Fig. 1

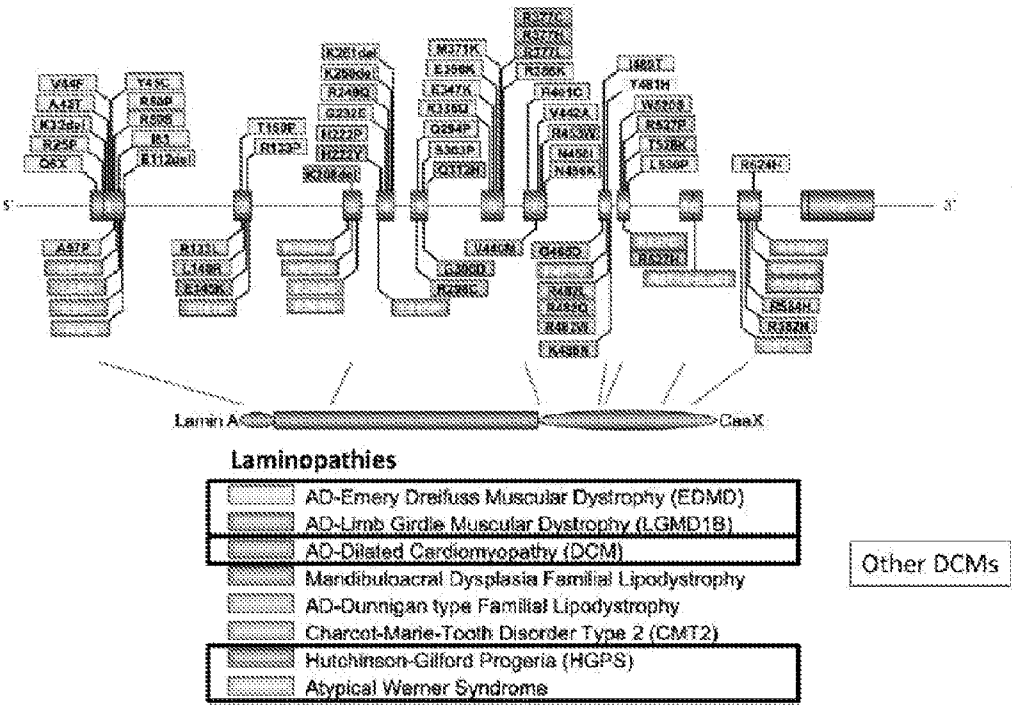

Lamin A
CaaX
Laminopathies
AD-Emery Dreifuss Muscular Dystrophy (EDMD)
AD-Limb Girdle Muscular Dystrophy (LGMD1B)
AD-Dilated Cardiomyopathy (DCM)
Mandibuloacral Dysplasia Familial Lipodystrophy
AD-Dunnigan type Familial Lipodystrophy
Charcot-Marie-Tooth Disorder Type 2 (CMT2)
Hutchinson-Gilford Progeria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

Fig. 2

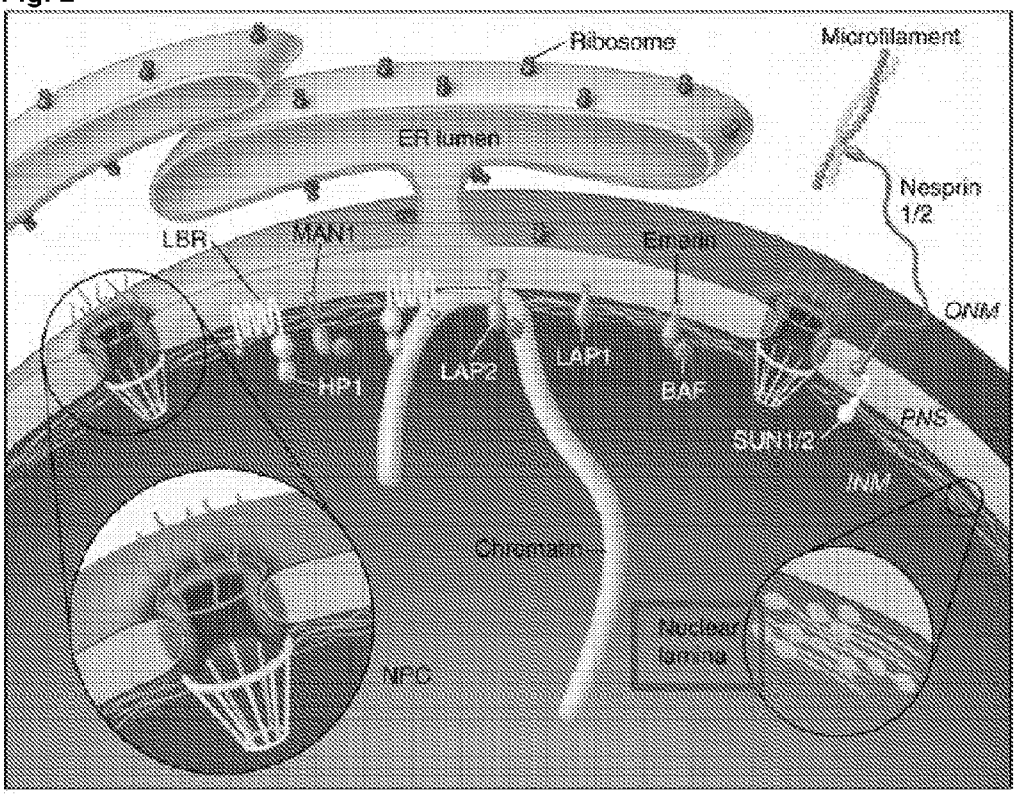

Ribosome
Microfilament
ER lumen
Nesprin 1/2
LBR
MAN1
Emerin
ONM
HP1
LAP2
LAP1
BAF
SUN1/2
PNS
INM
Chromatin
NPC
Nuclear lamina

Fig. 3

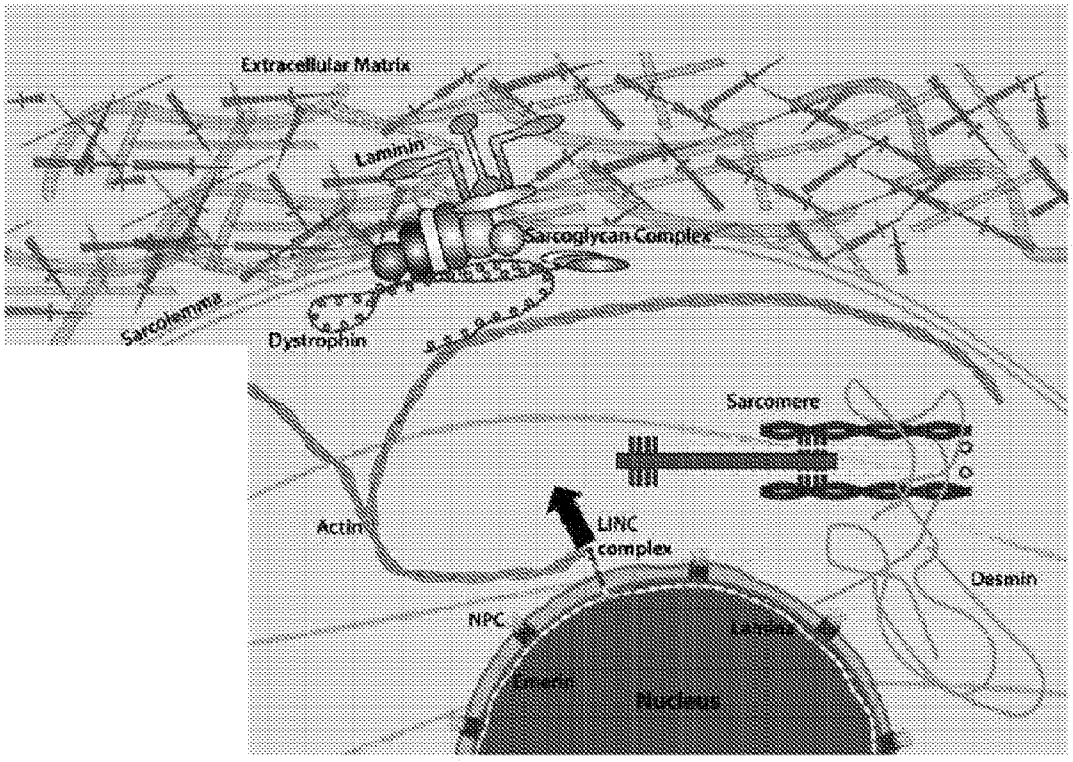

Extracellular Matrix
Laminin
Sarcoglycan Complex
Sarcolemma
Dystrophin
Sarcomere
Actin
LINC complex
Desmin
NPC
Lamina
Nucleus

Fig. 4

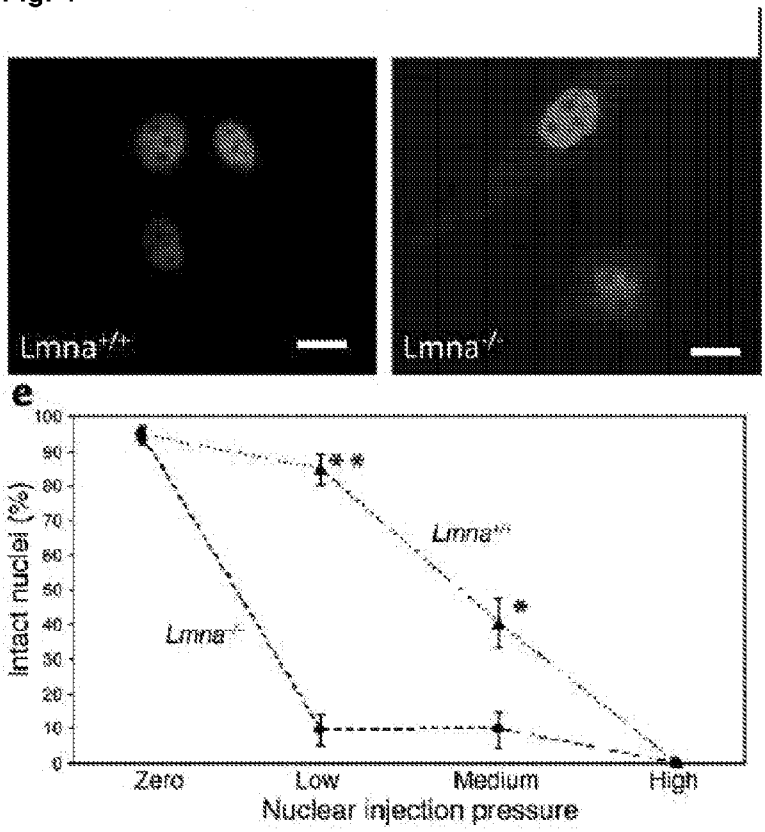

Lmna+/+
Lmna-/-
e
Intact nuclei (%)
Lmna+/+
Lmna-/-
Zero
Low
Medium
High
Nuclear injection pressure

