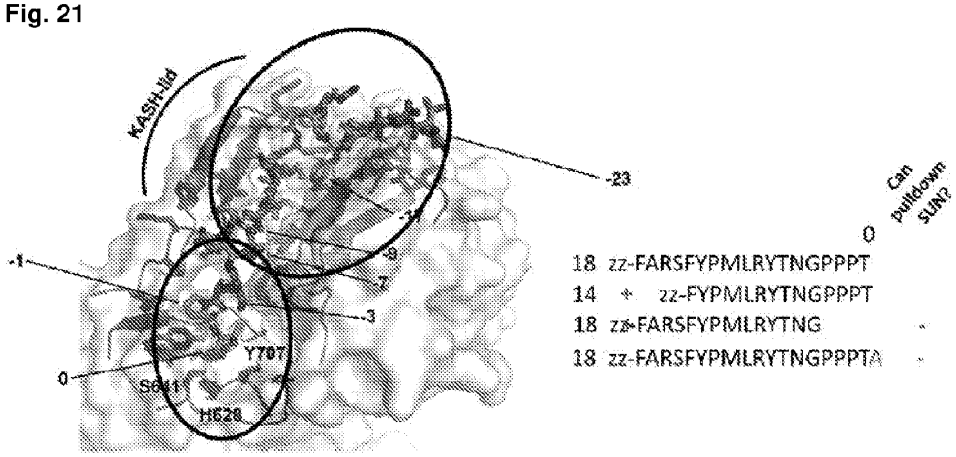

KASH-lid
-23
-11
-9
-7
-3
-1
0
Y707
S611
H628
Can pulldown SUN2
0
18 zz-FARSFYPMLRYTNGPPPT
14 zz-FYPMLRYTNGPPPT
18 zz-FARSFYPMLRYTNG -
18 zz-FARSFYPMLRYTNGPPPTA -

Fig. 22

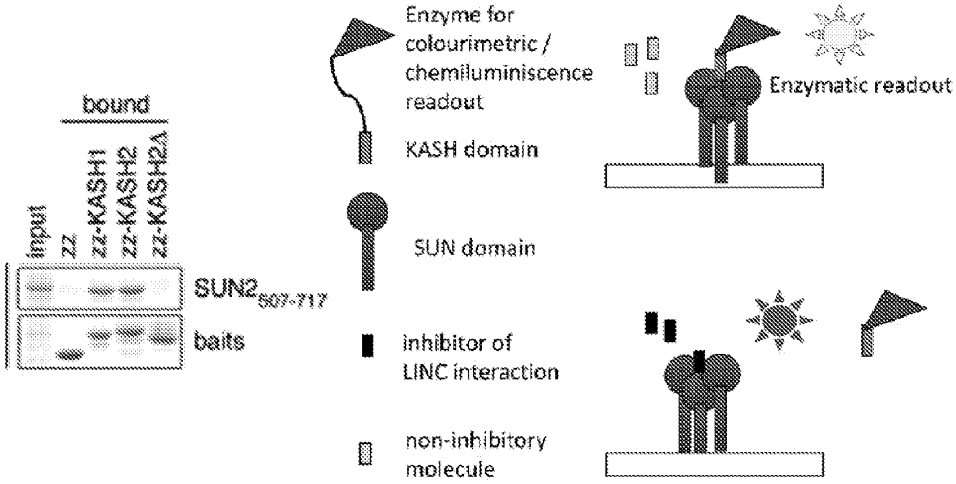

bound
input
zz
zz-KASH1
zz-KASH2
zz-KASH2Δ
SUN2507-717
baits
Enzyme for colourimetric / chemiluminiscence readout
KASH domain
SUN domain
inhibitor of LINC interaction
non-inhibitory molecule
Enzymatic readout

Fig. 23

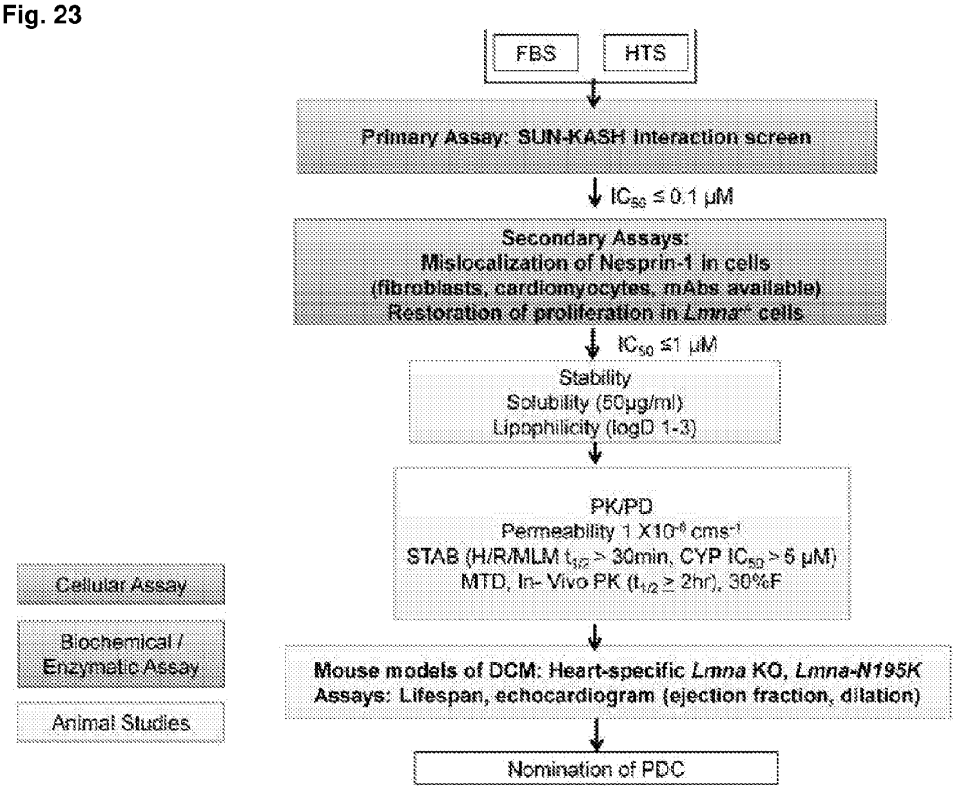

FBS
HTS
Primary Assay: SUN-KASH Interaction screen
IC50 ≤ 0.1 μM
Secondary Assays:
Mislocalization of Nesprin-1 in cells
(fibroblasts, cardiomyocytes, mAbs available)
Restoration of proliferation in Lmna-/- cells
IC50 ≤1 μM
Stability
Solubility (50μg/ml)
Lipophilicity (logD 1-3)
PK/PD
Permeability 1 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

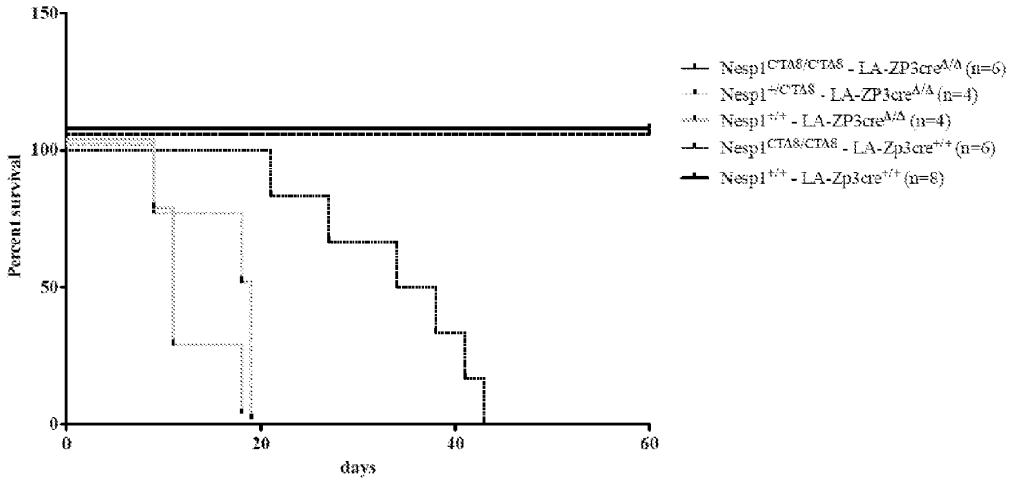

150
100
50
0
Percent survival
0
20
40
60
days
Nesp1CTΔ8/CTΔ8 - LA-ZP3creΔ/Δ (n=6)
Nesp1+/CTΔ8 - LA-ZP3creΔ/Δ (n=4)
Nesp1+/+ - LA-ZP3creΔ/Δ (n=4)
Nesp1CTΔ8/CTΔ8 - LA-Zp3cre+/+ (n=6)
Nesp1+/+ - LA-Zp3cre+/+ (n=8)

Fig. 25

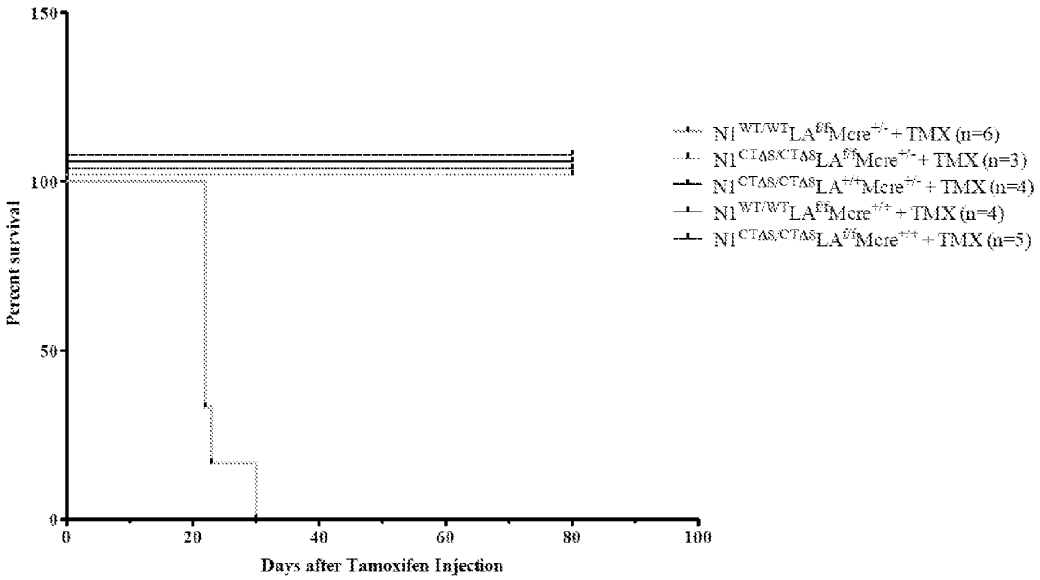

150
100
50
0
Percent survival
0
20
40
60
80
100
Days after Tamoxifen Injection
N1WT/WTLAfl/flMcre+/- + TMX (n=6)
N1CTΔS/CTΔSLAfl/flMcre+/- + TMX (n=3)
N1CTΔS/CTΔSLA+/+Mcre+/- + TMX (n=4)
N1WT/WTLAfl/flMcre+/+ + TMX (n=4)
N1CTΔS/CTΔSLAfl/flMcre+/+ + TMX (n=5)