Fig. 5A

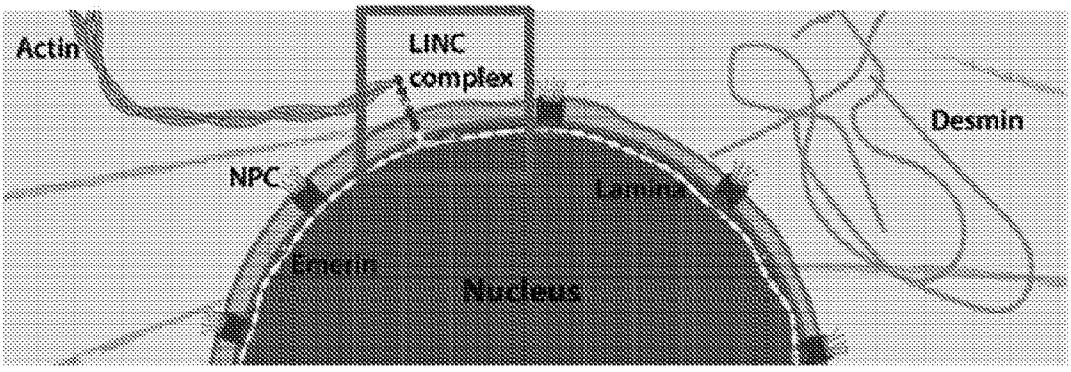

Actin
LINC complex
Desmin
NPC
Lamina
Emerin
Nucleus

Fig. 5B

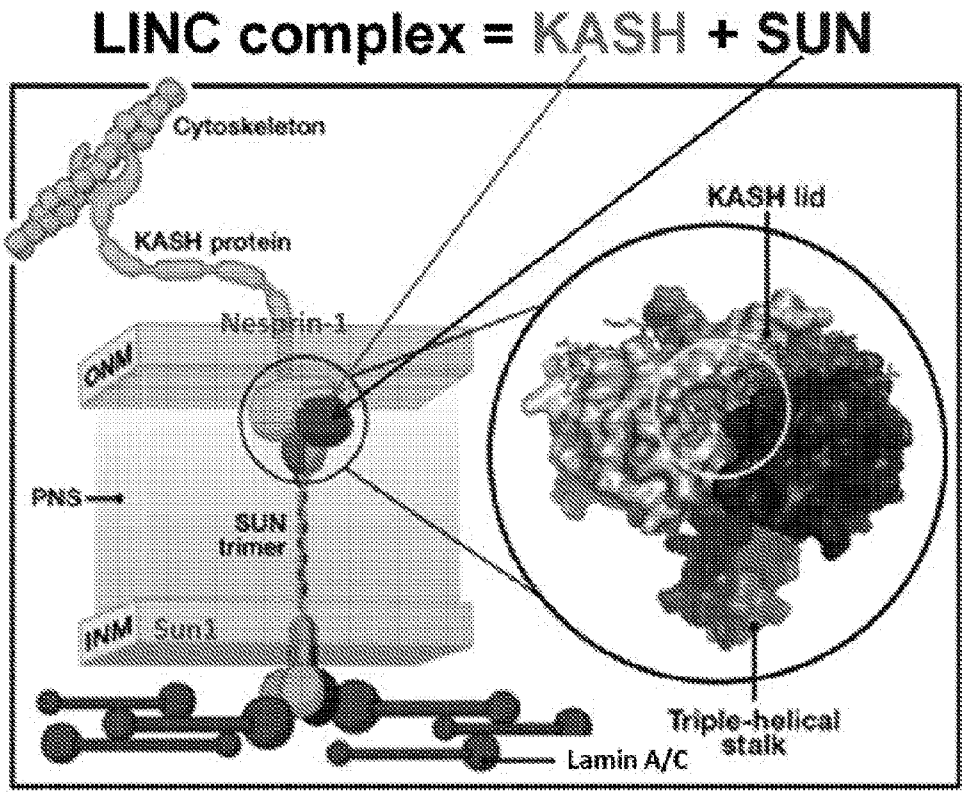

LINC complex = KASH + SUN
Cytoskeleton
KASH protein
KASH lid
Nesprin-1
ONM
PNS
SUN trimer
INM Sun1
Triple-helical stalk
Lamin A/C

Fig. 6A

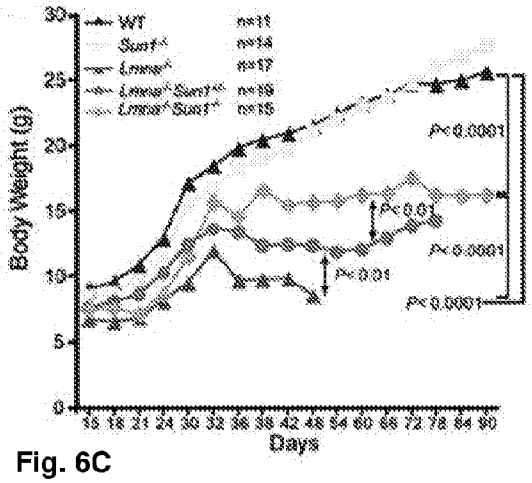


Fig. 6B

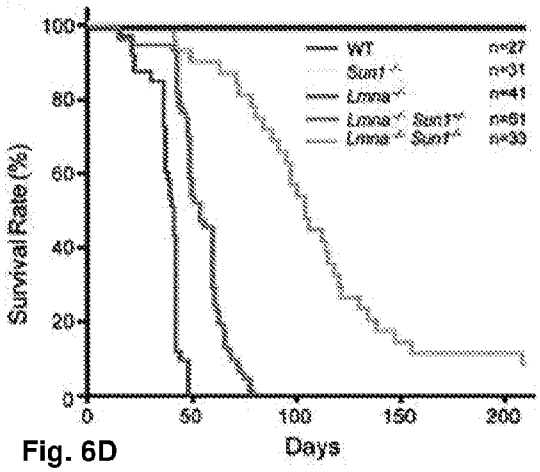


Fig. 6C

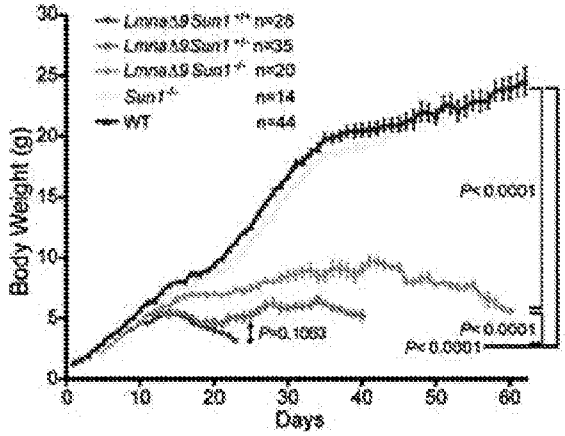


Fig. 6D

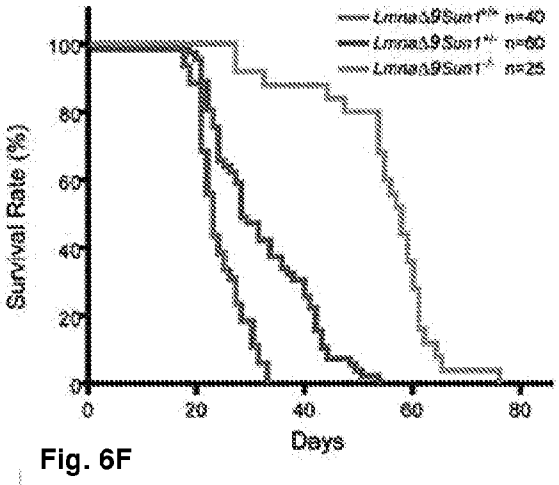


Fig. 6E

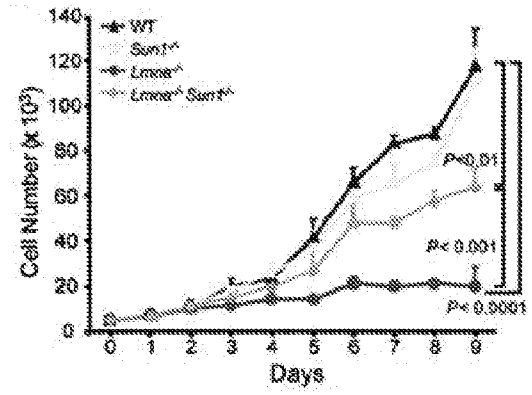


Fig. 6F

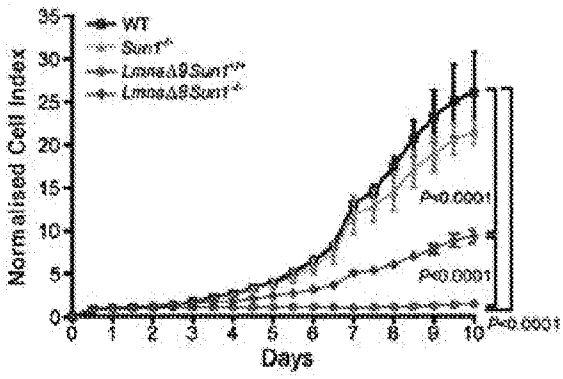


Fig. 7

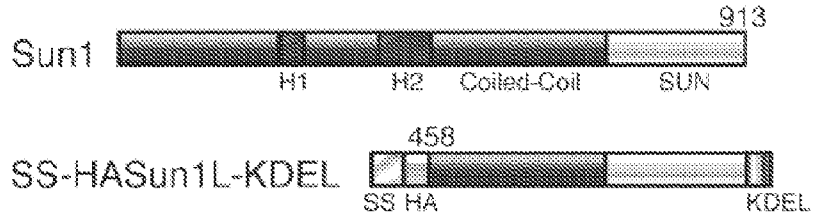

913
Sun1
H1
H2
Coiled-Coil
SUN
458
SS-HASun1L-KDEL
SS HA
KDEL

Fig. 8

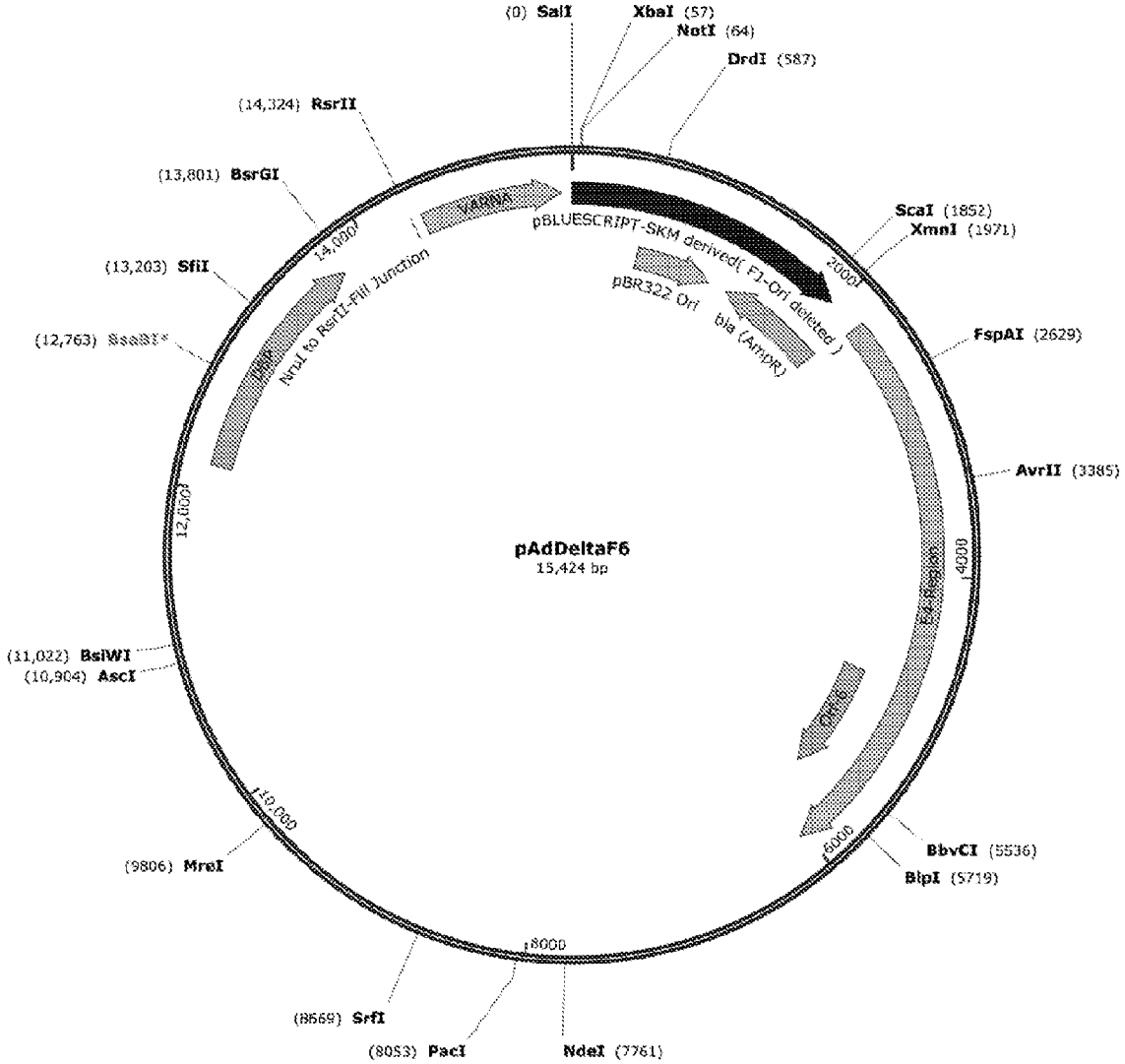

(0) SalI
XbaI (57)
NotI (64)
DrdI (587)
(14,324) RsrII
(13,801) BsrGI
(13,203) SfiI
(12,763) SspBI*
VA RNA
pBLUESCRIPT-SKM derived(F1-Ori deleted)
pBR322 Ori
bla (AmpR)
ScaI (1852)
XmnI (1971)
FspAI (2629)
AvrII (3385)
pAdDeltaF6
15,424 bp
(11,022) BsiWI
(10,904) AscI
(9806) MreI
(8669) SrfI
(8053) PacI
NdeI (7761)
BbvCI (5536)
BlpI (5719)

Fig. 9


(6983) NaeI
(6981) NgoMIV
NcoI (372)
SgrAI (706)
BciT* (711)
(6218) ScaI
BstBI (1371)
BstEII (1447)
ZraI (1617)
AatII (1619)
HindIII (1629)
pAAV 2/9 (p0008) Q
7330 bp
LacZ promoter
PasI (2788)
BsiWI (3007)
(4398) EcoRV
(4241) SbfI
(4232) PmeI
(4029) AfeI
(3972) XcmI