Fig. 26A

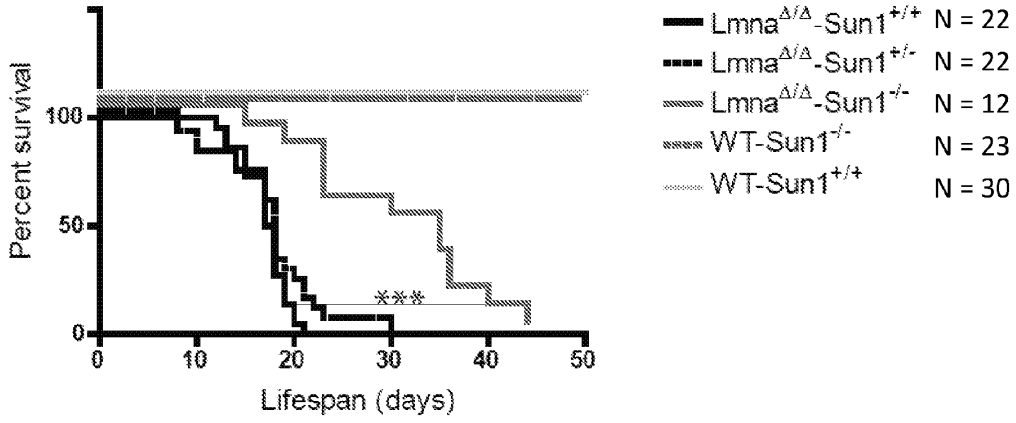


Fig. 26B

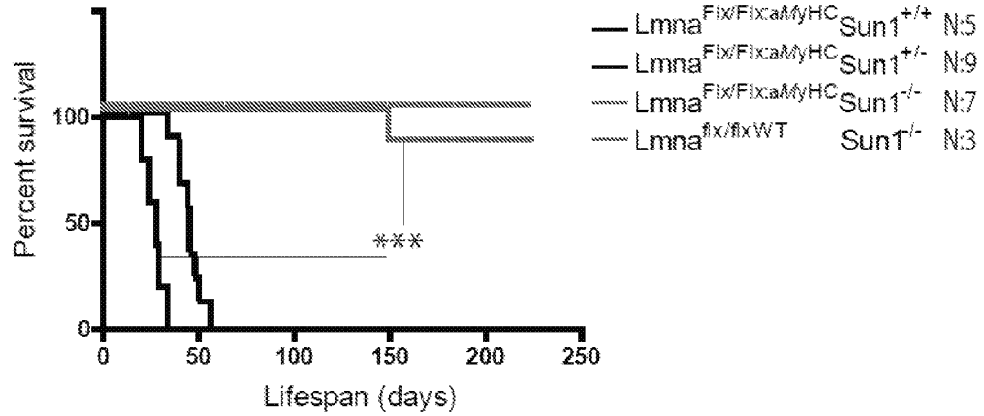


Fig. 26C

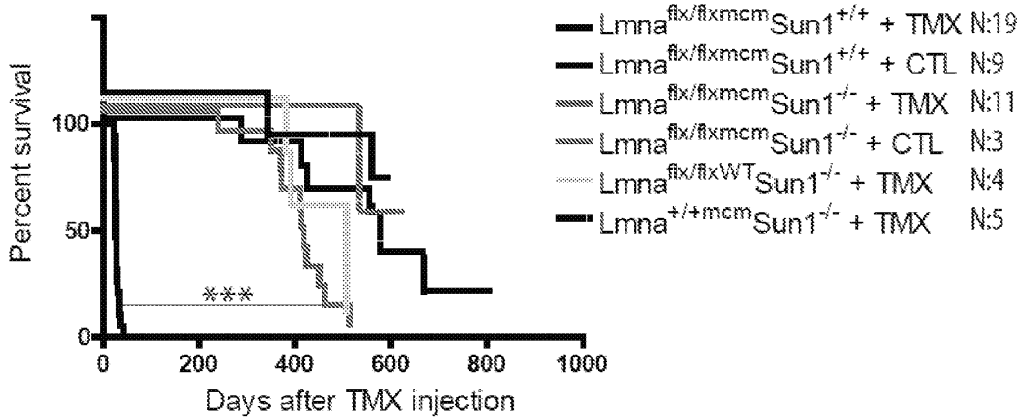


Fig. 26D

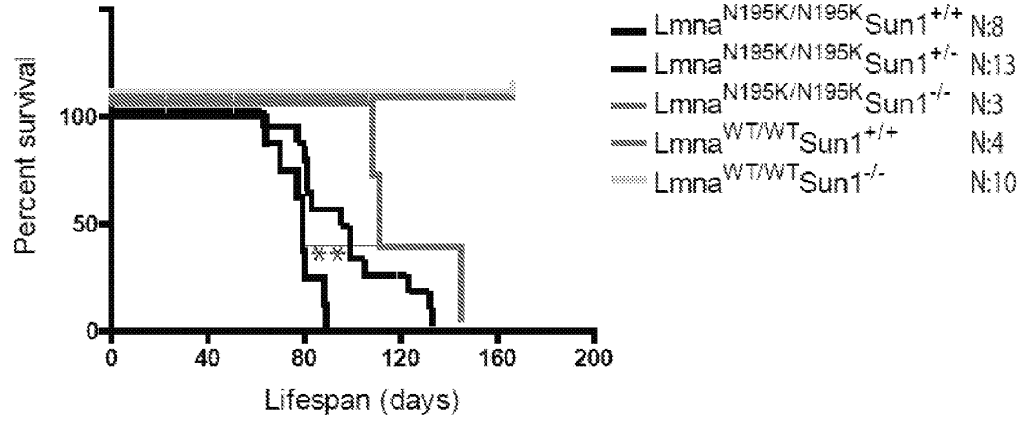


Fig. 27A

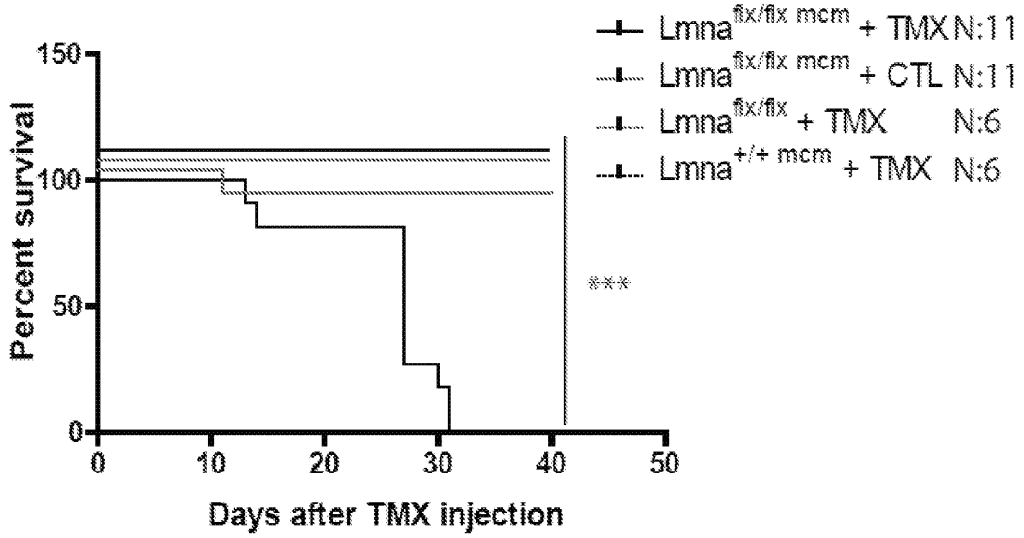


Fig. 27B

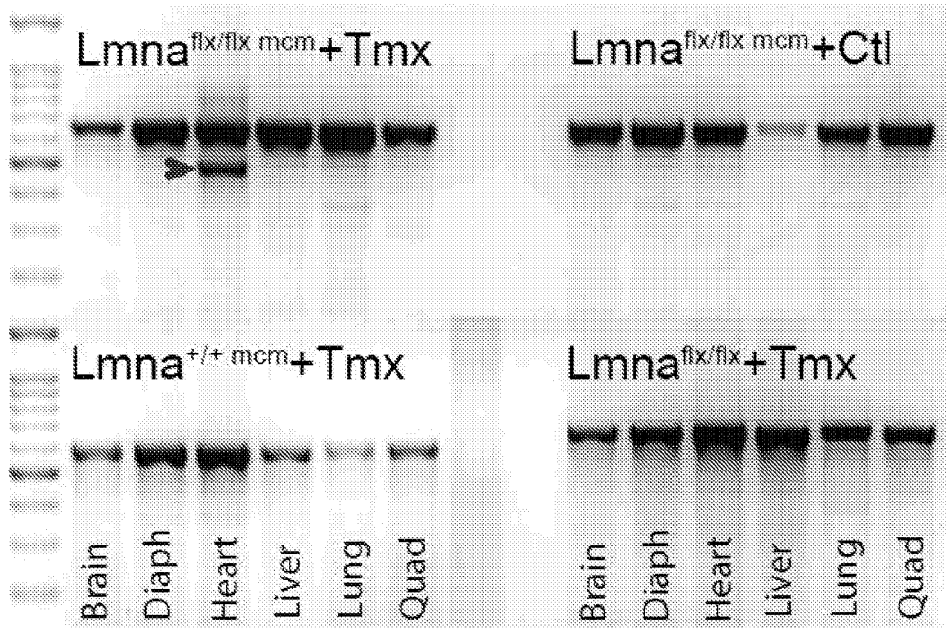


Fig. 27C

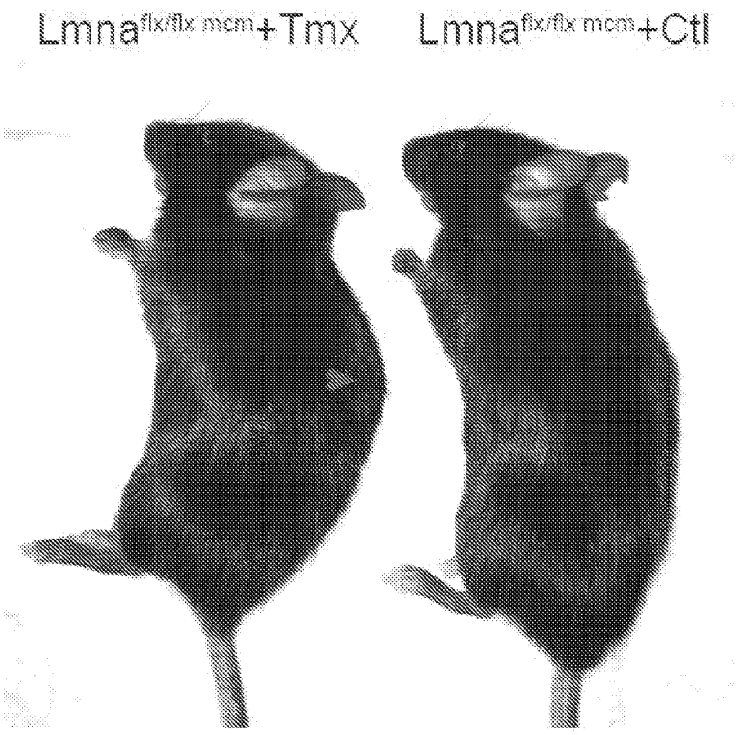


Fig. 27D

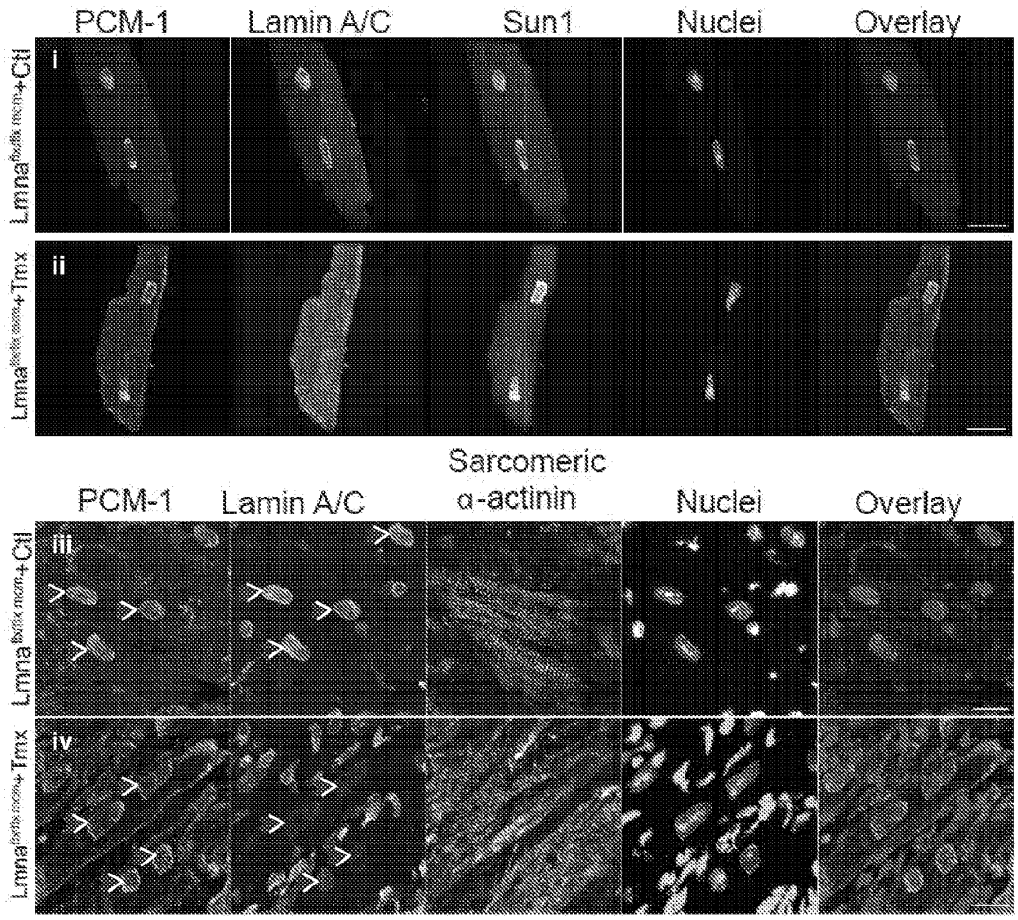

PCM-1
Lamin A/C
Sun1
Nuclei
Overlay
i
ii
Sarcomeric
α-actinin
iii
iv

Fig. 27E

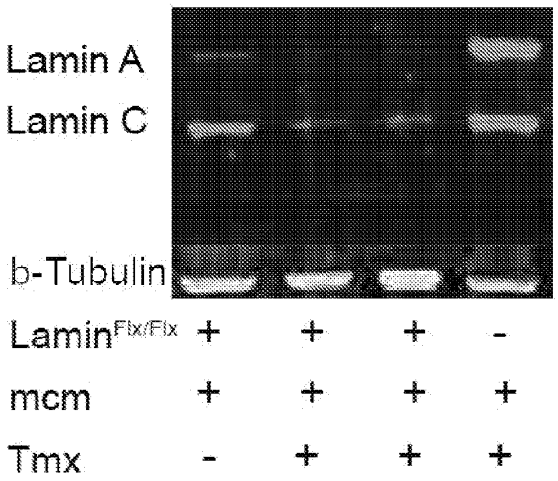


Fig. 27F

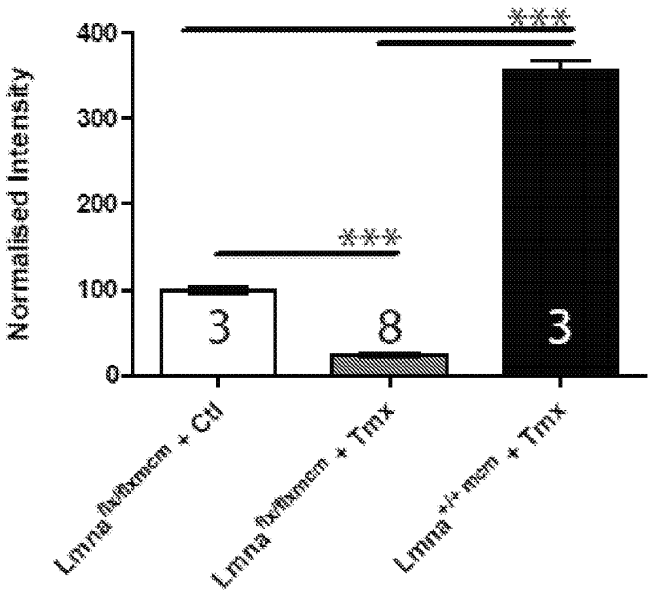


Fig. 28A

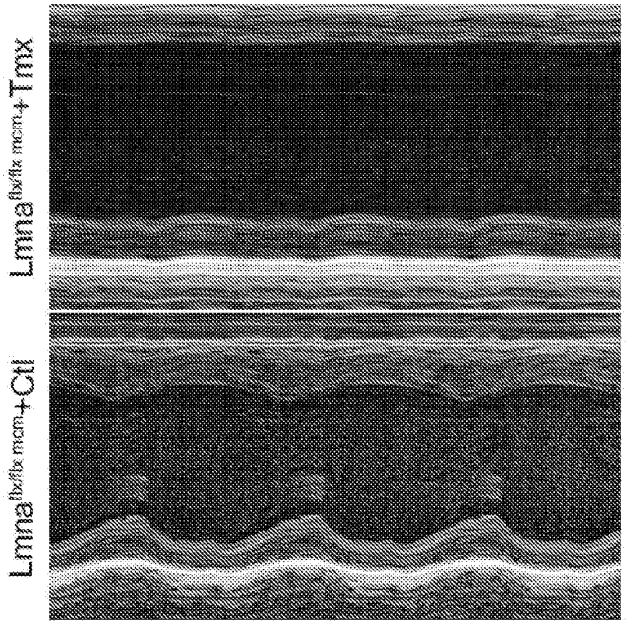


Fig. 28B

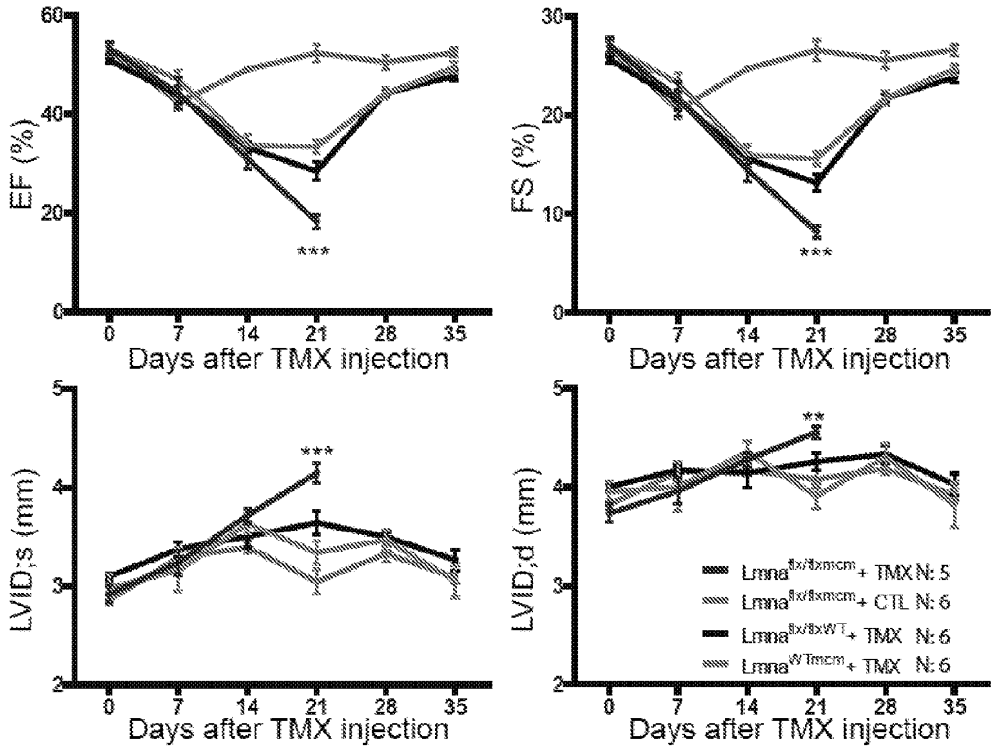


Fig. 28C

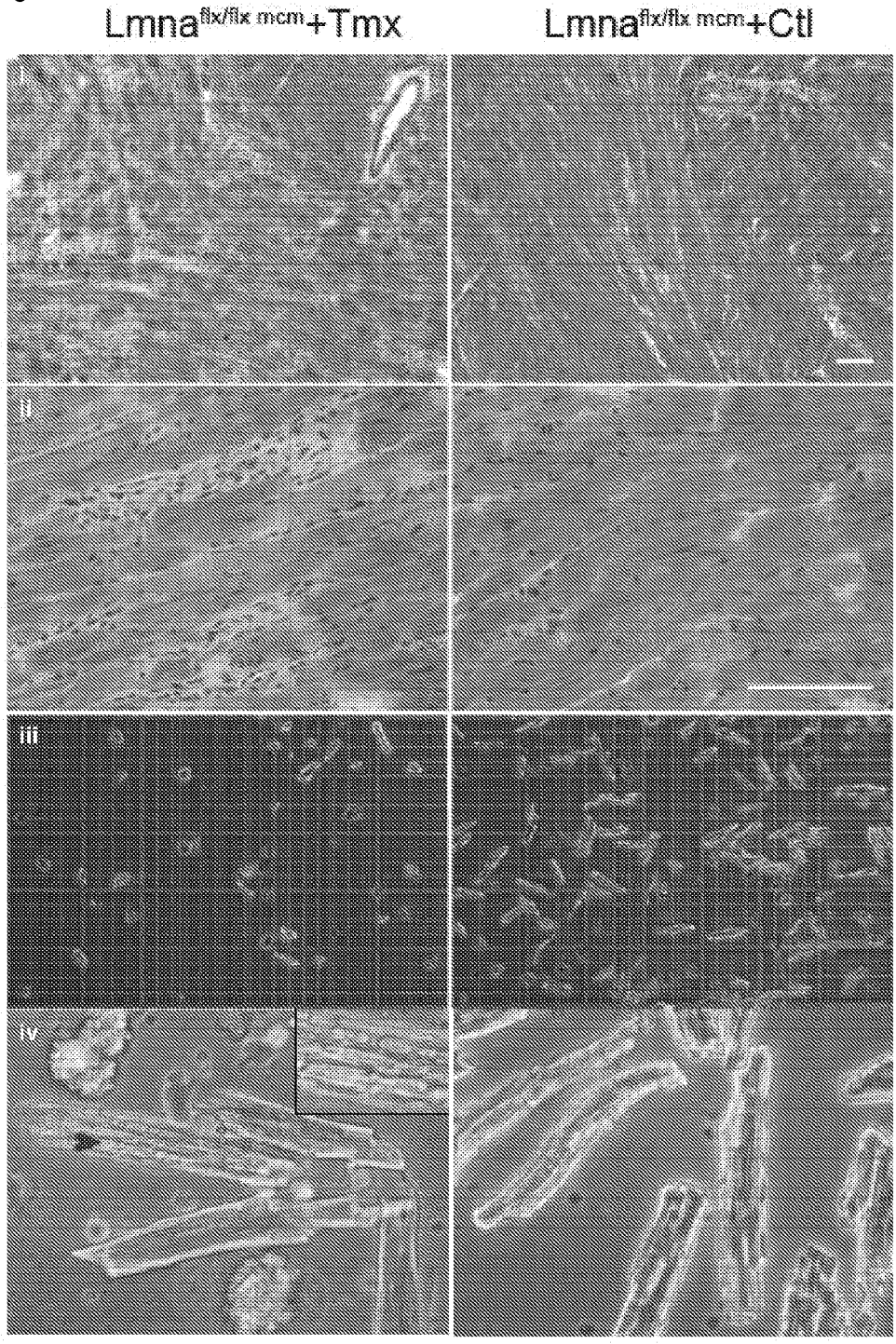


Fig. 28D

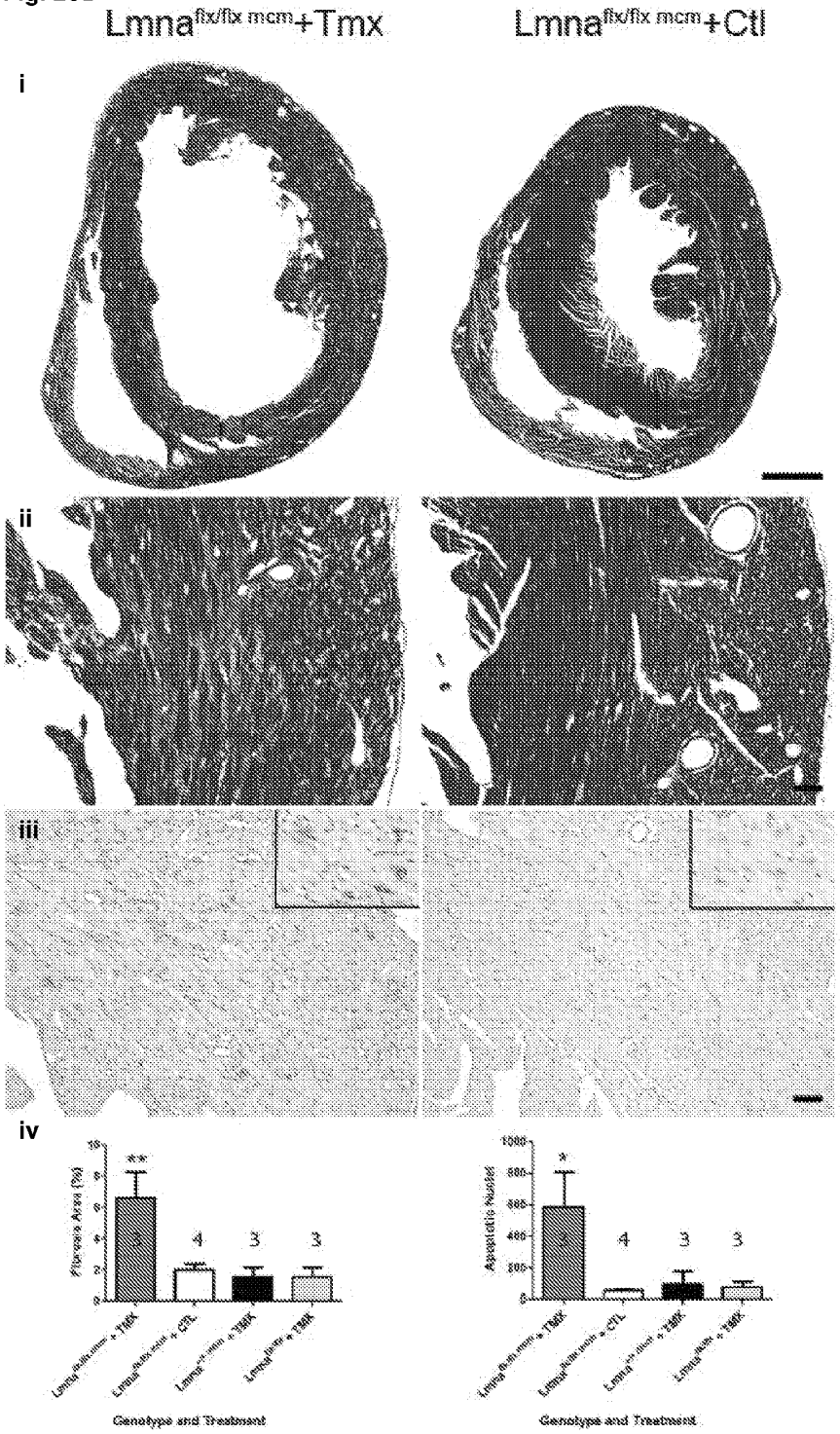

Lmna fix/fix mcm +Tmx
Lmna fix/fix mcm +Ctl
i
ii
iii
iv
Fibrosis Area (%)
Apoptotic Nuclei
4
3
3
Genotype and Treatment
Genotype and Treatment

Fig. 29A

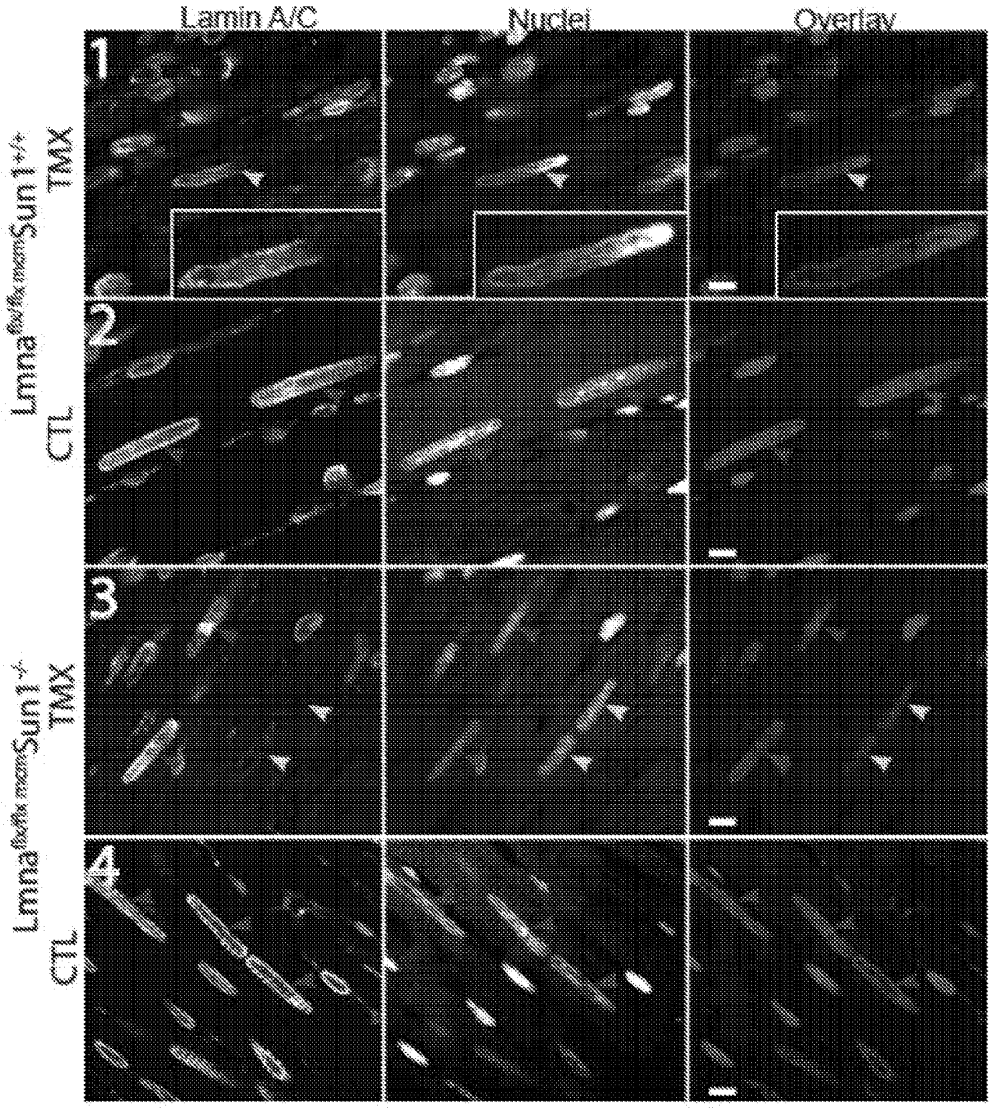
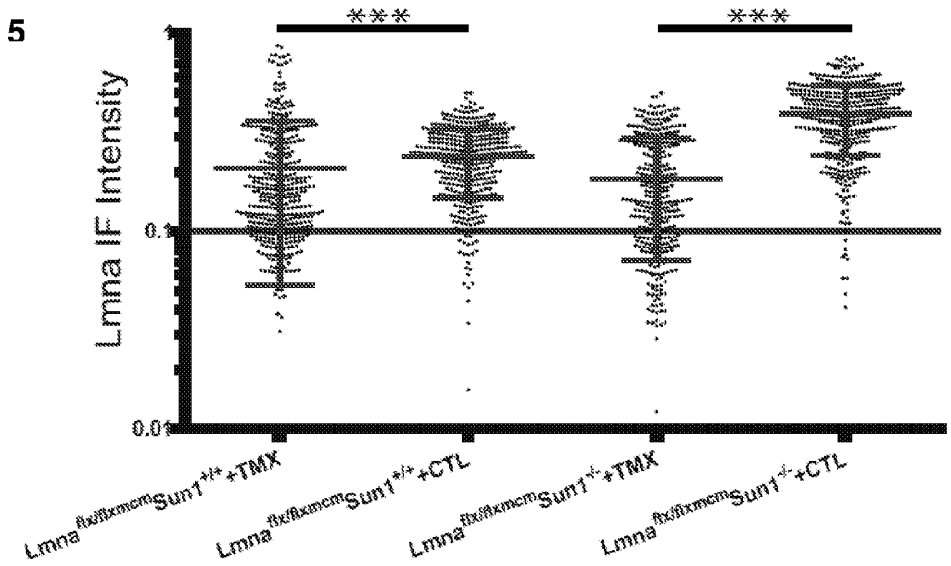