Fig. 10


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

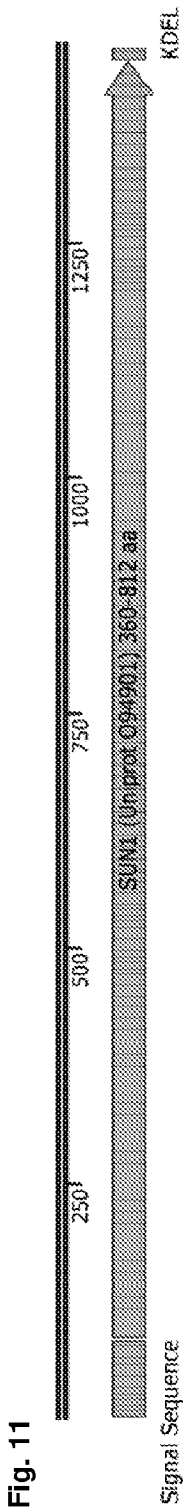

250
500
750
1000
1250
KDEL
SUN1 (Uniprot Q9H9Q1) 360-812 aa
Signal Sequence

Fig. 11

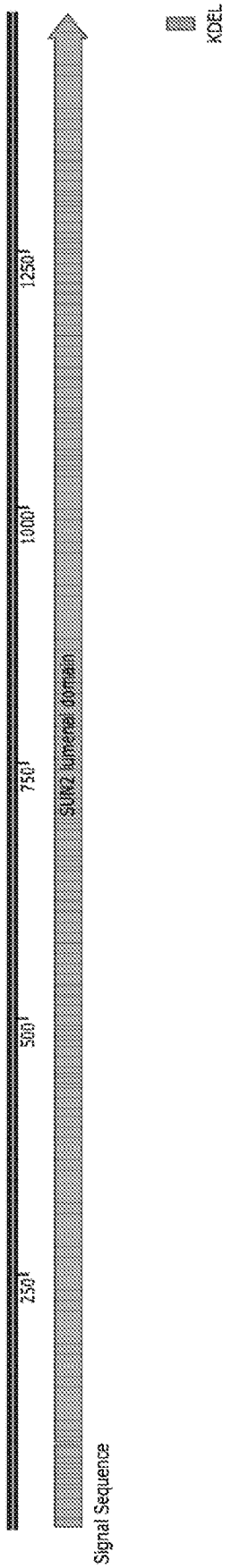

250
500
750
1000
1250
KDEL
SUN2 Lumenal domain
Signal Sequence

Fig. 12

Fig. 13

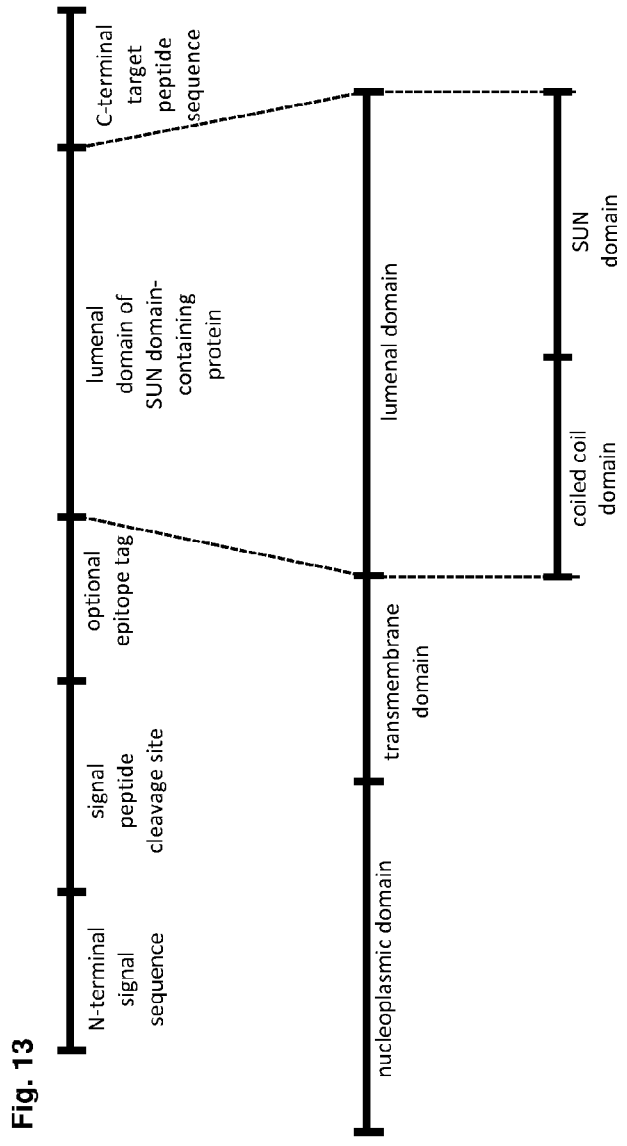


Fig. 14

```
SEQ ID NO:7  KASH1_aminoacid      RGFLFRVLRA-AIP----LQLLLLLLIGIACLVPMSEEDYSCALSNNFARSFHPMLRYTNG
SEQ ID NO:9  KASH2_aminoacid      RSFLSRVVRA-AIP----LQLLLLLLLLLACLLPSSEEDYSCTQANNFARSFYPMLRYTNG
SEQ ID NO:11 KASH3_aminoacid      GSLFRRACCV-ALP----LQLLLLLFLLLLFLLPIREEDRSCTLANNFARSFTLMLRYNGP
SEQ ID NO:13 KASH4_aminoacid      -----DPASRQPLTFLLLFLLFLLLVGAMFLLP--ASGGPCCSHARIPRTFYLVLSYVNG