Lamin A/C
Nuclei
Overlay
1
2
3
4
Lmna flox/flox mcmSun1 +/+
TMX
CTL
Lmna flox/flox mcmSun1 -/-
TMX
CTL

Fig. 29A (continued)



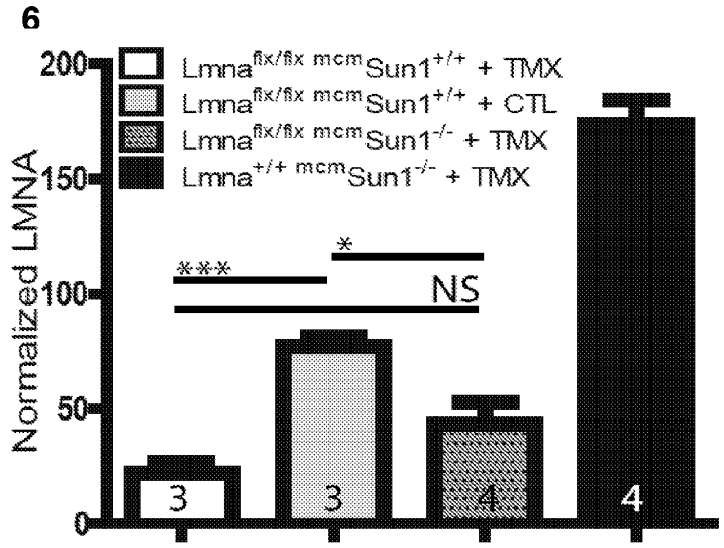


Fig. 29B

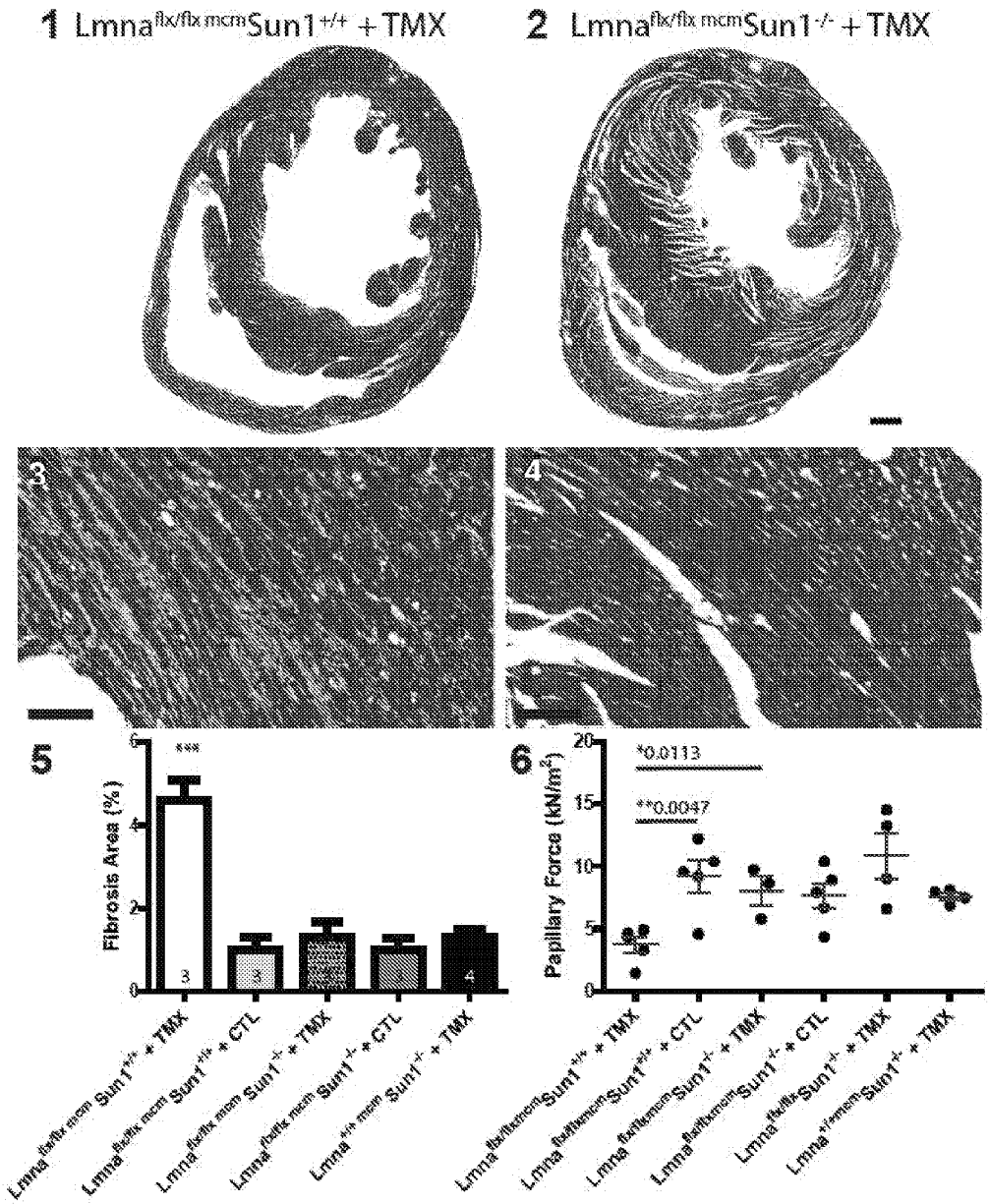

1 Lmna flx/flx mcm Sun1 +/+ + TMX
2 Lmna flx/flx mcm Sun1 -/- + TMX
3
4
5
Fibrosis Area (%)
***
6
Papillary Force (kN/m²)
*0.0113
**0.0047
Lmna flx/flx mcm Sun1 +/+ + TMX
Lmna flx/flx mcm Sun1 +/+ + CTL
Lmna flx/flx mcm Sun1 -/- + TMX
Lmna flx/flx mcm Sun1 -/- + CTL
Lmna +/+ mcm Sun1 -/- + TMX

Fig. 29C

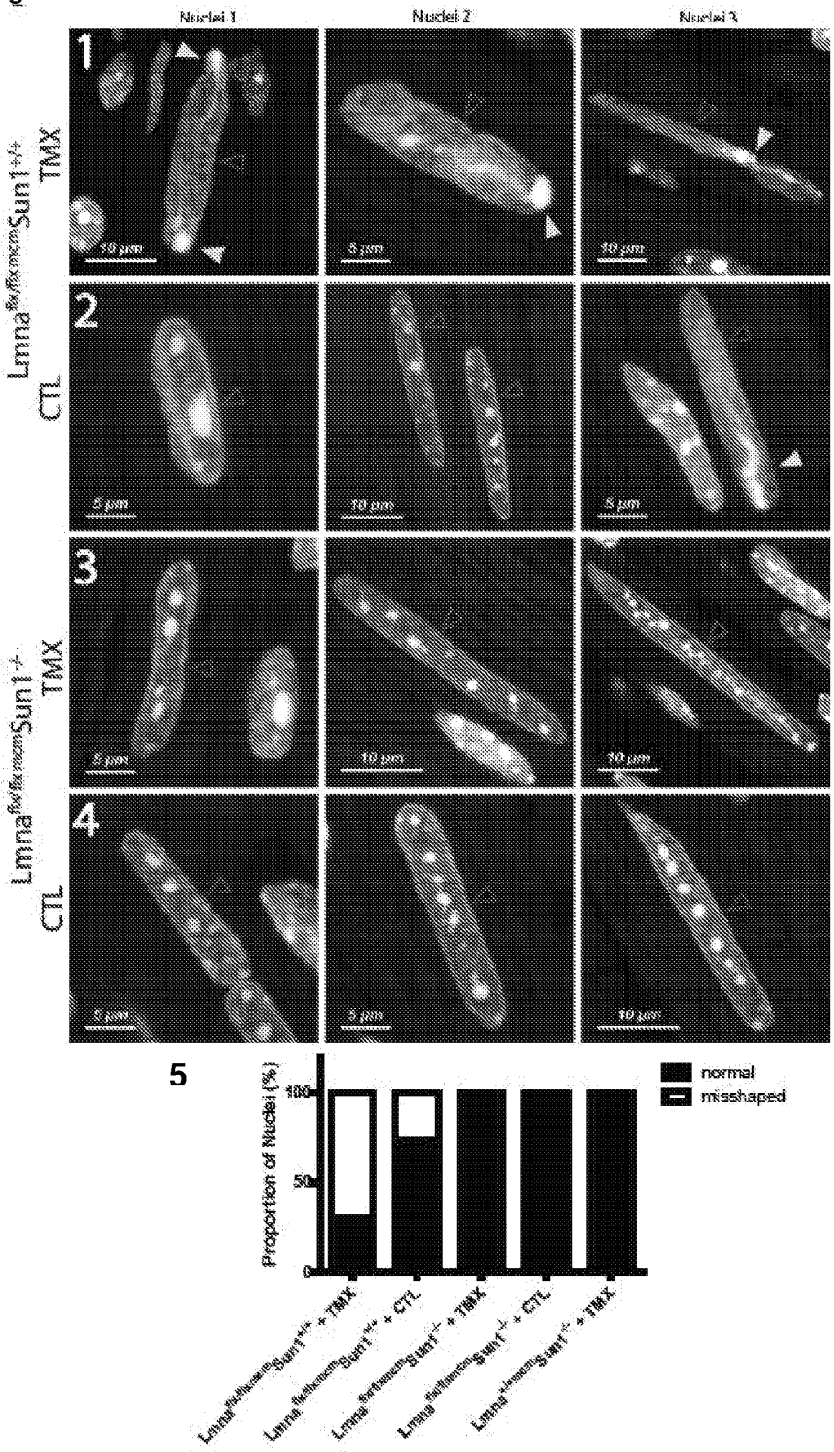

Nuclei 1
Nuclei 2
Nuclei 3
1
2
3
4
5
TMX
CTL
TMX
CTL
10 µm
5 µm
Proportion of Nuclei (%)
100
50
0
normal
misshaped
Lmna+/flox-mcm Sun1+/+ + TMX
Lmna+/flox-mcm Sun1+/+ + CTL
Lmna+/flox-mcm Sun1-/- + TMX
Lmna+/flox-mcm Sun1-/- + CTL
Lmna+/mcm Sun1-/- + TMX

Fig. 29D

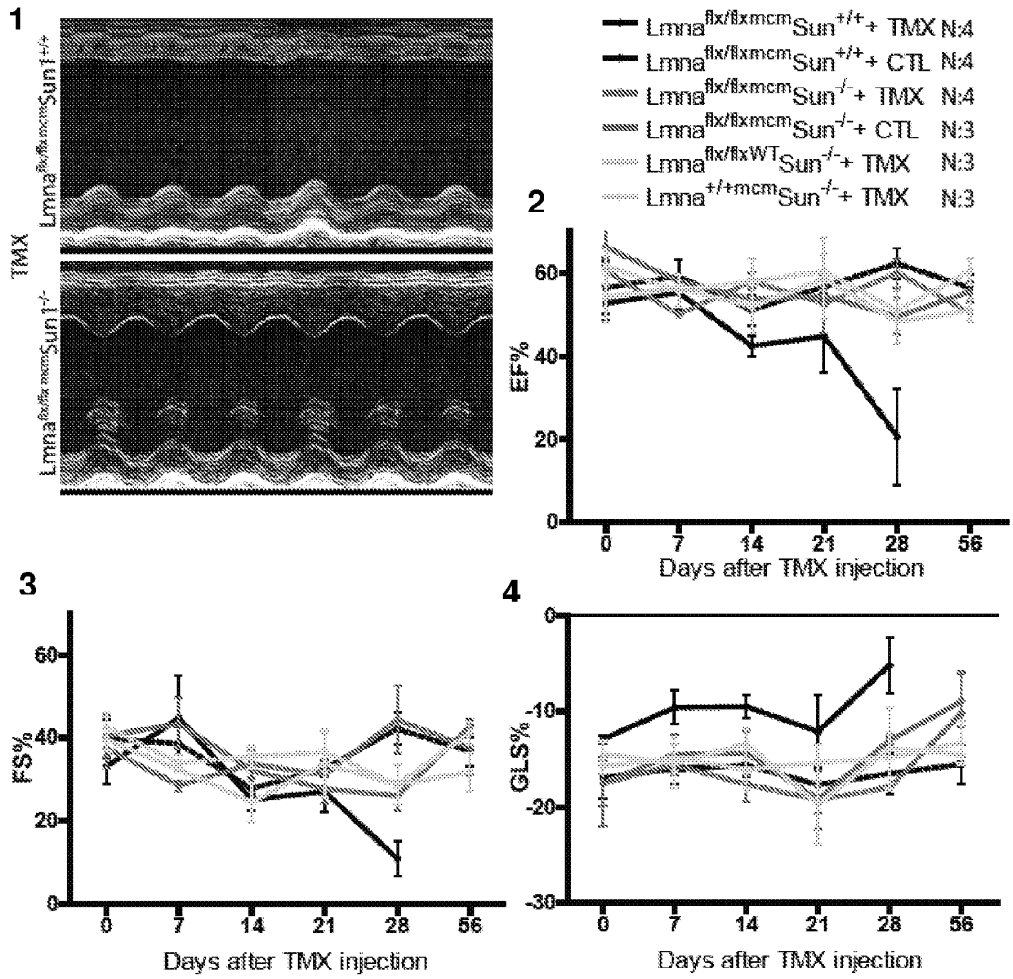

1
TMX
Lmna flx/flxmcm Sun 1+/+
Lmna flx/flxmcm Sun 1-/-
2
Lmna flx/flxmcm Sun +/+ + TMX N:4
Lmna flx/flxmcm Sun +/+ + CTL N:4
Lmna flx/flxmcm Sun -/- + TMX N:4
Lmna flx/flxmcm Sun -/- + CTL N:3
Lmna flx/flxWT Sun -/- + TMX N:3
Lmna +/+mcm Sun -/- + TMX N:3
EF%
Days after TMX injection
3
FS%
Days after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

Fig. 30A

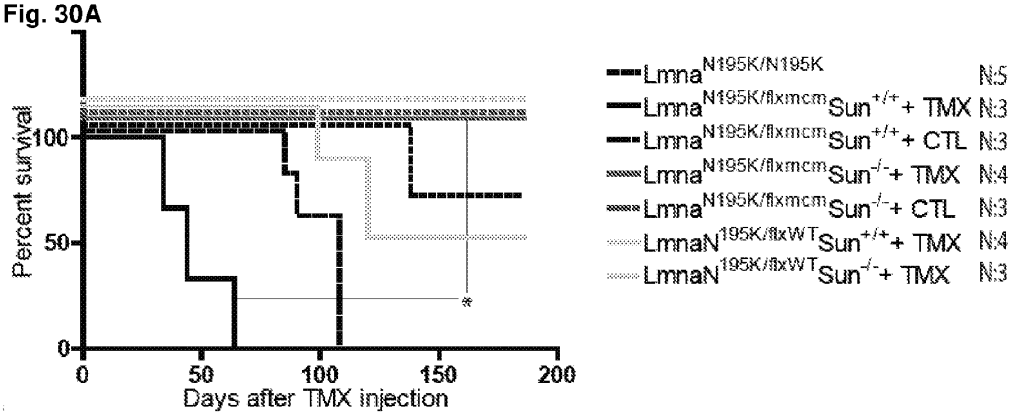


Fig. 30B

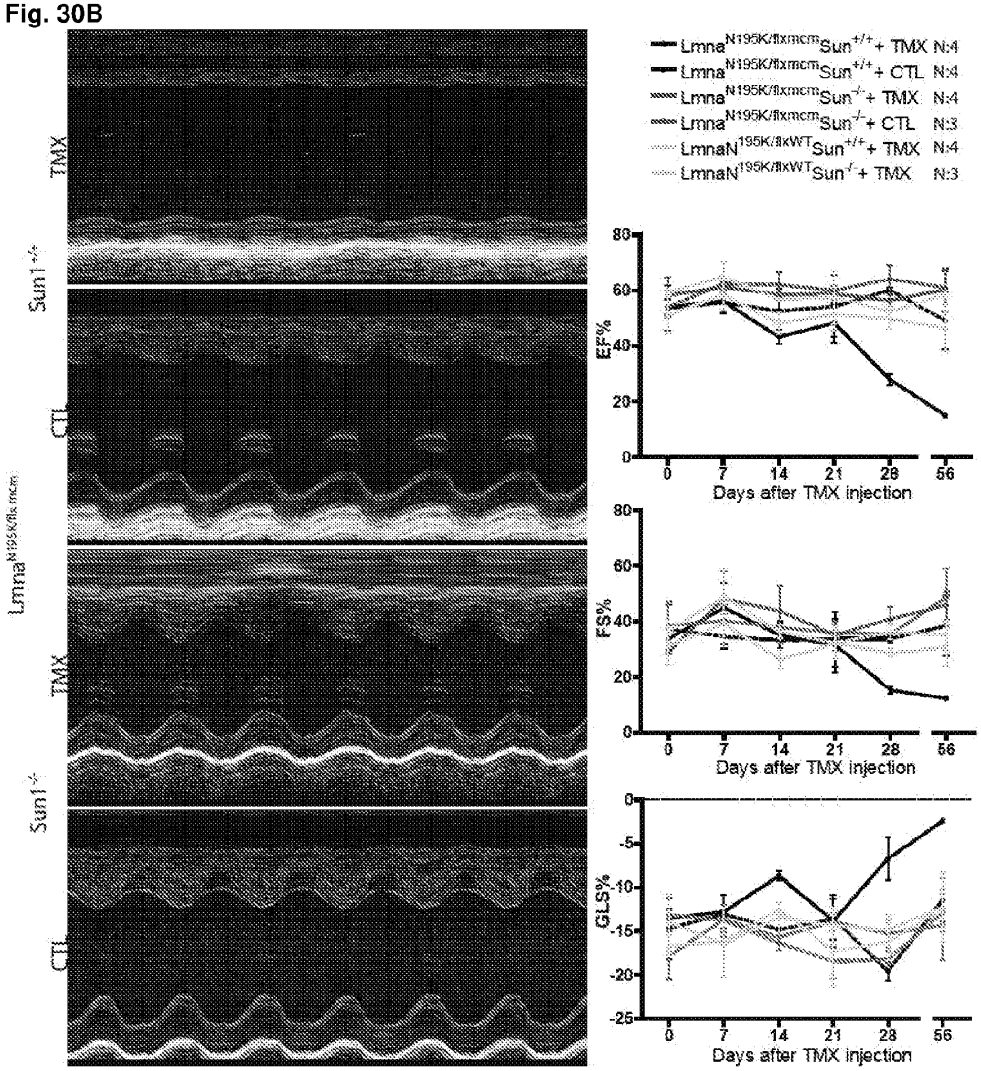


Fig. 31A

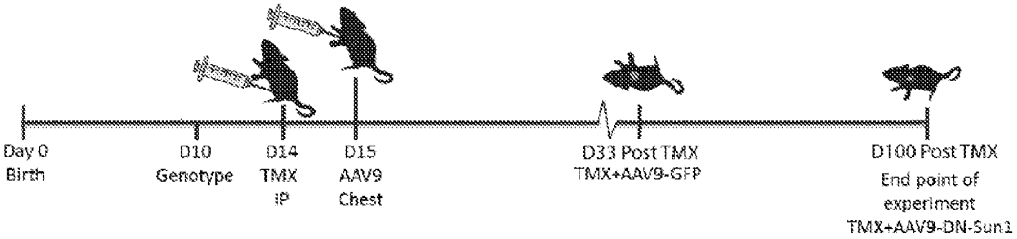


Fig. 31B

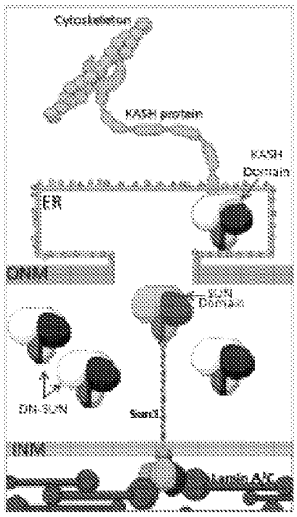


Fig. 31C

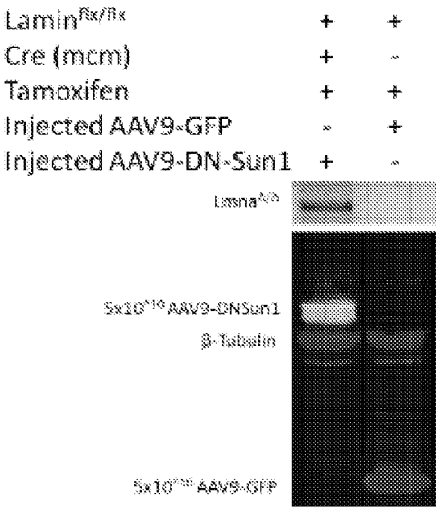


Fig. 31D

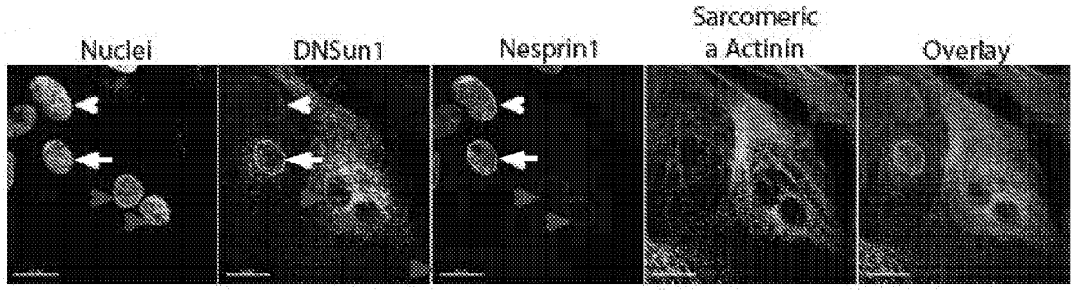


Fig. 31E (i)

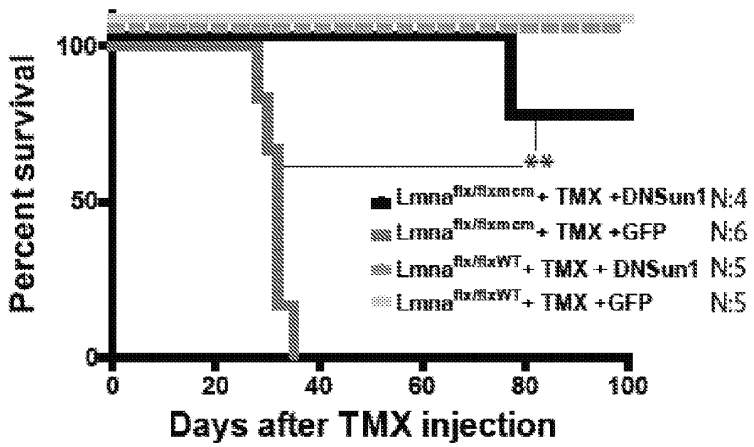


Fig. 31E (ii)