SEQ ID NO:15 KASH5_aminoacid      ----LRVTRHPLIPAFVLGLLLLLLLSVLLLGPSPPPTWP----------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

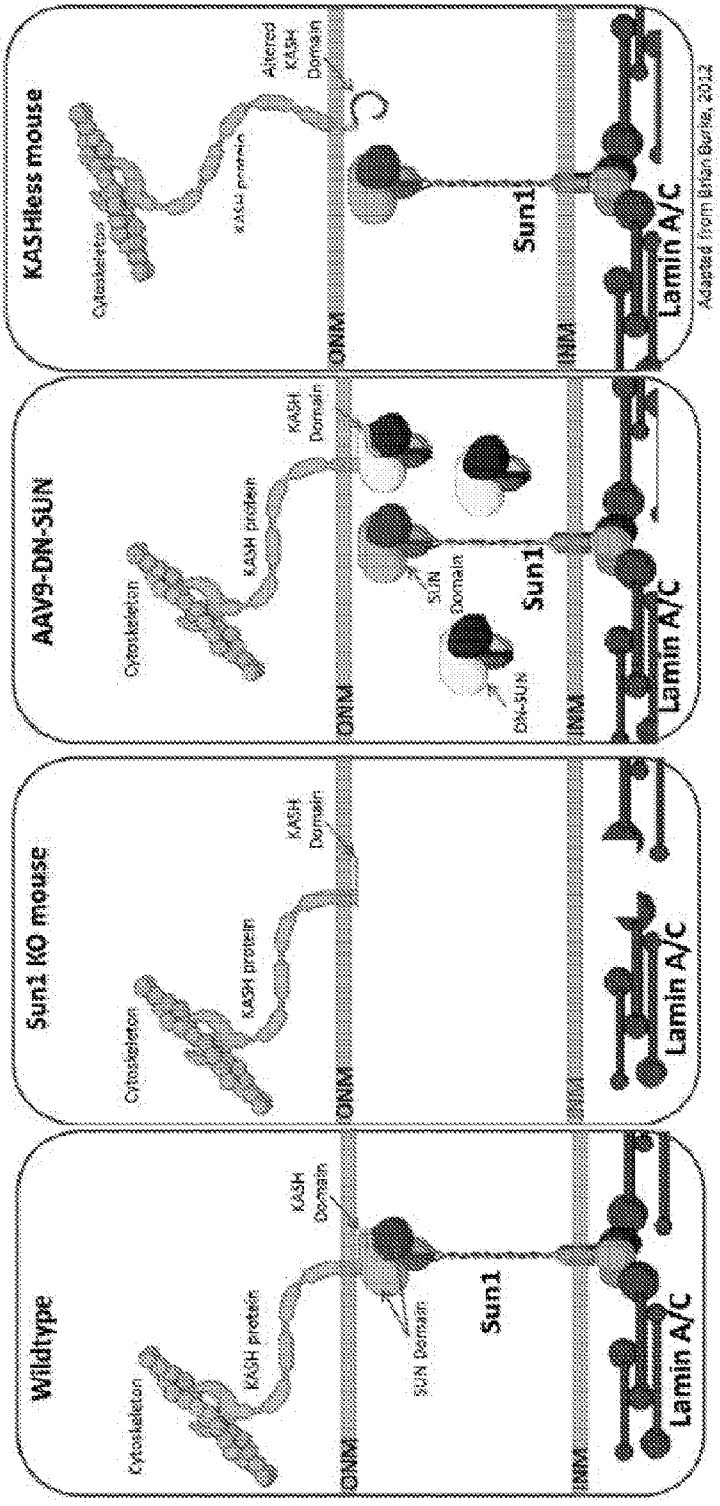

Wildtype
Cytoskeleton
KASH protein
KASH Domain
SUN Domain
Sun1
Lamin A/C
ONM
INM
Sun1 KO mouse
AAV9-DN-SUN
DN-SUN
KASHless mouse
Altered KASH Domain
Adapted from Brian Burke, 2013

Fig. 16

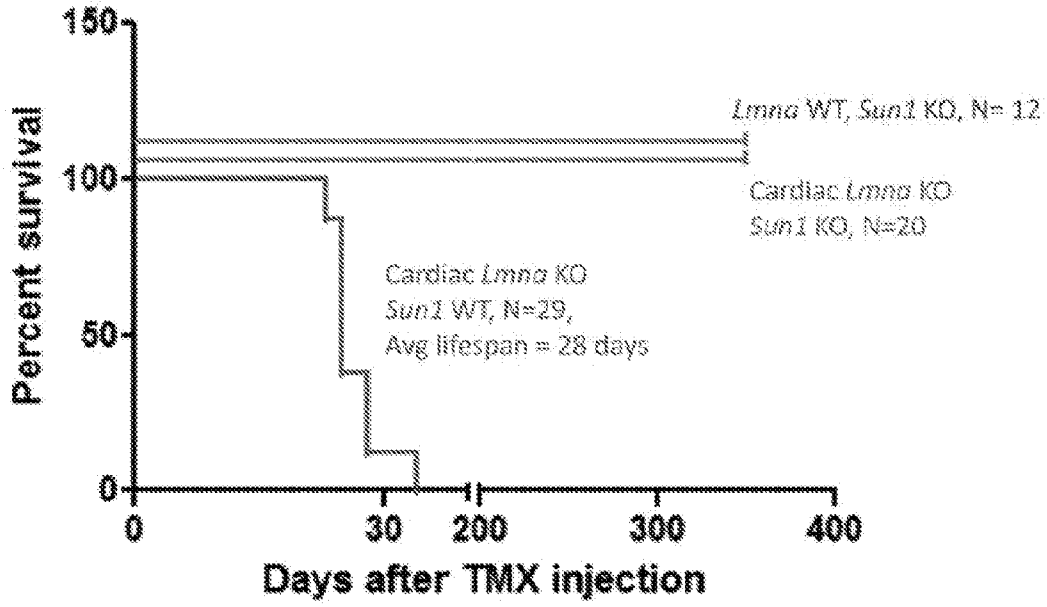

150
100
50
0
Percent survival
Lmna WT, Sun1 KO, N= 12
Cardiac Lmna KO
Sun1 KO, N=20
Cardiac Lmna KO
Sun1 WT, N=29,
Avg lifespan = 28 days
0
30
200
300
400
Days after TMX injection

Fig. 17

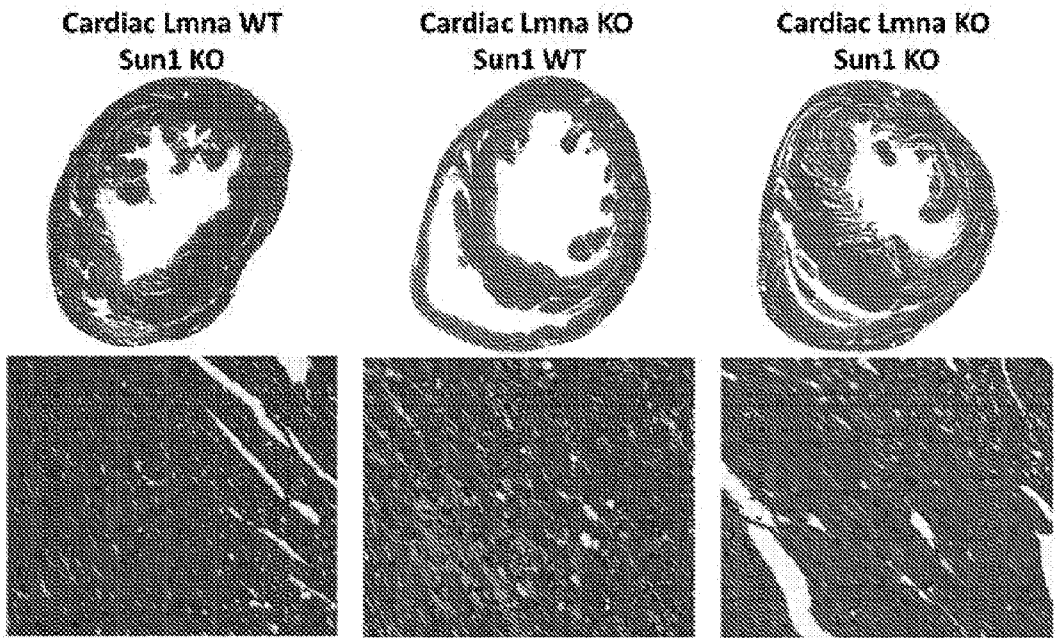

Cardiac Lmna WT
Sun1 KO
Cardiac Lmna KO
Sun1 WT
Cardiac Lmna KO
Sun1 KO

Fig. 18

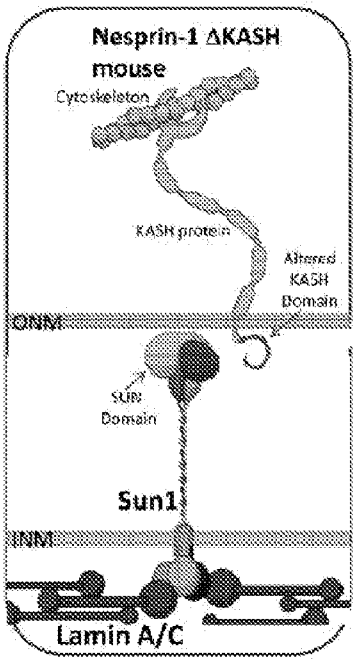


Fig. 19A

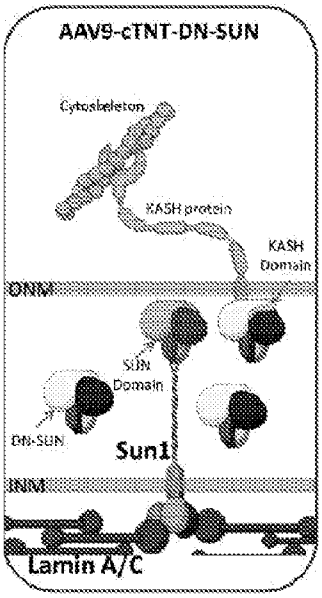


Fig. 19B

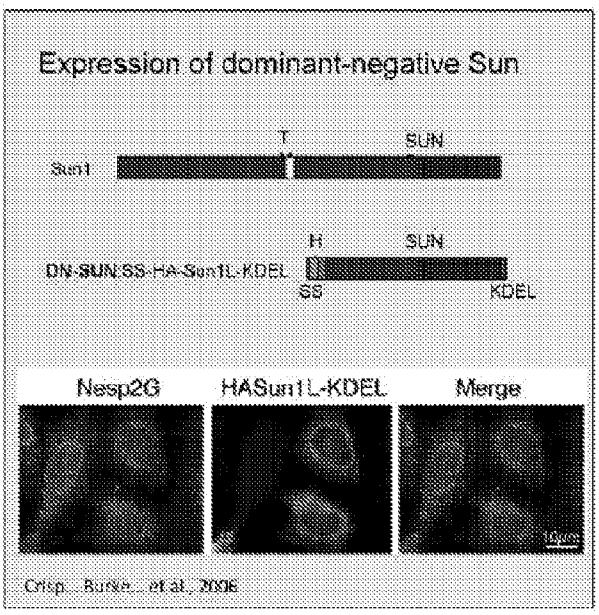


Fig. 20

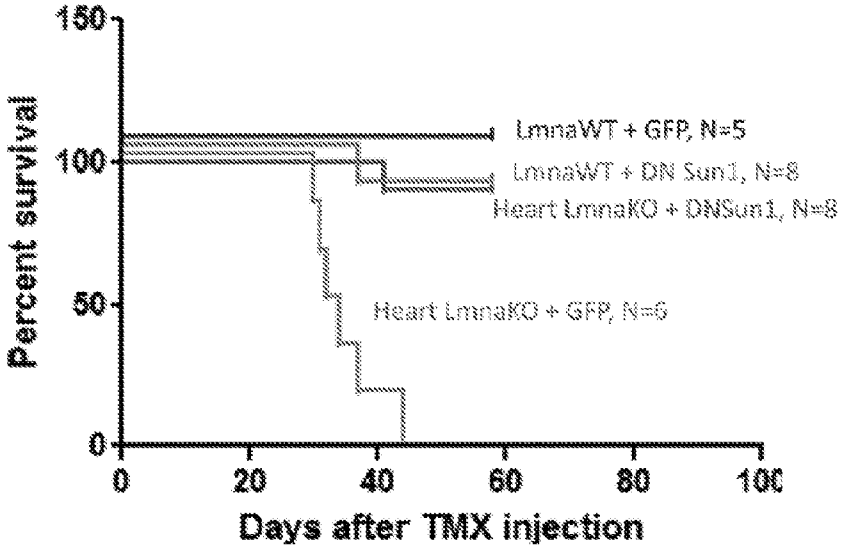

150
100
50
0
Percent survival
0
20
40
60
80
100
Days after TMX injection
LmnaWT + GFP, N=5
LmnaWT + DN Sun1, N=8
Heart LmnaKO + DNSun1, N=8
Heart LmnaKO + GFP, N=6

Fig. 21

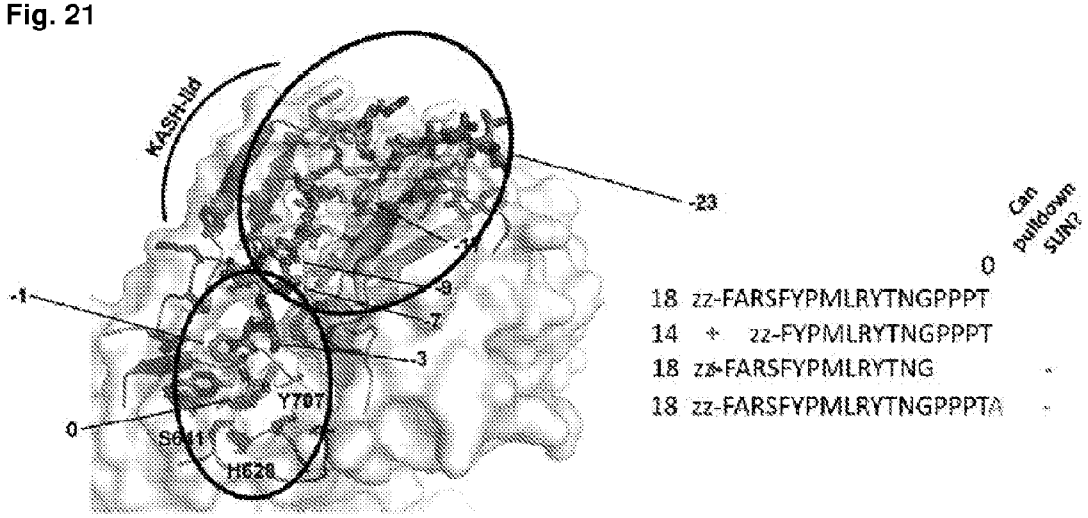

KASH-lid
-23
-1
0
Y787
H628
Can pulldown SUN2
18 zz-FARSFYPMLRYTNGPPPT
14 + zz-FYPMLRYTNGPPPT
18 zz-FARSFYPMLRYTNG -
18 zz-FARSFYPMLRYTNGPPPTA -

Fig. 22

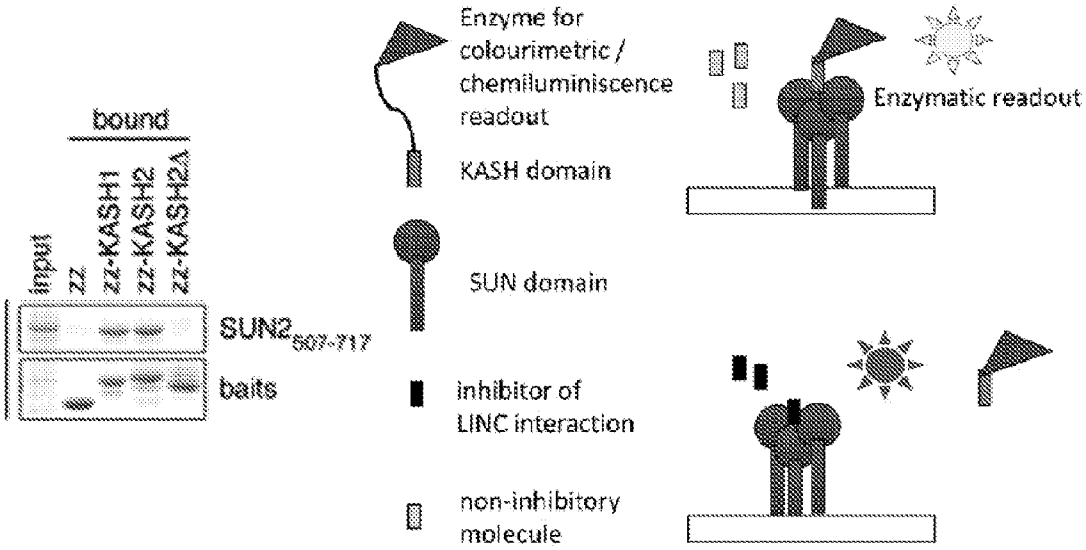

bound
input
zz
zz-KASH1
zz-KASH2
zz-KASH2Δ
SUN2507-717
baits
Enzyme for colourimetric / chemiluminiscence readout
KASH domain
SUN domain
inhibitor of LINC interaction
non-inhibitory molecule
Enzymatic readout

Fig. 23

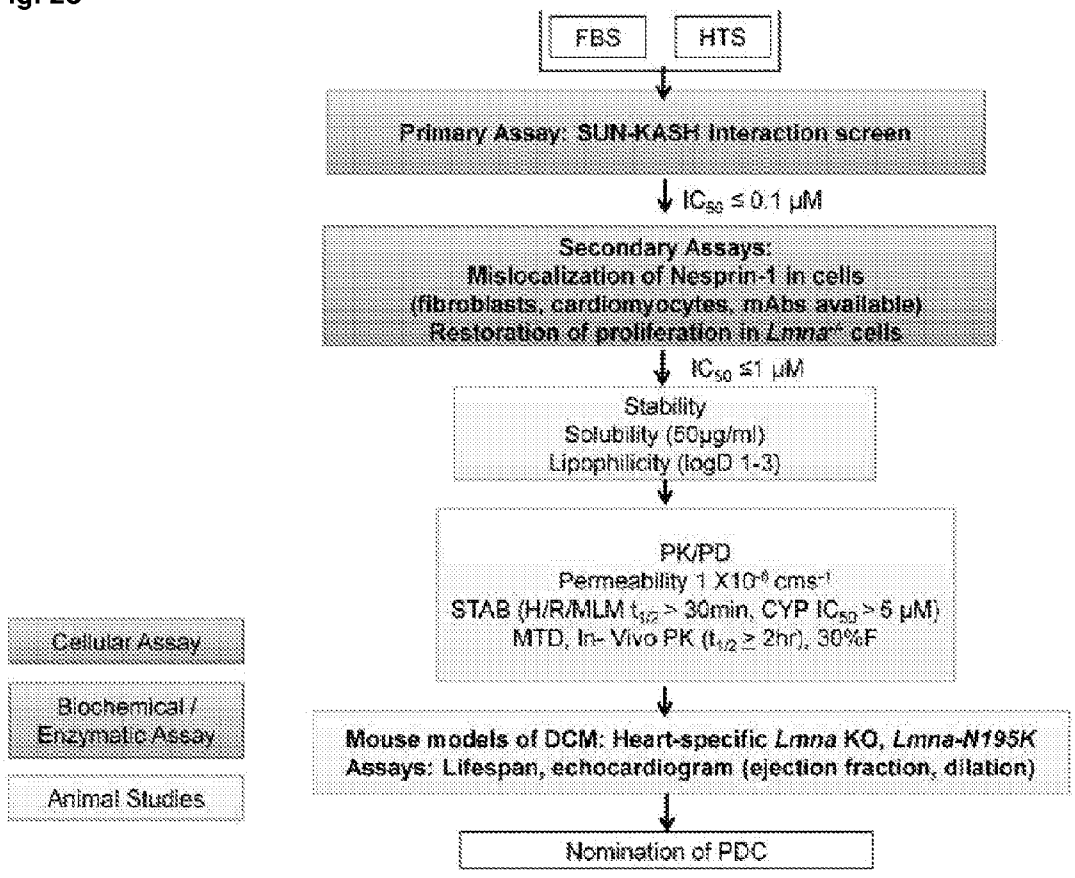

FBS
HTS