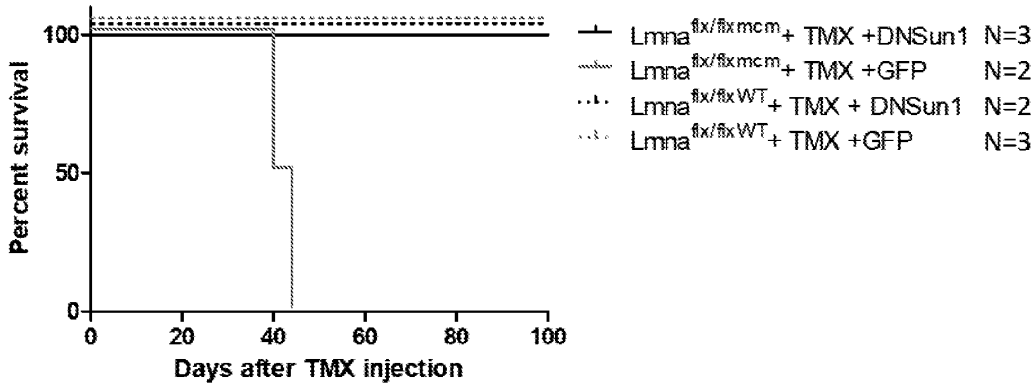


Fig. 31F

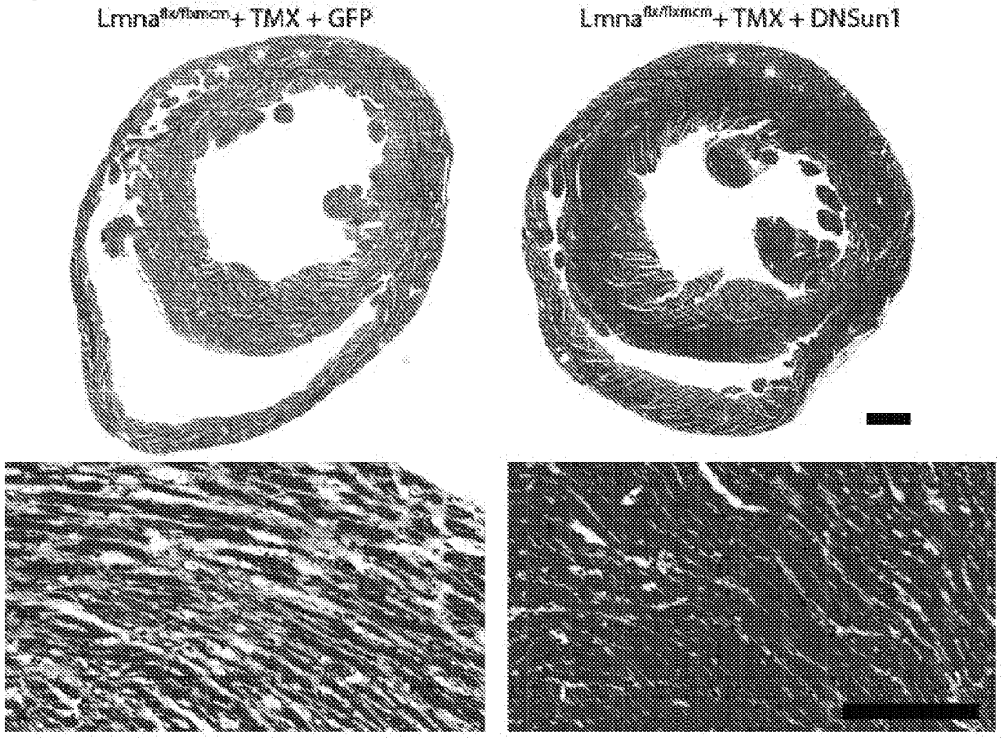


Fig. 31G

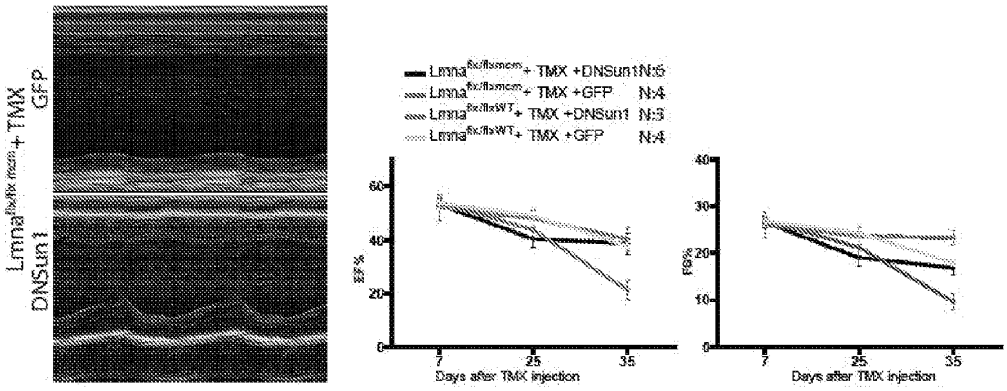


Fig. 32A

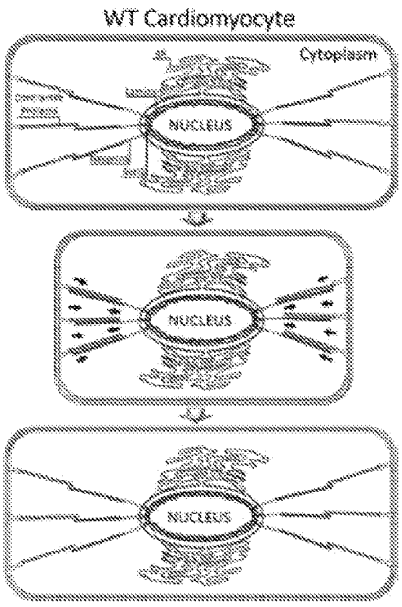


Fig. 32B

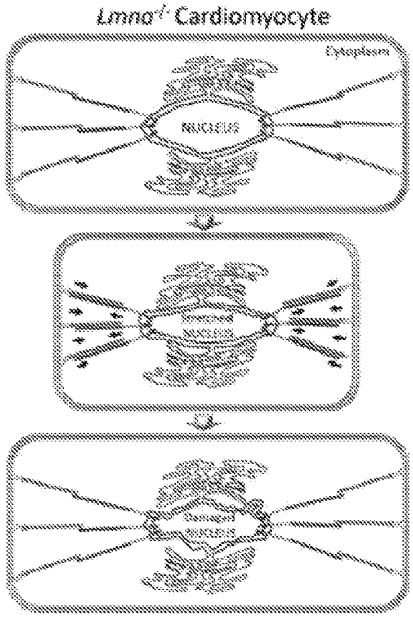


Fig. 32C

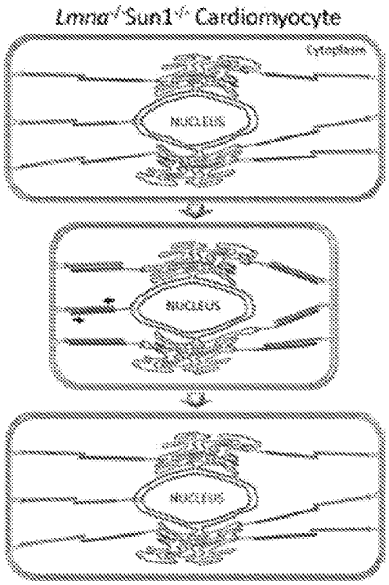


Fig. 32D

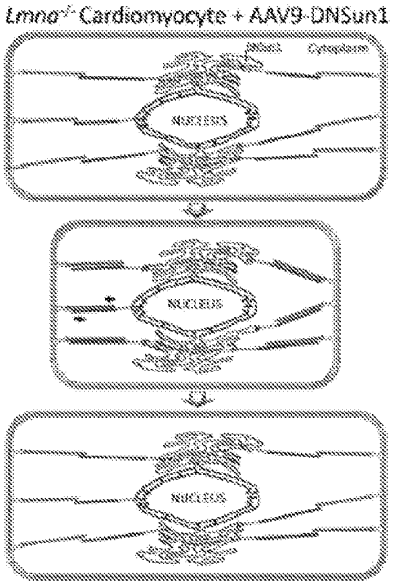


Fig. 33

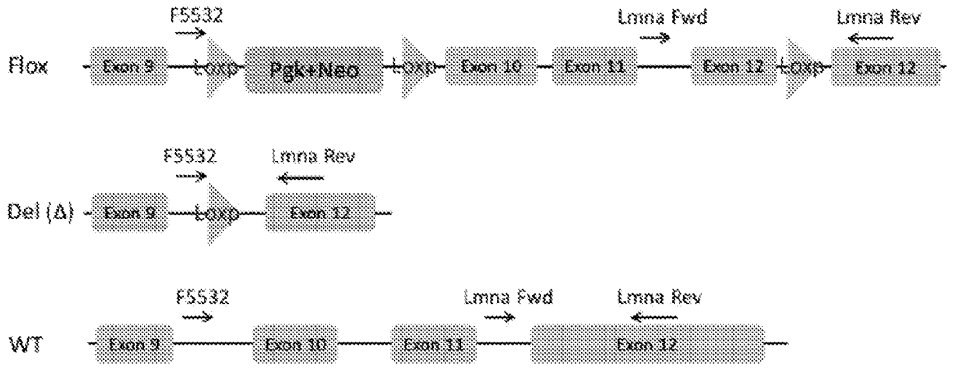


Fig. 34

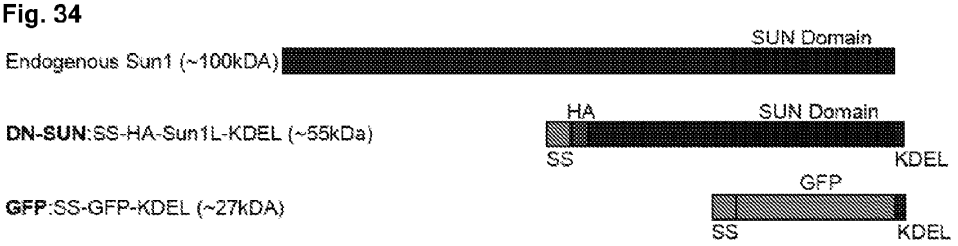


Fig. 35

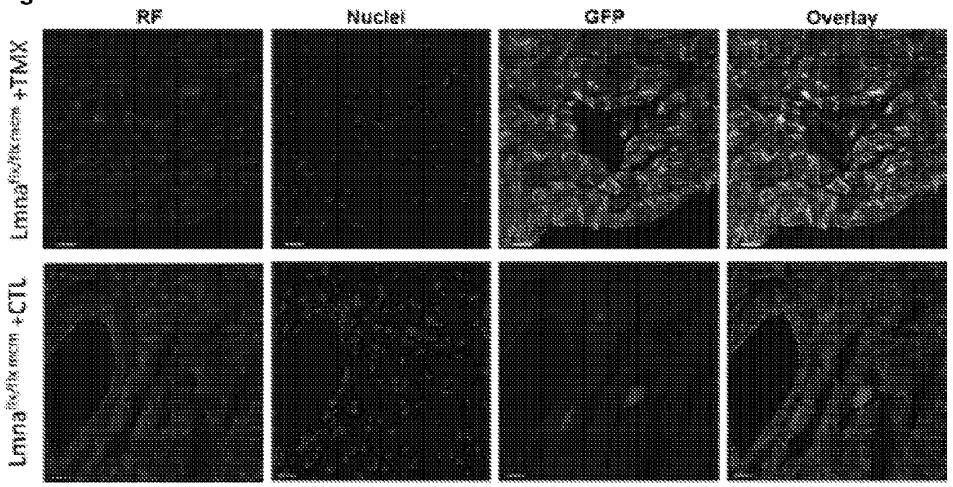
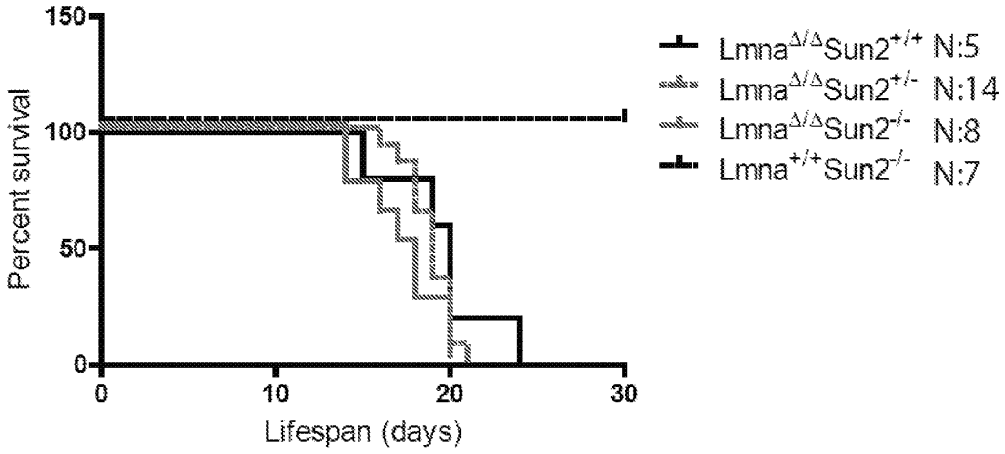


Fig. 36


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

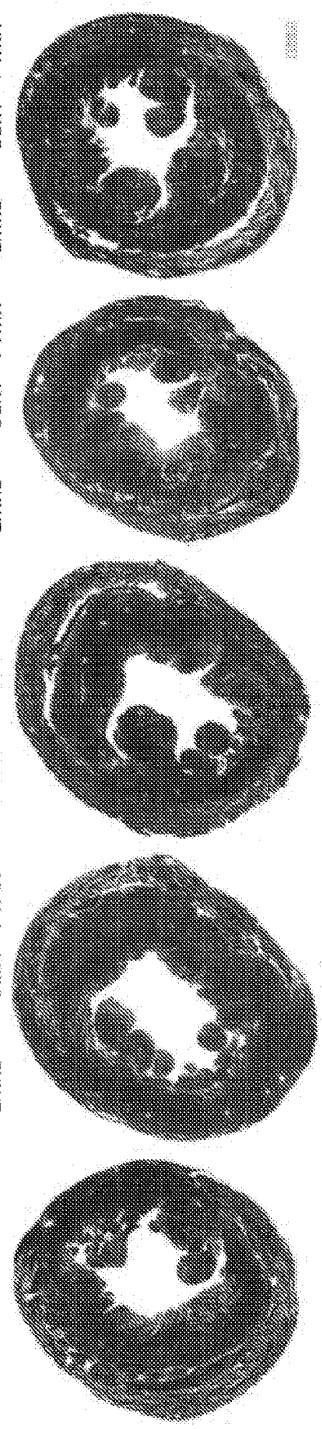

Lmna$^{+/+ \text{ nicm}}$Sun1$^{-/-}$ + TMX
Lmna$^{flox/flox\ WT}$Sun1$^{-/-}$ + TMX
Lmna$^{flox/flox\ nicm}$Sun1$^{-/-}$ + CTL
Lmna$^{flox/flox\ nicm}$Sun1$^{-/-}$ + TMX

Fig. 37A

Fig. 37C

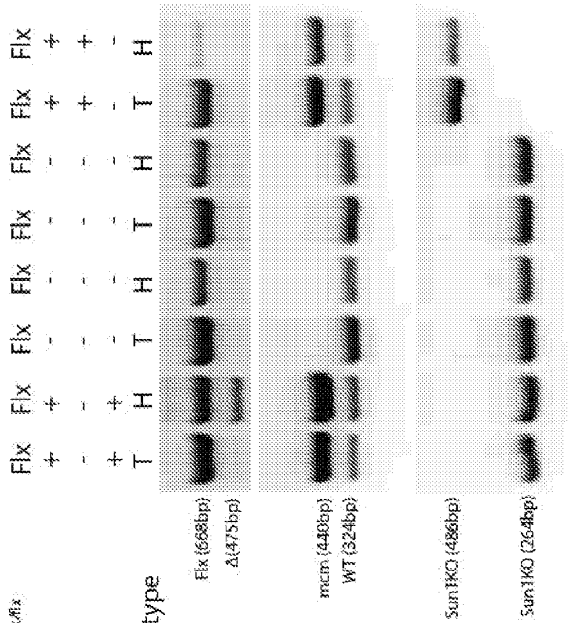


Fig. 37B

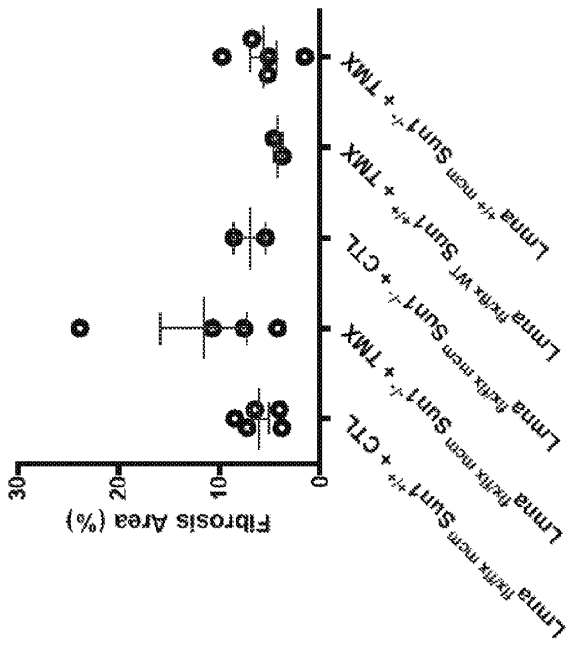


Fig. 37D

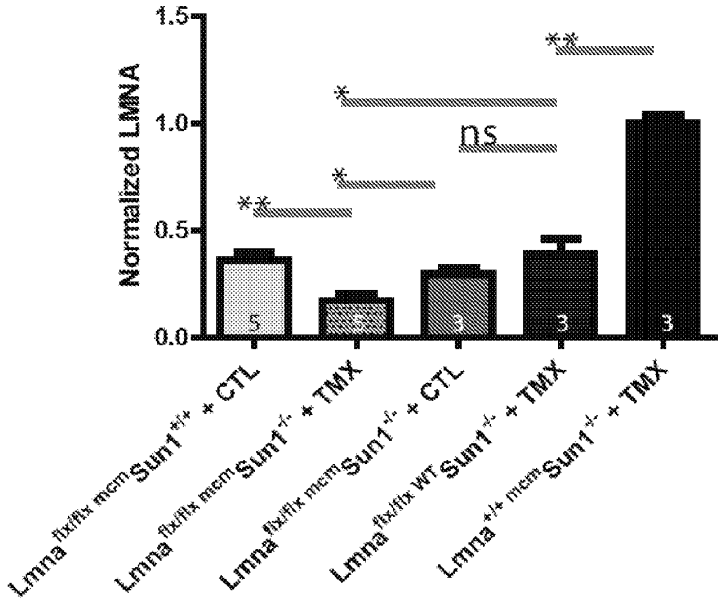

Normalized LMNA
1.5
1.0
0.5
0.0
**
*
*
ns
**
5
5
3
3
3
Lmna flx/flx mem Sun1 +/+ + CTL
Lmna flx/flx mem Sun1 -/- + TMX
Lmna flx/flx mem Sun1 -/- + CTL
Lmna flx/flx WT Sun1 -/- + TMX
Lmna +/+ mem Sun1 -/- + TMX