Primary Assay: SUN-KASH Interaction screen
IC50 ≤ 0.1 μM
Secondary Assays:
Mislocalization of Nesprin-1 in cells
(fibroblasts, cardiomyocytes, mAbs available)
Restoration of proliferation in Lmna-/- cells
IC50 ≤1 μM
Stability
Solubility (50μg/ml)
Lipophilicity (logD 1-3)
PK/PD
Permeability 1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

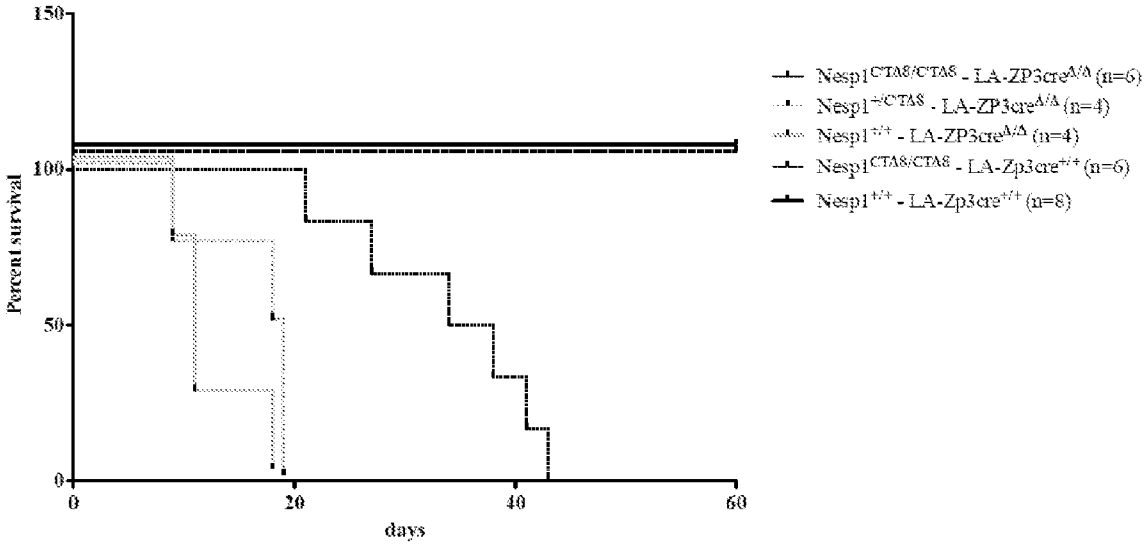

150
100
50
0
Percent survival
0
20
40
60
days
Nesp1CTAδ/CTAδ - LA-ZP3creΔ/Δ (n=6)
Nesp1+/CTAδ - LA-ZP3creΔ/Δ (n=4)
Nesp1+/+ - LA-ZP3creΔ/Δ (n=4)
Nesp1CTAδ/CTAδ - LA-Zp3cre+/+ (n=6)
Nesp1+/+ - LA-Zp3cre+/+ (n=8)

Fig. 25

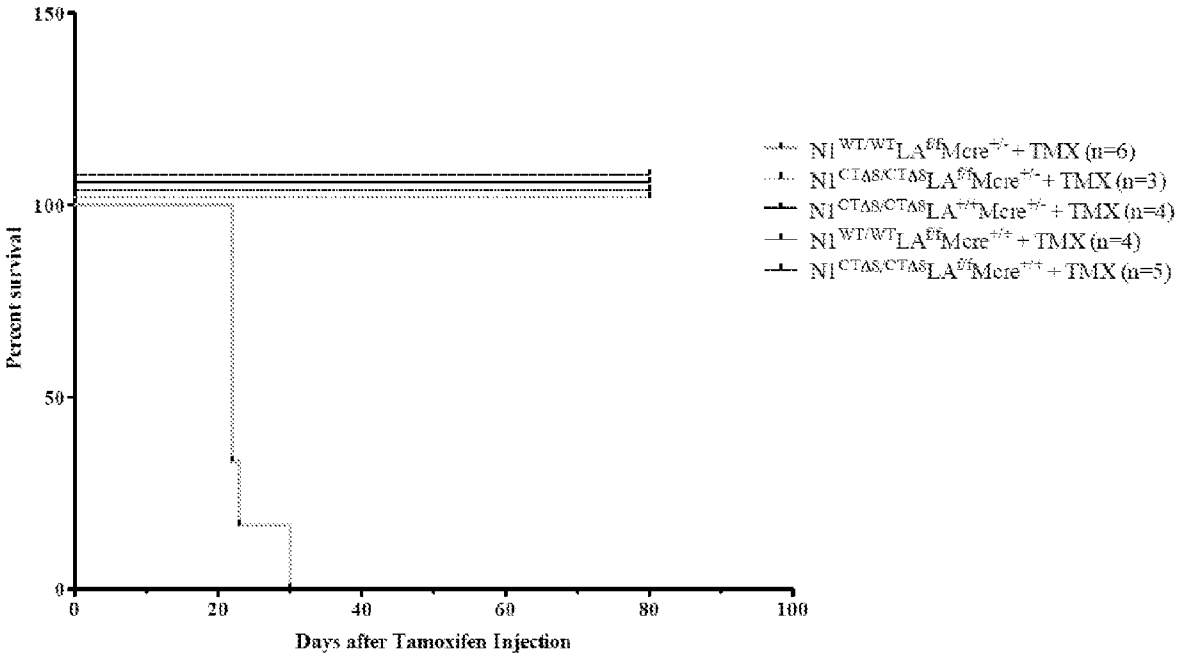

150
100
50
0
Percent survival
0
20
40
60
80
100
Days after Tamoxifen Injection
N1WT/WTLAf/fMcre+/- + TMX (n=6)
N1CTΔS/CTΔSLAf/fMcre+/- + TMX (n=3)
N1CTΔS/CTΔSLA+/+Mcre+/- + TMX (n=4)
N1WT/WTLAf/fMcre+/+ + TMX (n=4)
N1CTΔS/CTΔSLAf/fMcre+/+ + TMX (n=5)

Fig. 26A

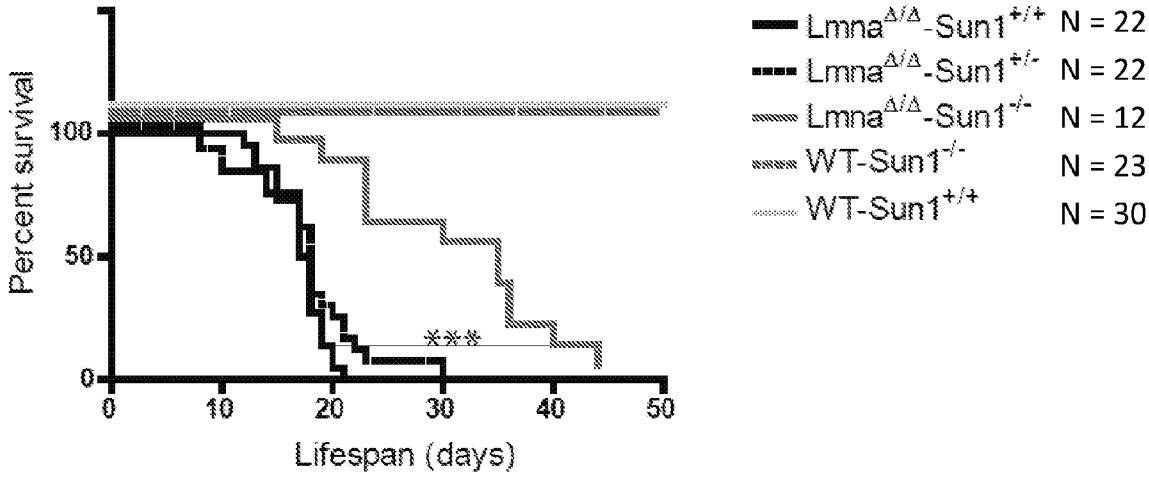


Fig. 26B

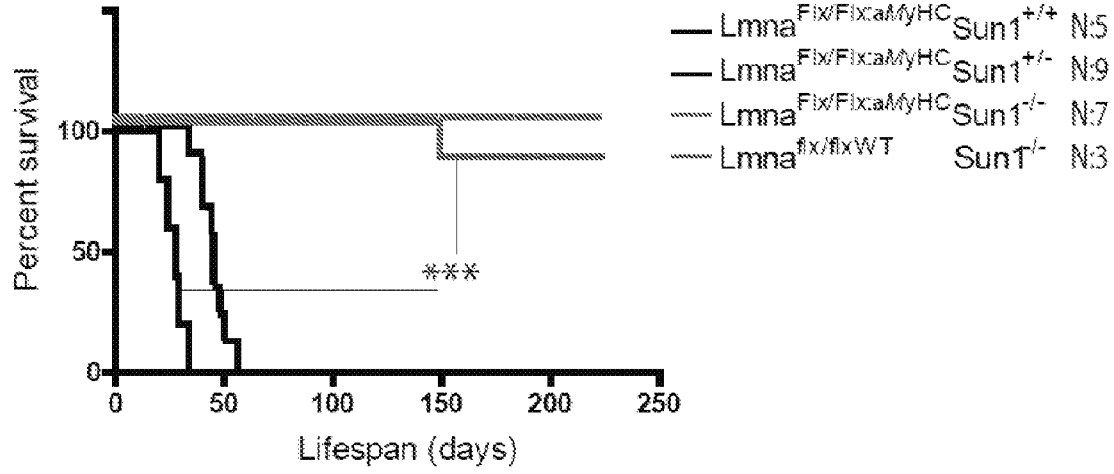


Fig. 26C

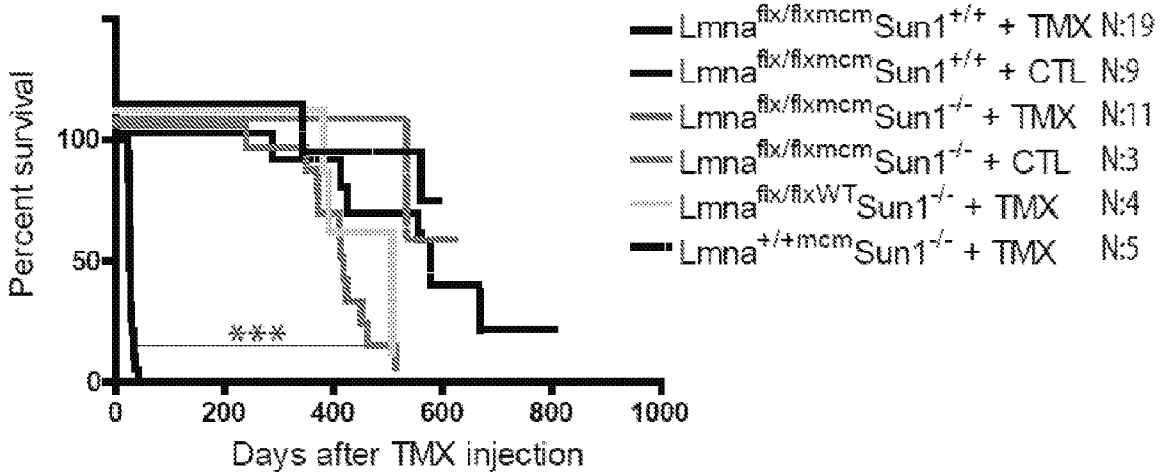


Fig. 26D

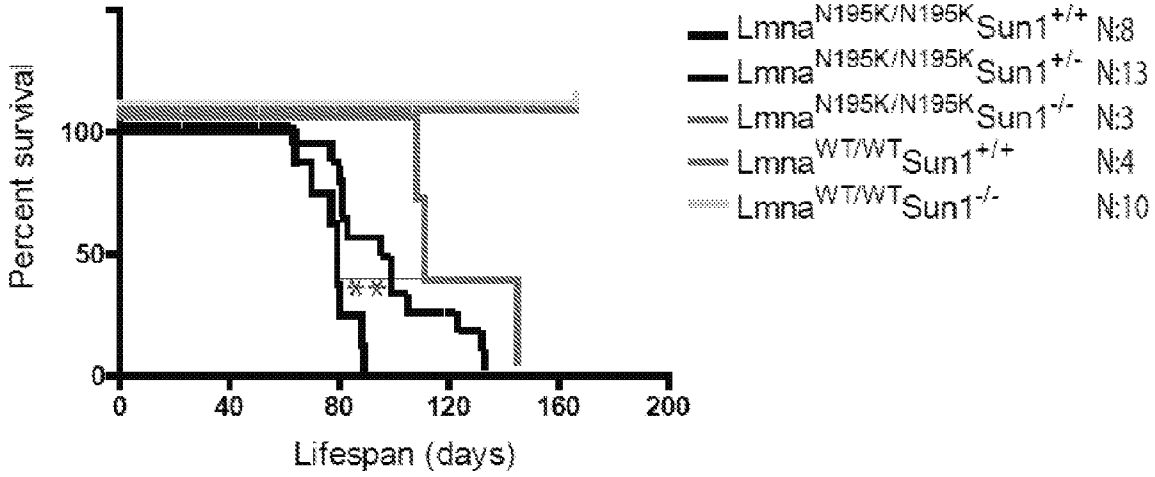


Fig. 27A

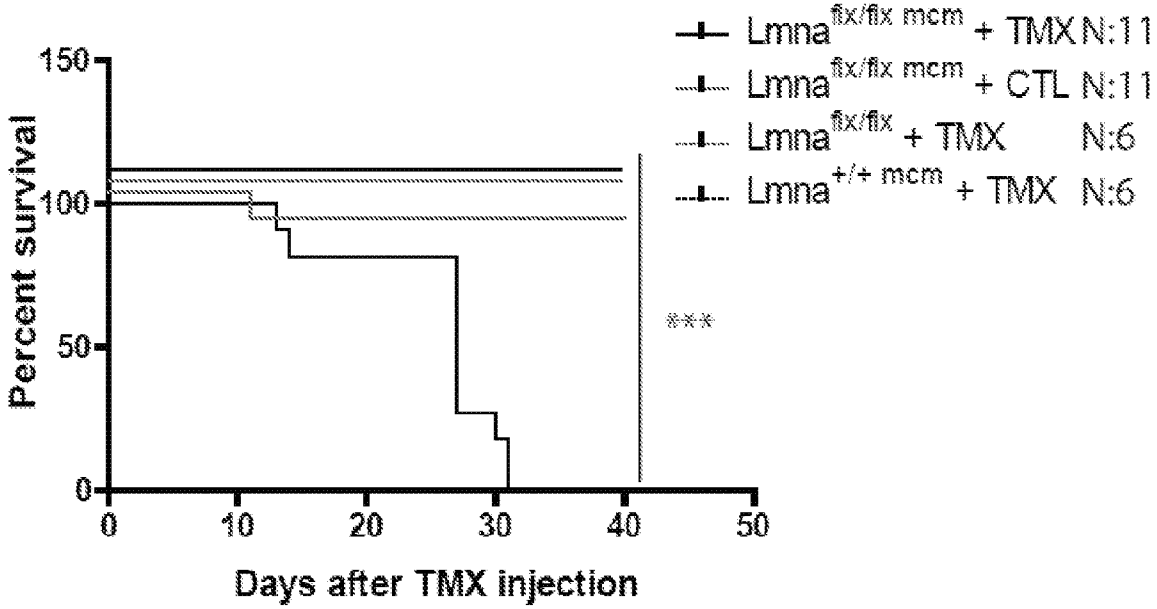


Fig. 27B

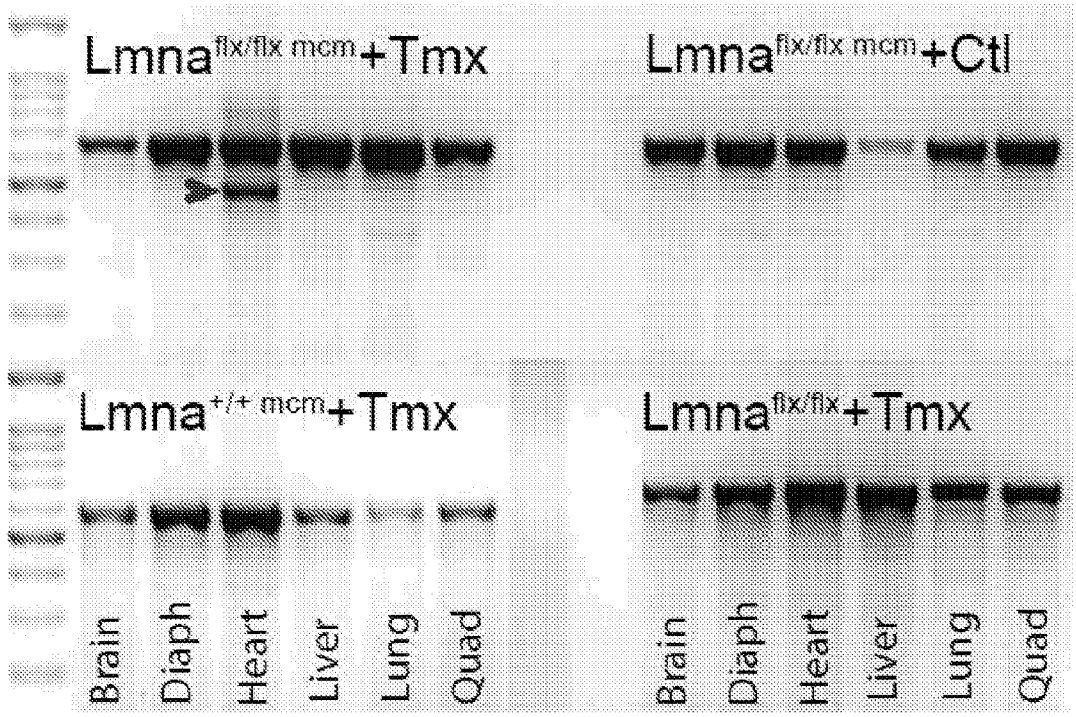


Fig. 27C

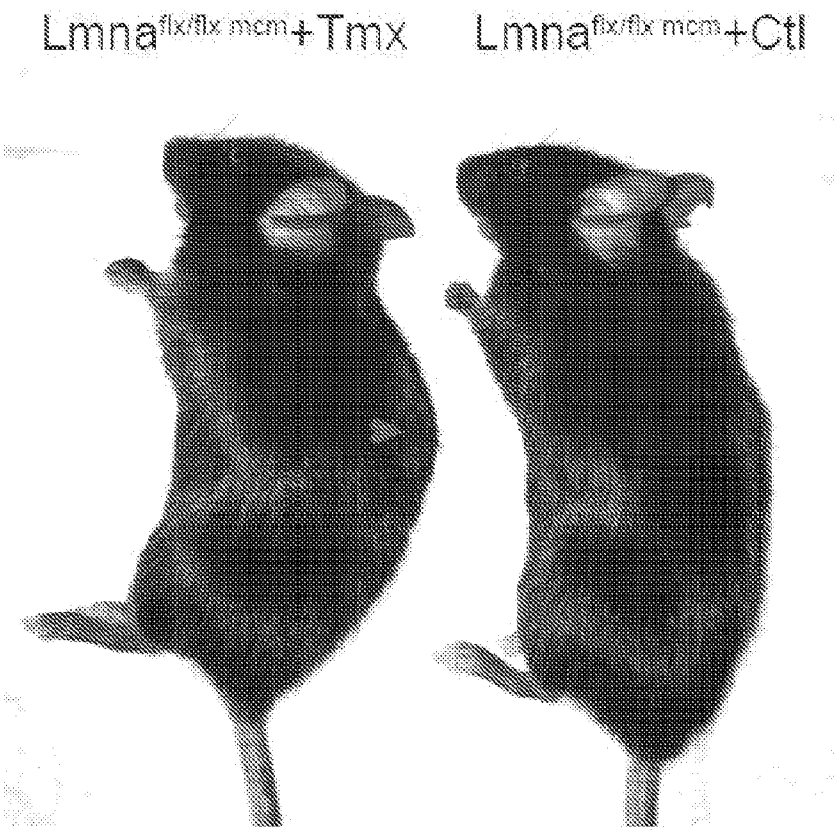


Fig. 27D

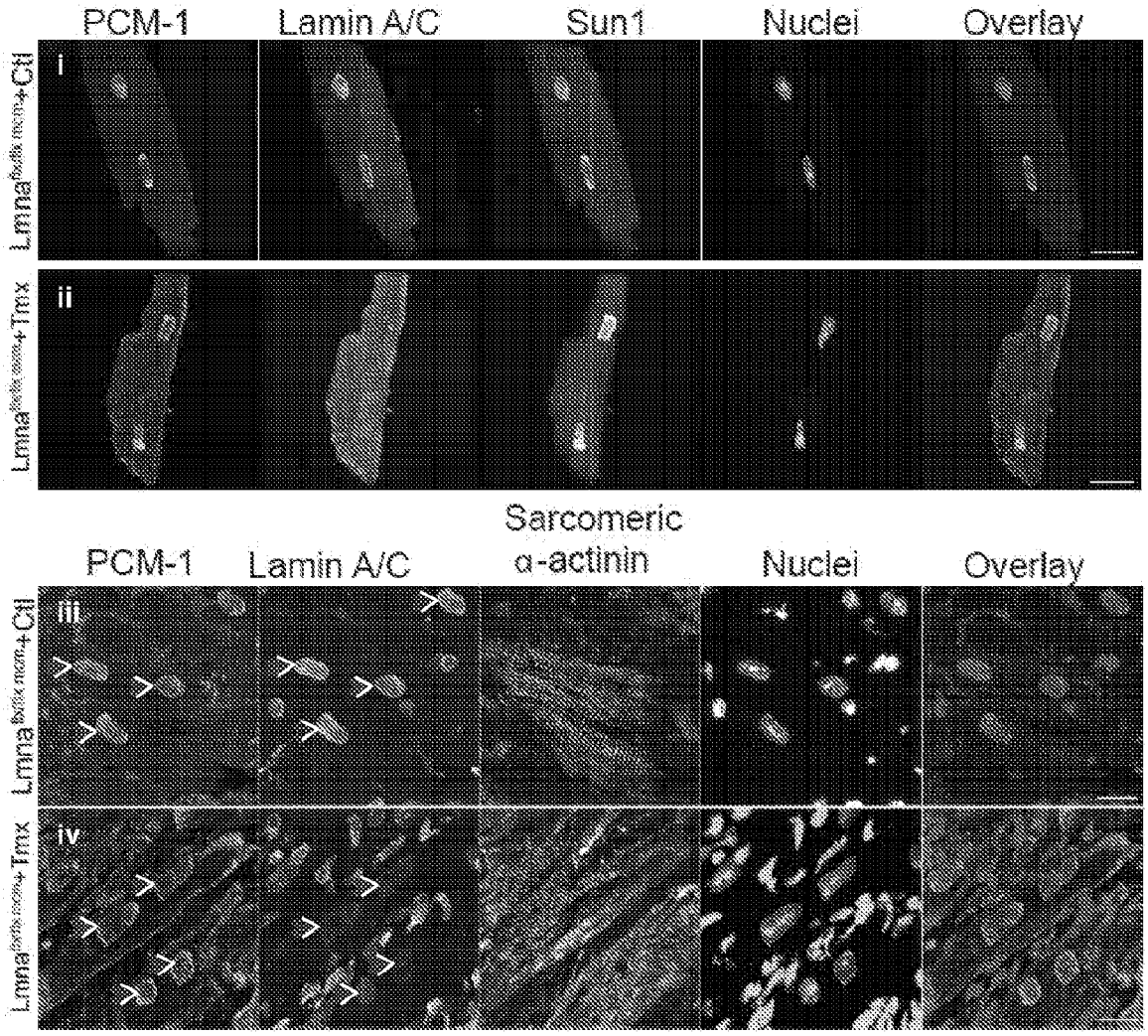

PCM-1
Lamin A/C
Sun1
Nuclei
Overlay
i
ii
Sarcomeric
α-actinin
iii
iv

Fig. 27E

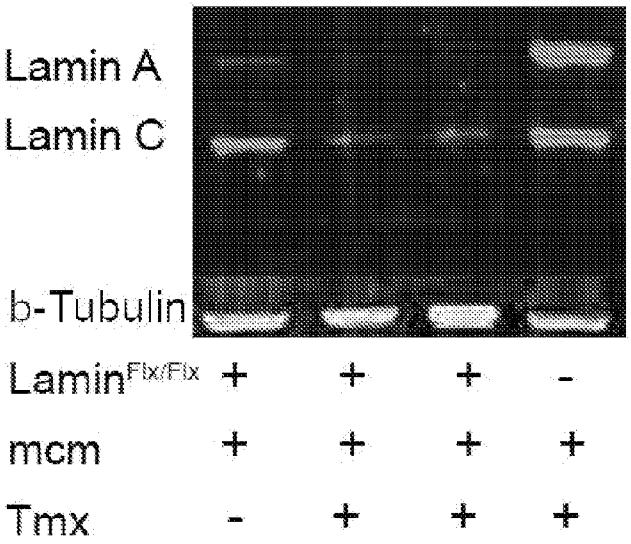


Fig. 27F

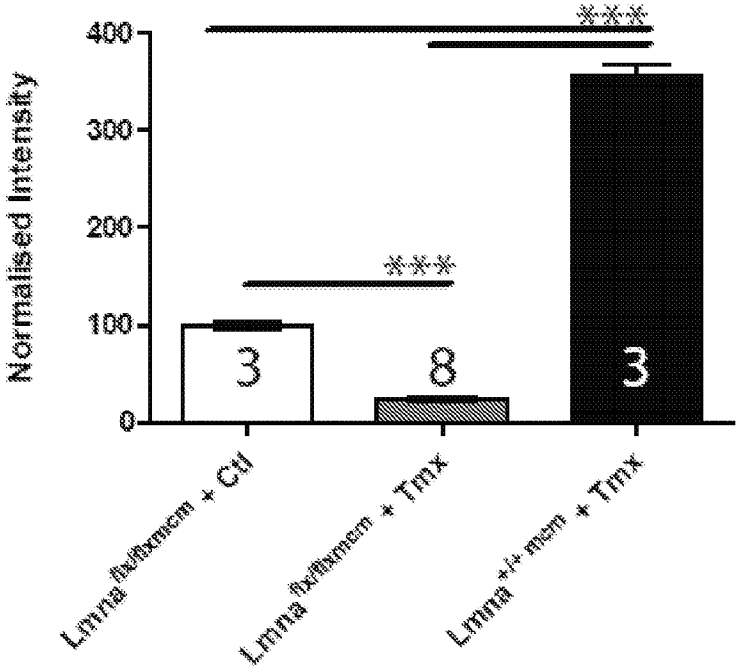


Fig. 28A

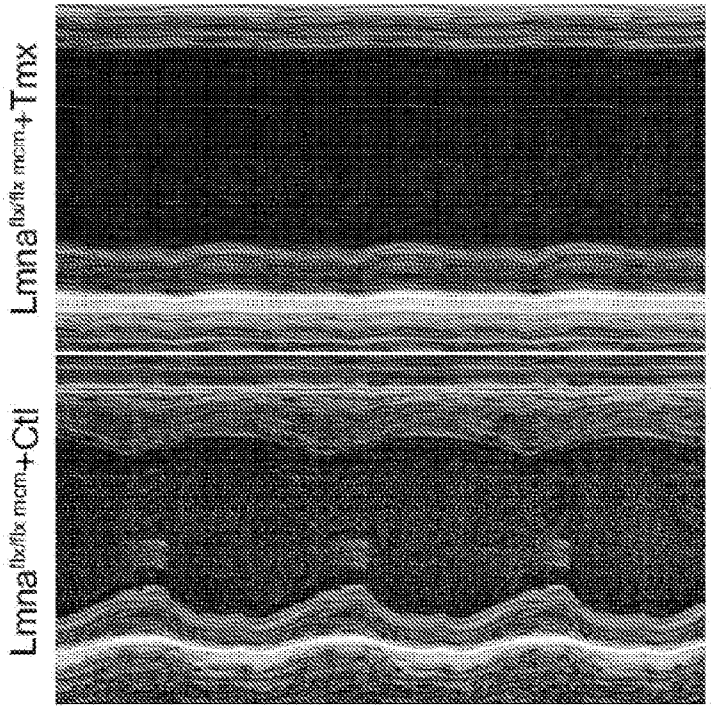


Fig. 28B

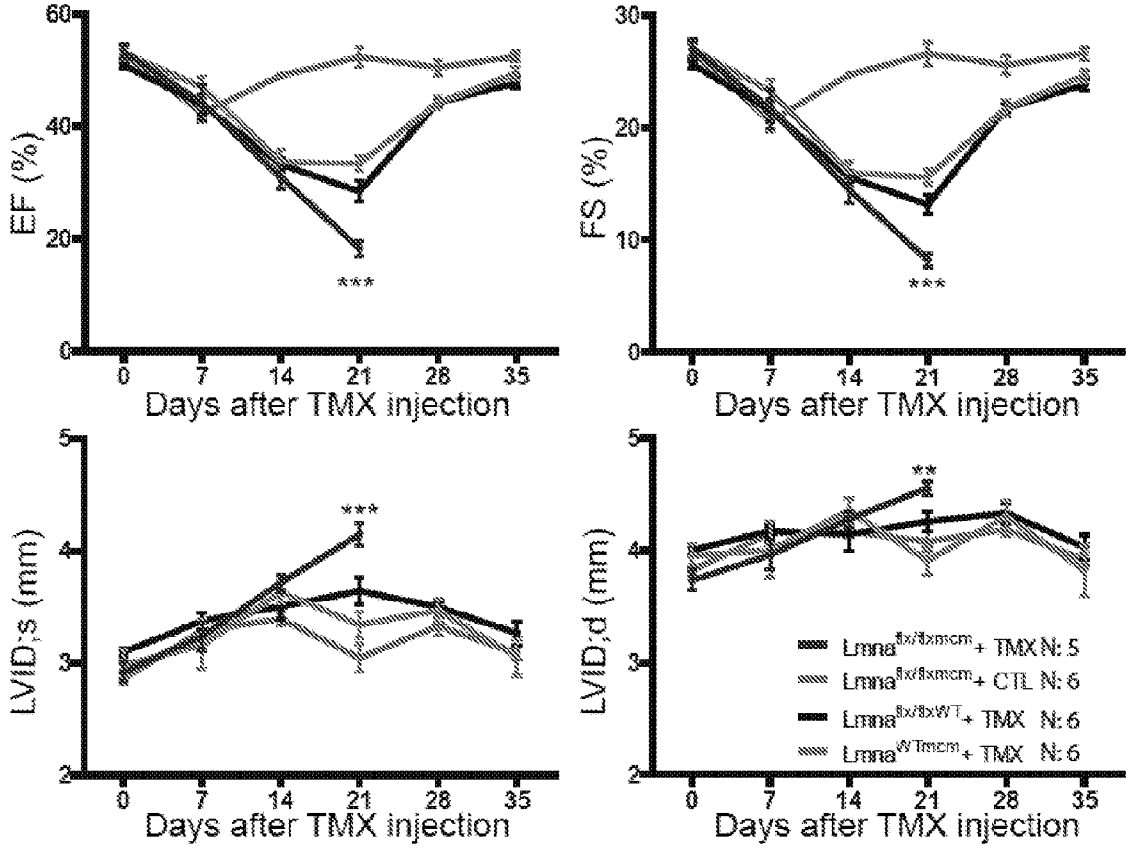