Fig. 37E

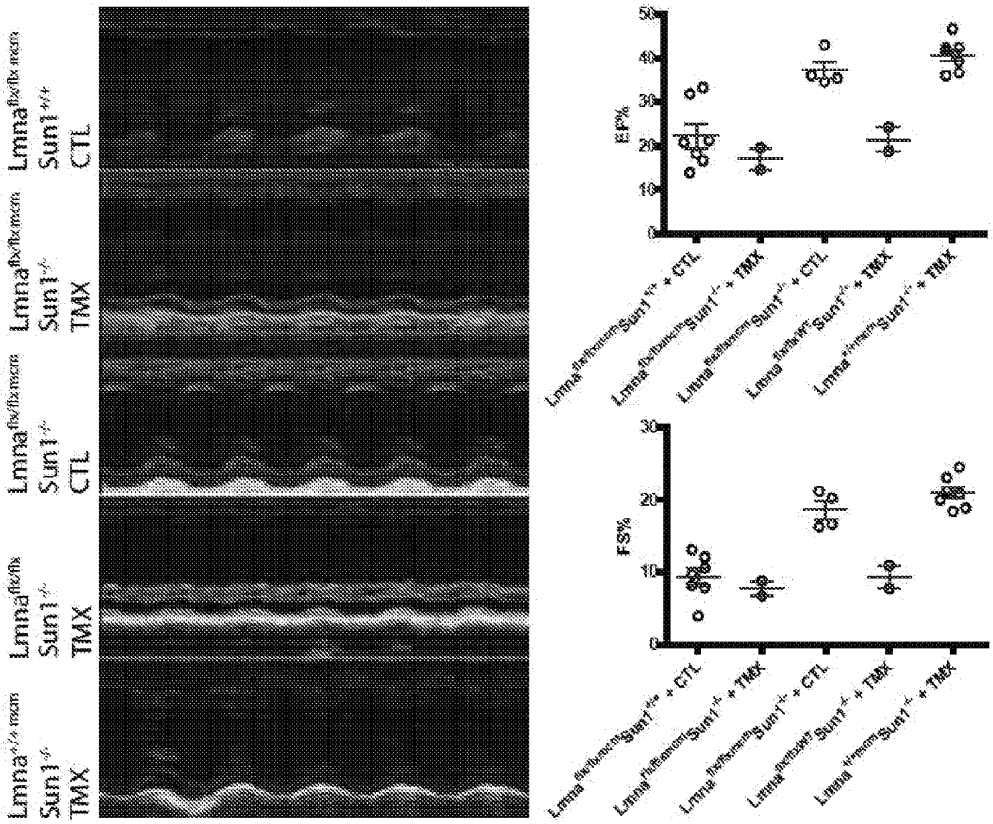

Lmna fl/fl mcm Sun1 +/+ CTL
Lmna fl/fl mcm Sun1 -/- TMX
Lmna fl/fl mcm Sun1 -/- CTL
Lmna fl/fl Sun1 -/- TMX
Lmna +/+ mcm Sun1 -/- TMX
EF%
FS%

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

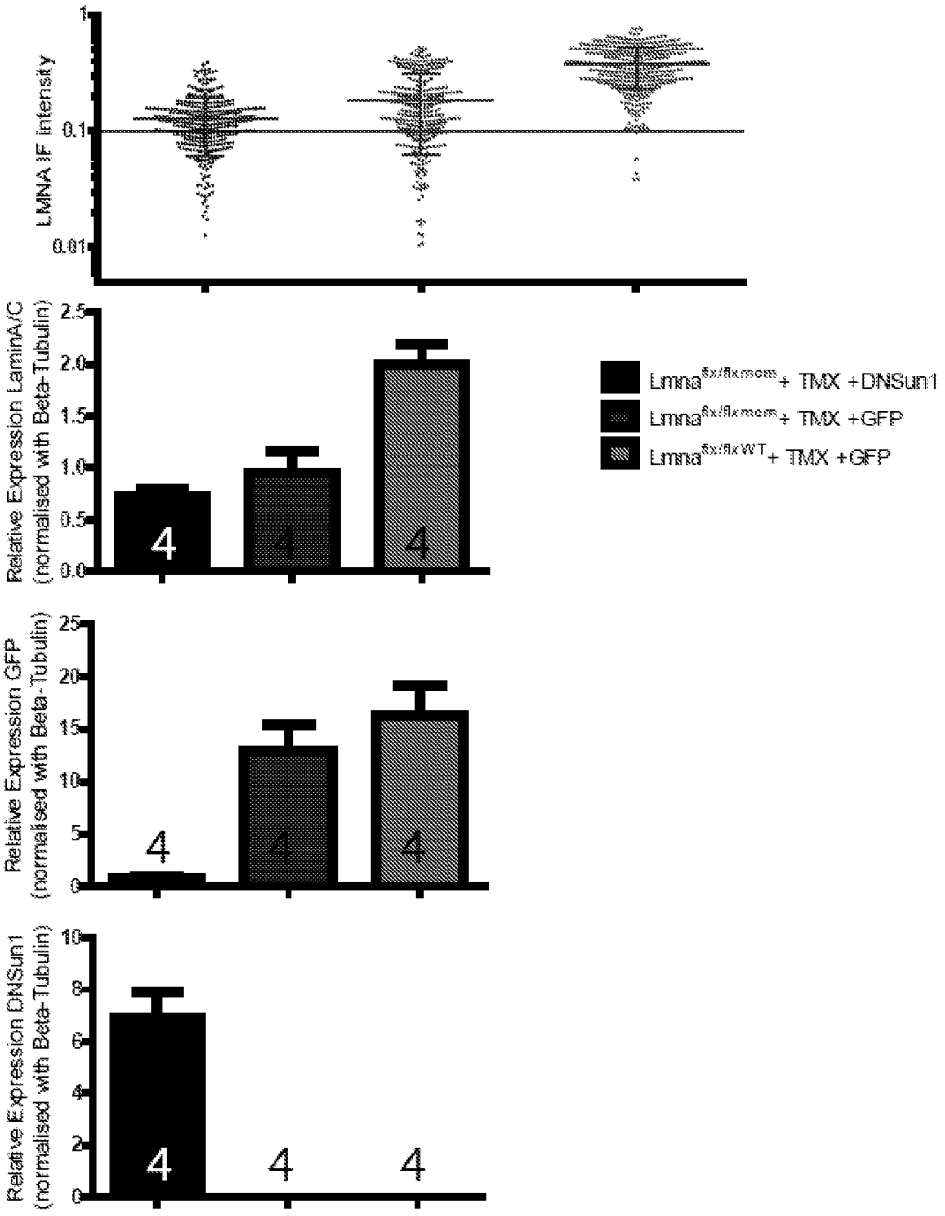

LMNA IF intensity
1
0.1
0.01
Relative Expression LaminA/C (normalised with Beta-Tubulin)
2.5
2.0
1.5
1.0
0.5
0.0
4
4
4
Lmna$^{fx/fxmom}$ + TMX +DNSun1
Lmna$^{fx/fxmom}$ + TMX +GFP
Lmna$^{fx/fxWT}$ + TMX +GFP
Relative Expression GFP (normalised with Beta-Tubulin)
25
20
15
10
5
0
4
4
4
Relative Expression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

Fig. 39B

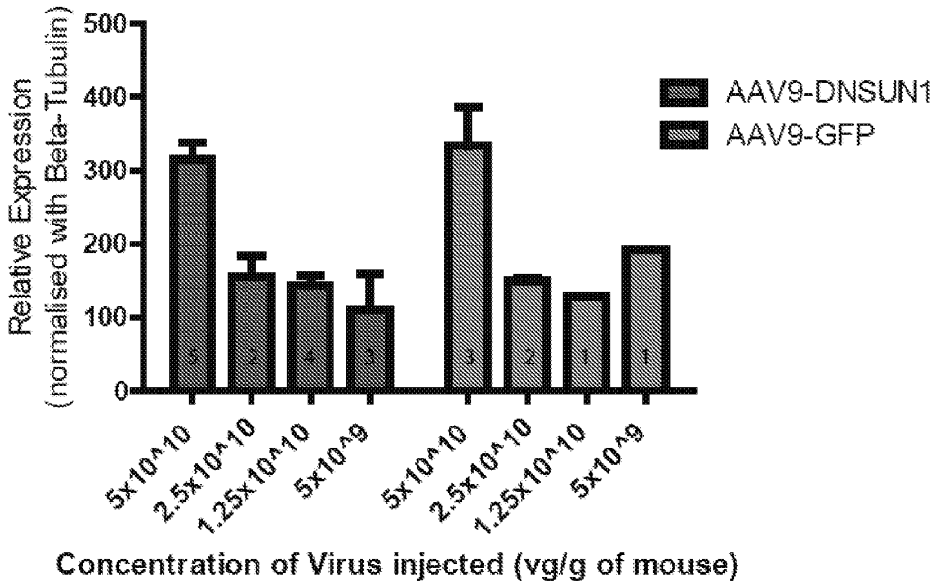


Fig. 39C

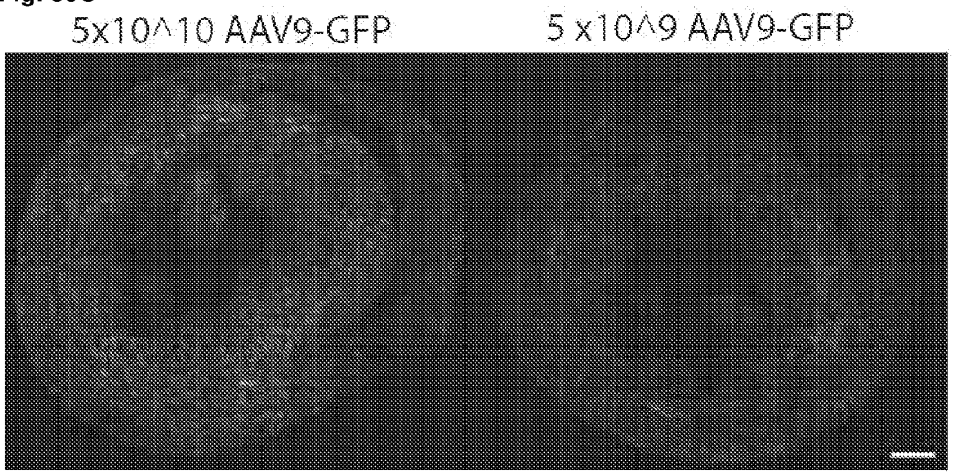


Fig. 40A

```
SEQ ID NO:69  wildtype   CATGACCTTGAGCTGAAACTGCTGCAGAATATCACACACCACATCACCGTGACAGGACAG  2100
SEQ ID NO:70  Sun1_plus4 CATGACCTTGAGCTGAAACTGCTGCAGAATATCACACACCACATCACCGTGACAGGACAG  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  Sunl_plus4  WLLEKLSSRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPRHIRGYCVCRESGRDFRN  720
SEQ ID NO:74  Sunl_del7  WLLEKLSSRFVSKDELQVLLHDLELKLLQNITHHITVTGQAPRLLCLP---*---------  708
                         ********************************************

SEQ ID NO:72  wildtype  EAQAHIIVNNALKLYSQDKTGMVDFALESGGGSILSTPCSETYETKTALLSLFGVPLWYF  780
SEQ ID NO:73  Sunl_plus4  --------HRSASTYHCEQCSEAVLPRQDGDGGL---C-----------------SGVWRW  753
SEQ ID NO:74  Sunl_del7  ----------------------------------------------------------  708

SEQ ID NO:72  wildtype  SQSPRVVIQPDIYPGNCWAFKGSQGYLVVRLSMKIYPTTFTMEHIPKTLSPTGNISSAPK  840
SEQ ID NO:73  Sunl_plus4  QHPKHSVL*-------------------------------------------------  761
SEQ ID NO:74  Sunl_del7  ..........................................................  708

SEQ ID NO:72  wildtype  DFAVYGLETEYQEEGQPLGRFTYDQEGDSLQMFHTLERPDQAFQIVELRVLSNWGHPEYT  900
SEQ ID NO:73  Sunl_plus4  ----------------------------------------------------------  761
SEQ ID NO:74  Sunl_del7  ----------------------------------------------------------  708

SEQ ID NO:72  wildtype  CLYRFRVHGEPIQ*  913
SEQ ID NO:73  Sunl_plus4  -------------  761
SEQ ID NO:74  Sunl_del7  -------------  708
```

Fig. 40C

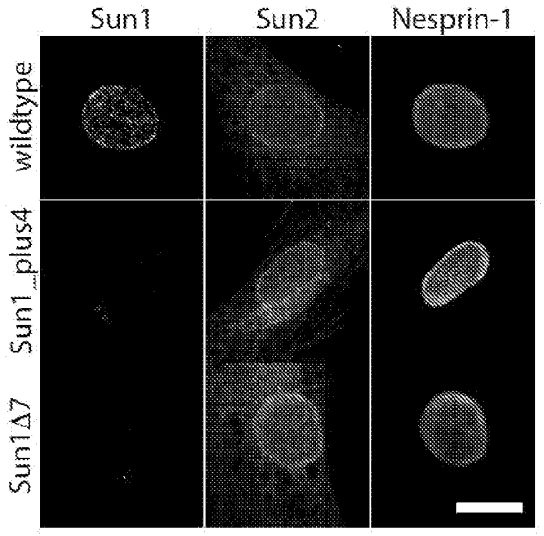


Fig. 41

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
                            ************************************************************
```

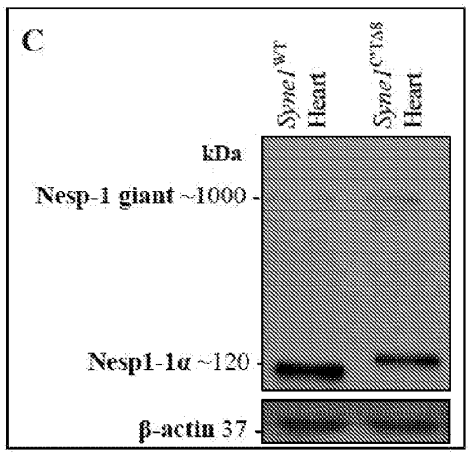
B

```
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)



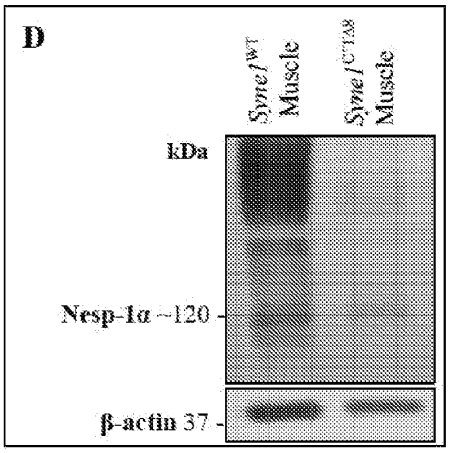


Fig. 42

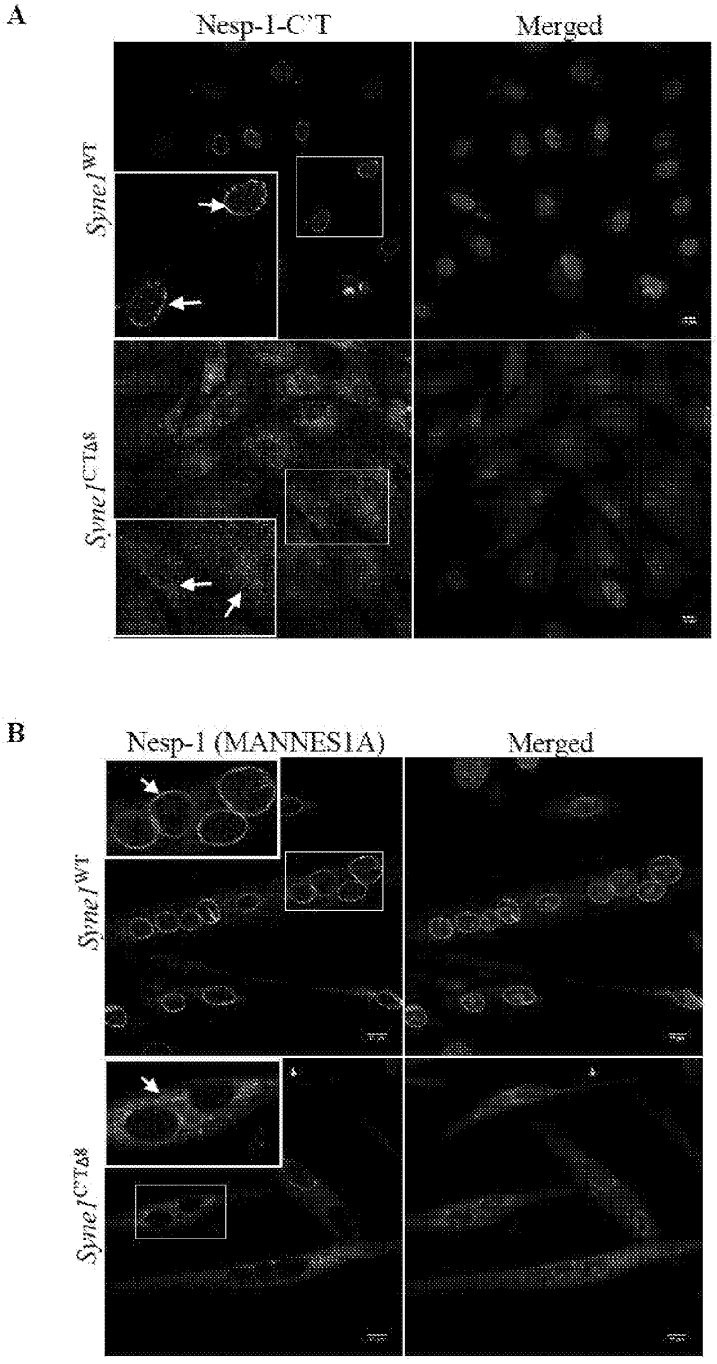

A
Nesp-1-C'T
Merged
Syne1WT
Syne1CTΔ8
B
Nesp-1 (MANNES1A)
Merged
Syne1WT
Syne1CTΔ8

Fig. 43

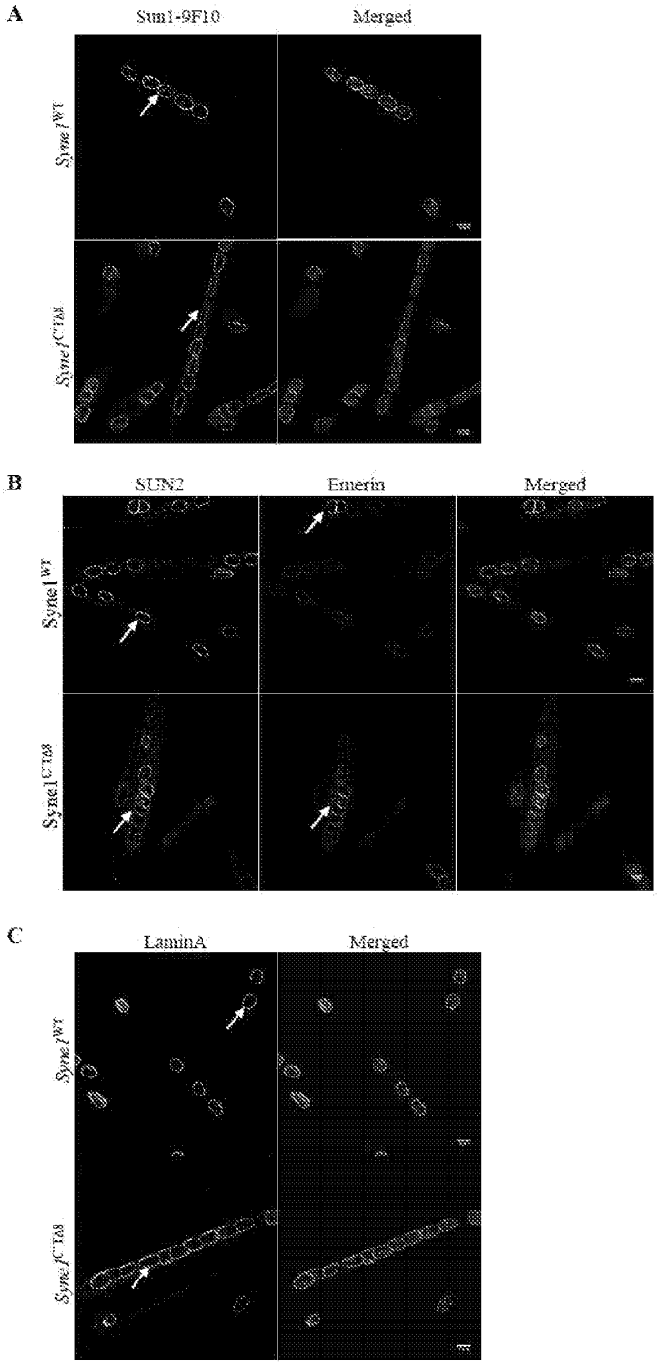

A
Sun1-9F10
Merged
Syne1WT
Syne1CTΔ8
B
SUN2
Emerin
Merged
Syne1WT
Syne1CTΔ8
C
LaminA
Merged
Syne1WT
Syne1CTΔ8

Fig. 44

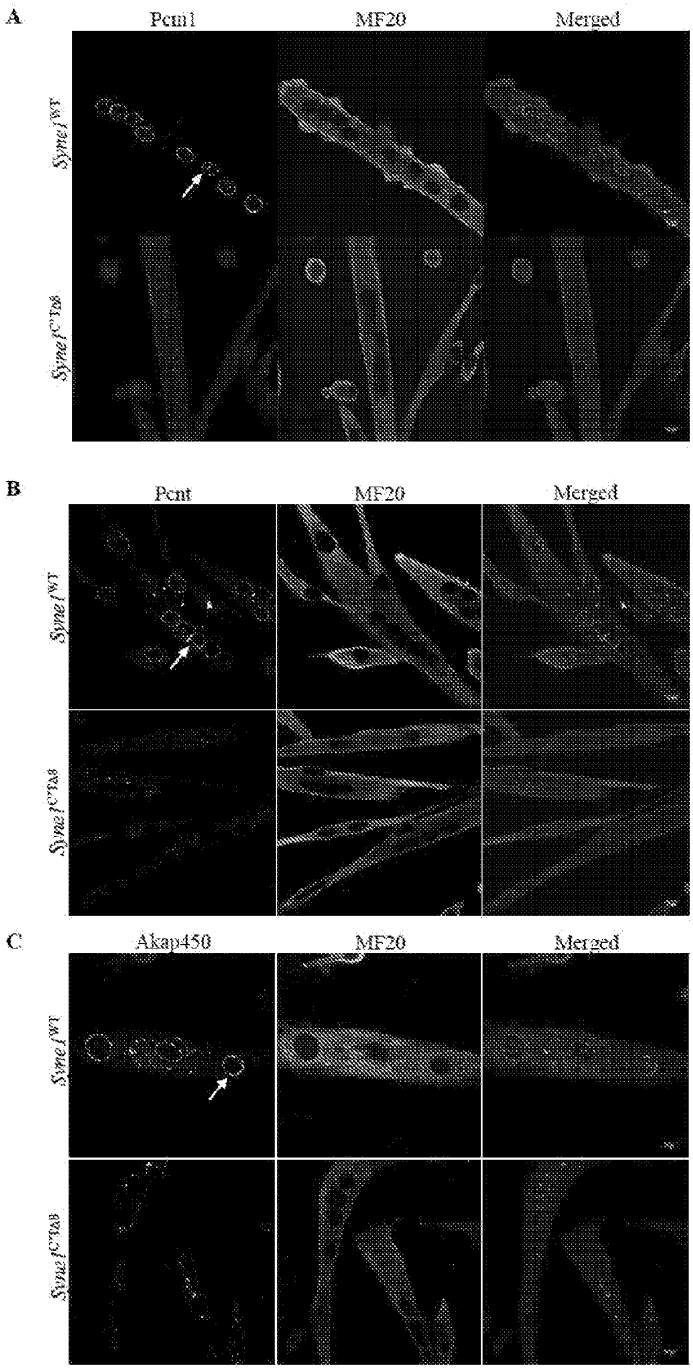
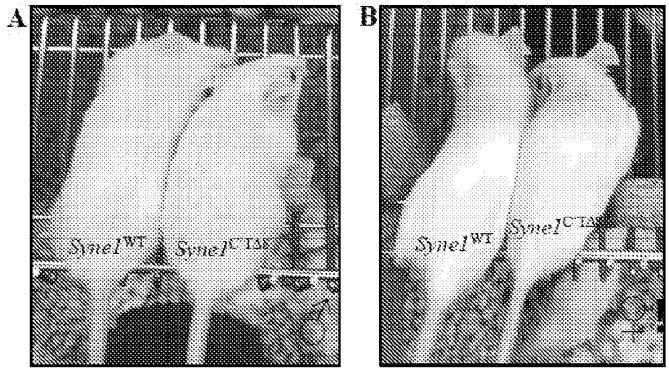

A
Pcm1
MF20
Merged
Syne1WT
Syne1CTa8
B
Pcnt
MF20
Merged
Syne1WT
Syne1CTa8
C
Akap450
MF20
Merged
Syne1WT
Syne1CTa8

Fig. 45


A
B
Syne1WT
Syne1C-TΔ8
Syne1WT
Syne1C-TΔ8

C

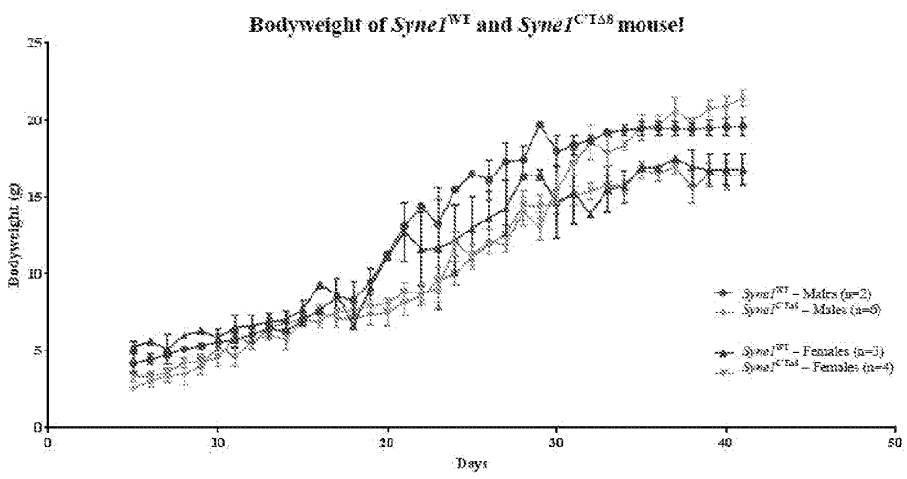

Bodyweight of Syne1WT and Syne1C-TΔ8 mouse!
Bodyweight (g)
Days
0
5
10
15
20
25
0
10
20
30
40
50
Syne1WT – Males (n=2)
Syne1C-TΔ8 – Males (n=6)
Syne1WT – Females (n=3)
Syne1C-TΔ8 – Females (n=4)

Fig. 46A

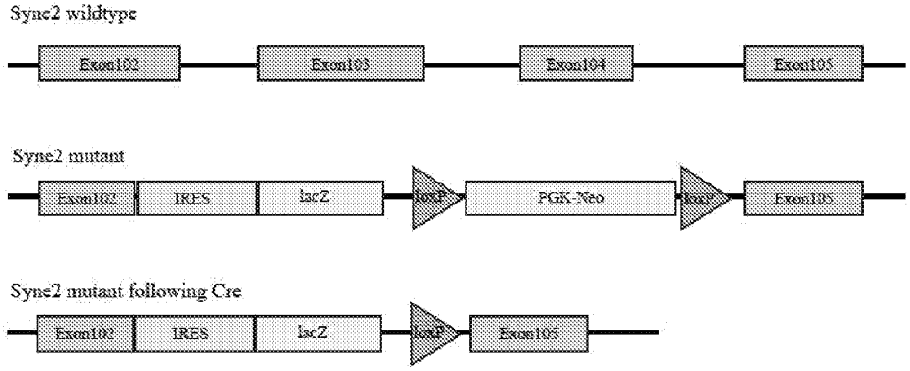


Fig. 46B

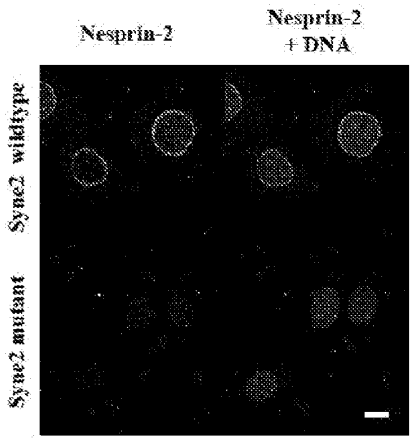


Fig. 46C

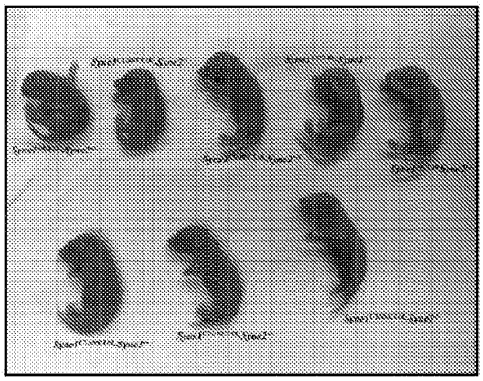
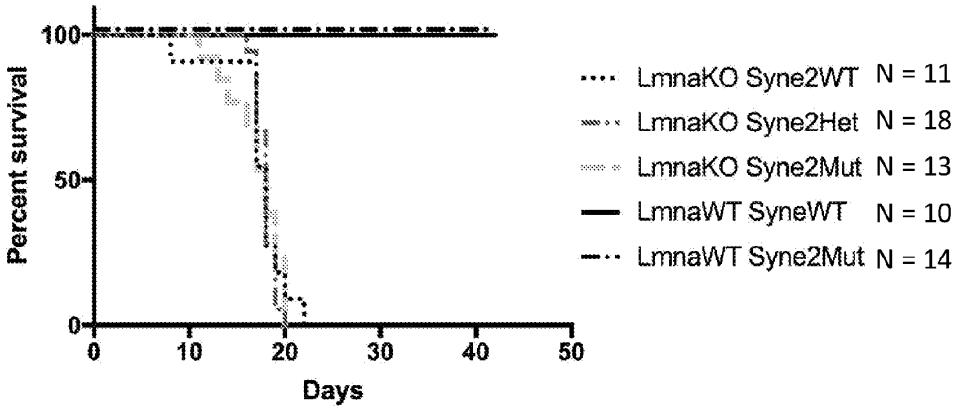


Fig. 47


100
50
0
Percent survival
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