Fig. 28C

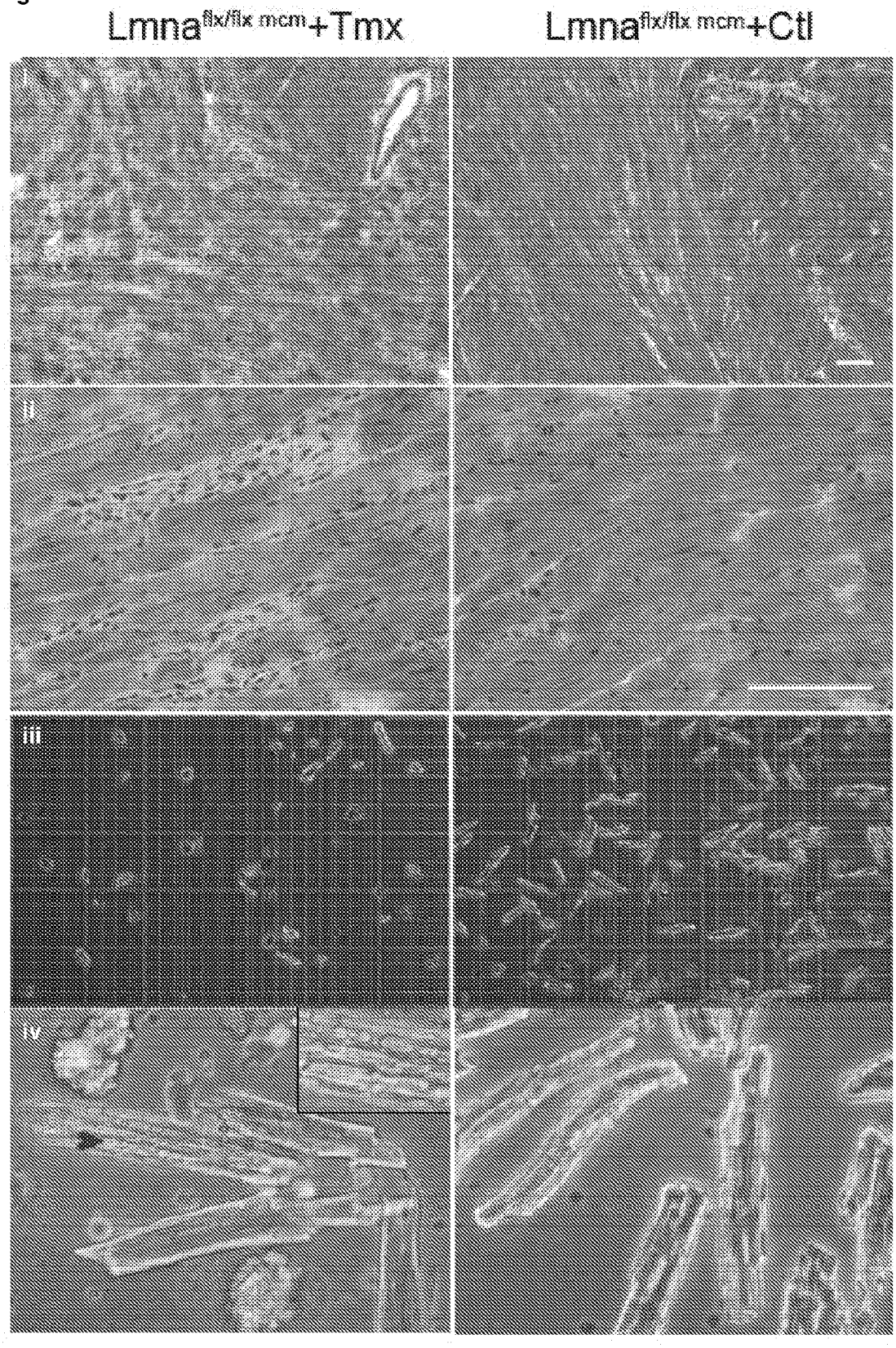


Fig. 28D

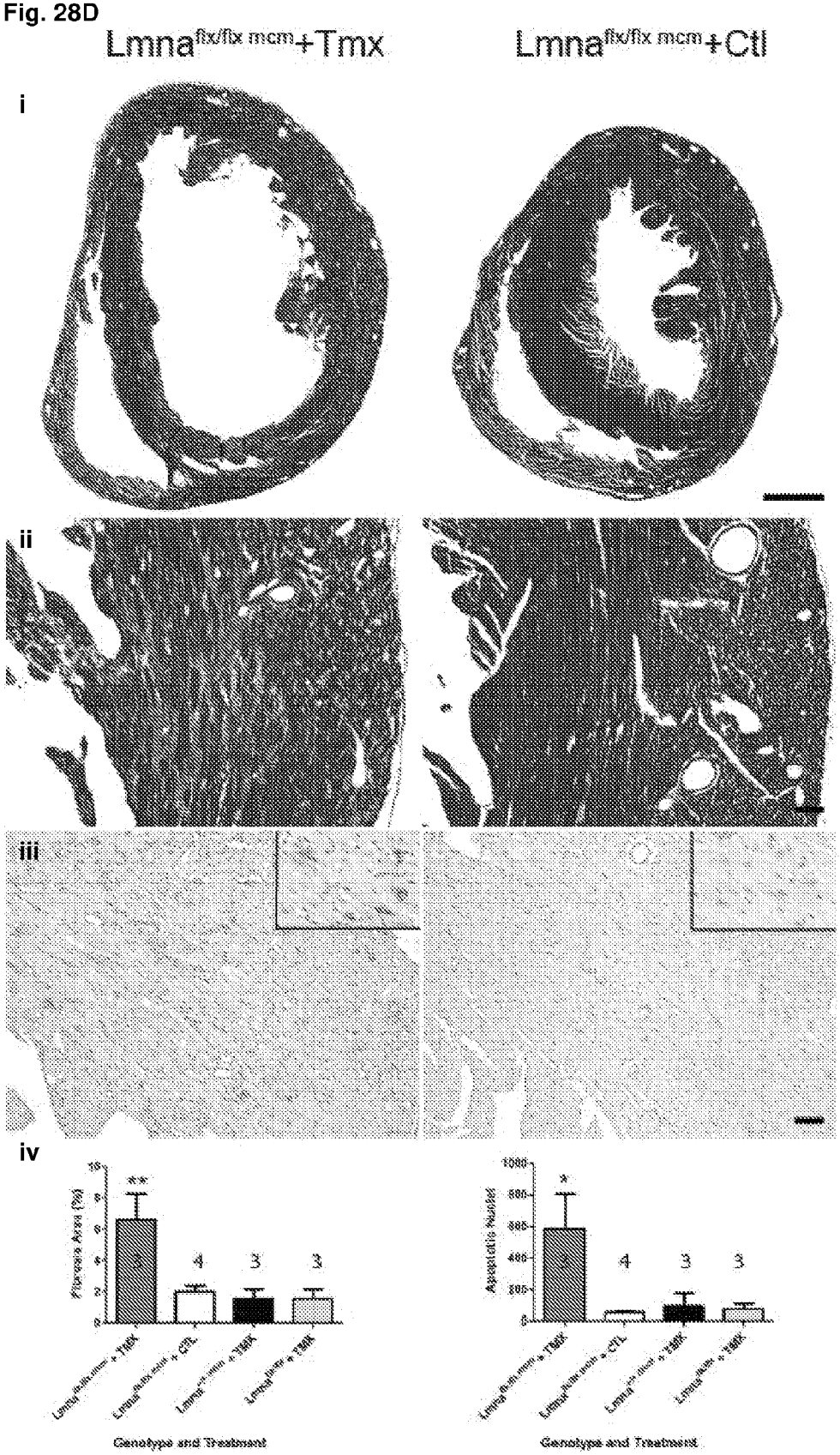

Lmna fix/fix mcm +Tmx
Lmna fix/fix mcm +Ctl
i
ii
iii
iv
Genotype and Treatment
Genotype and Treatment

Fig. 29A

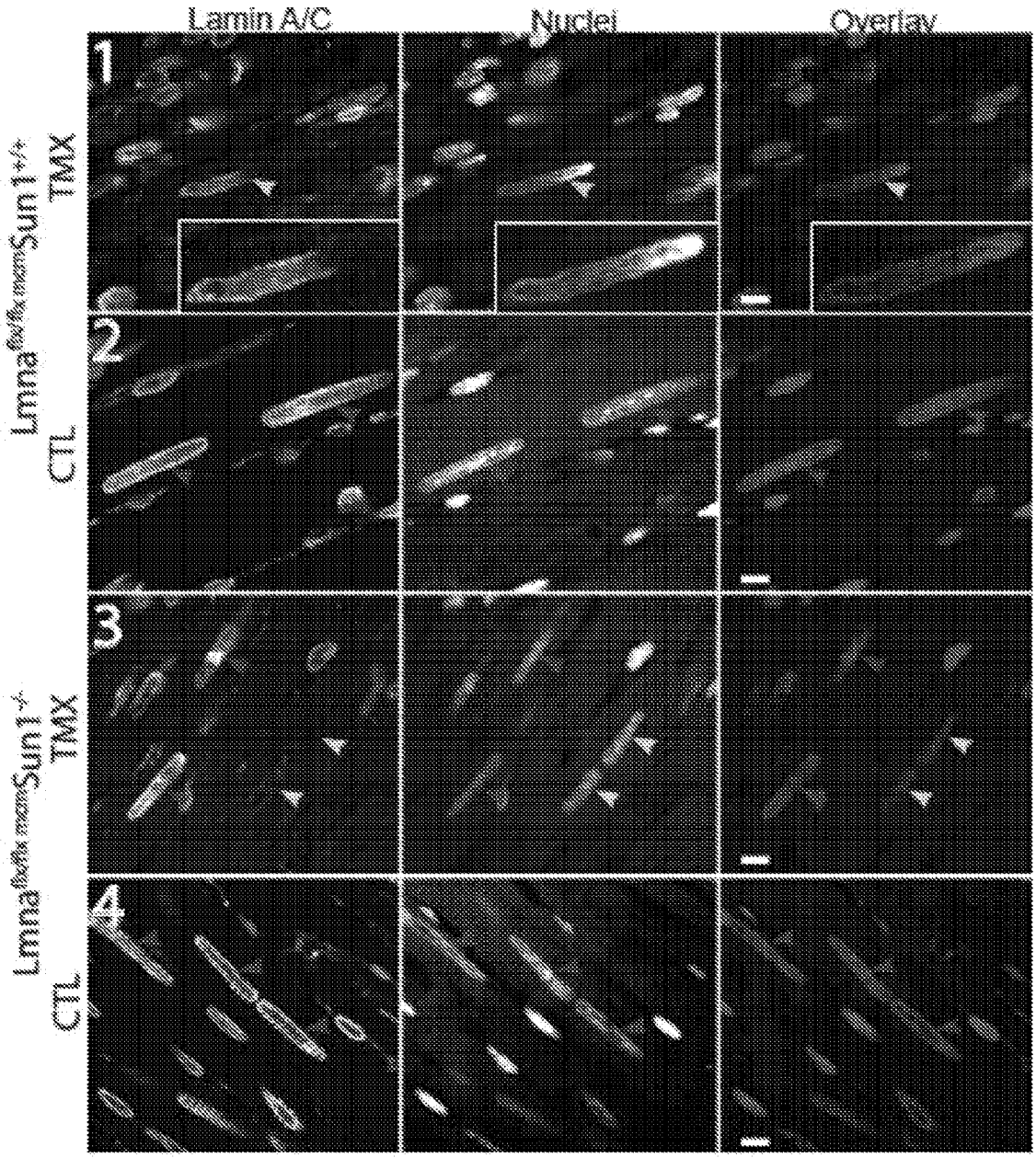
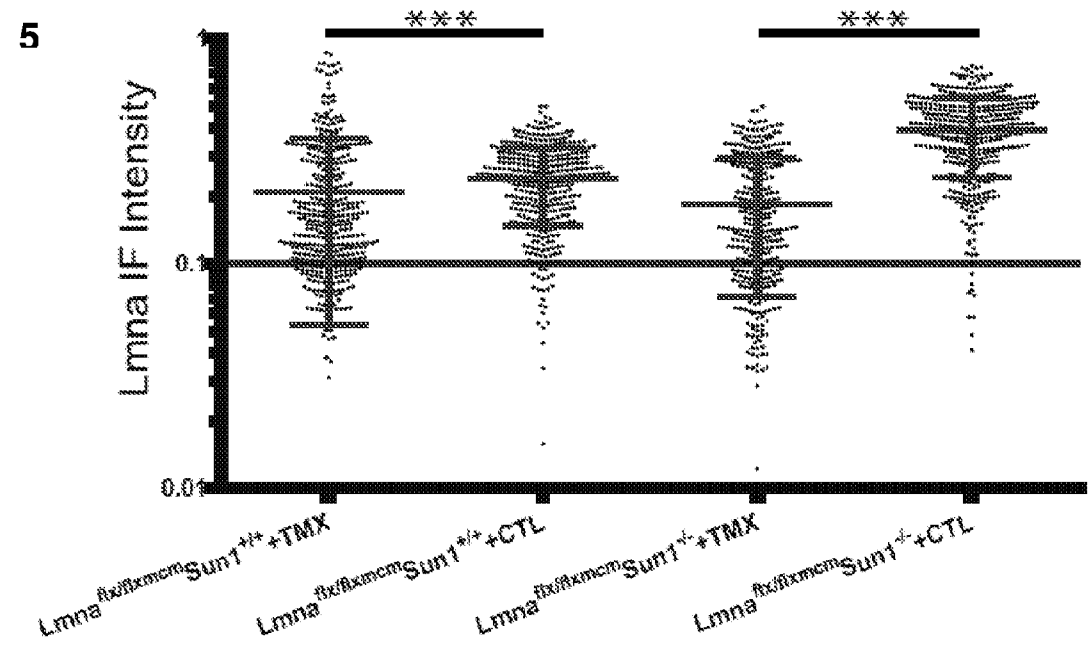

Lamin A/C
Nuclei
Overlay
1
2
3
4
Lmna flx/flx, tmxSun1 +/+
TMX
CTL
Lmna flx/flx, tmxSun1 -/-
TMX
CTL

Fig. 29A (continued)



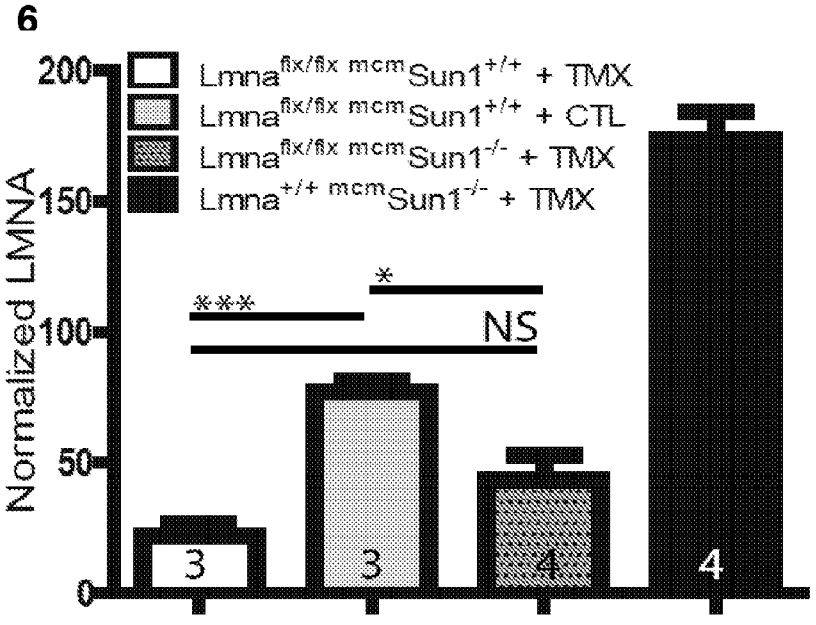


Fig. 29B

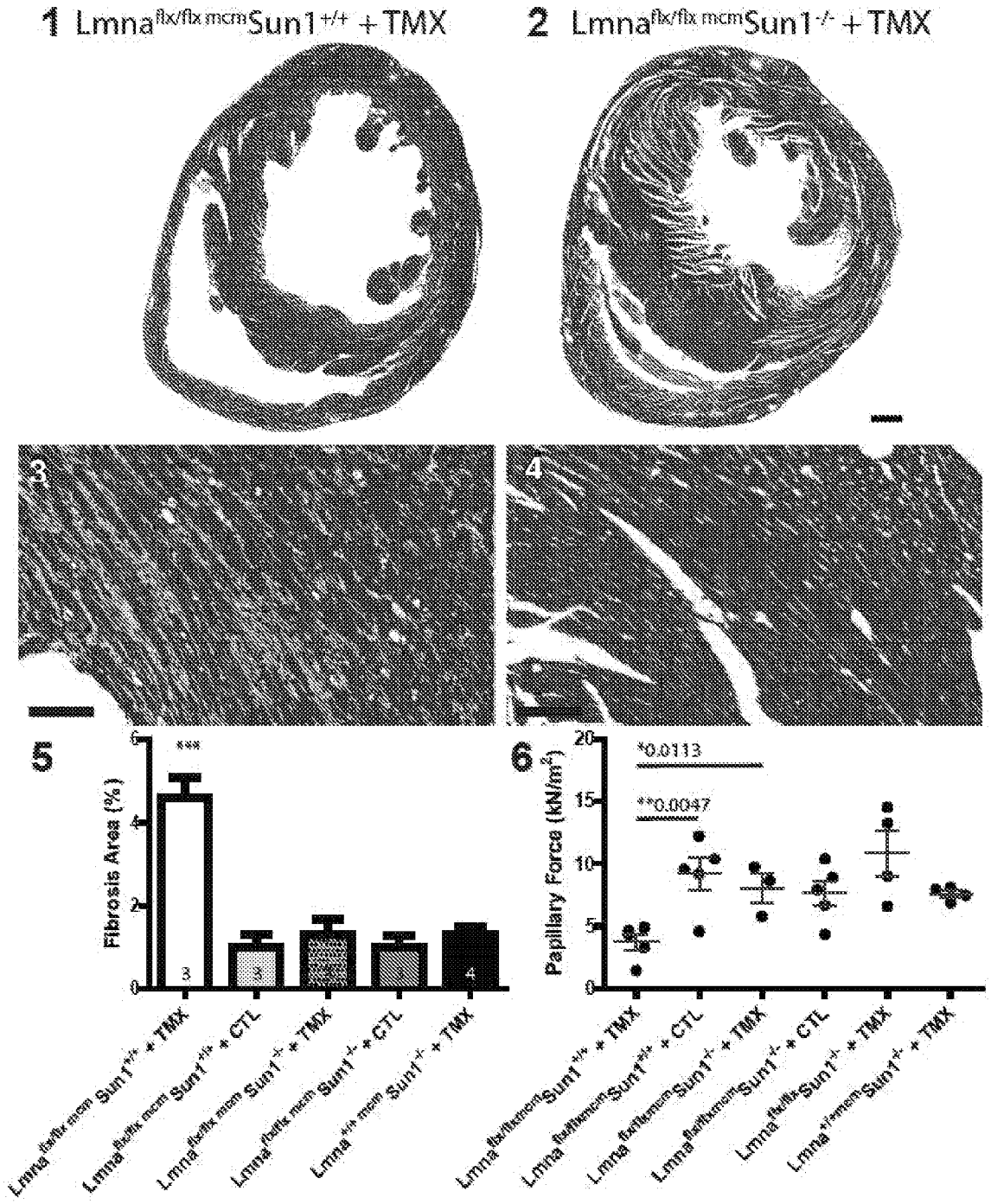

1 Lmna flx/flx mcm Sun1 +/+ + TMX
2 Lmna flx/flx mcm Sun1 -/- + TMX
3
4
5
Fibrosis Area (%)
***
6
Papillary Force (kN/m²)
*0.0113
**0.0047
Lmna flx/flx mcm Sun1 +/+ + TMX
Lmna flx/flx mcm Sun1 +/+ + CTL
Lmna flx/flx mcm Sun1 -/- + TMX
Lmna flx/flx mcm Sun1 -/- + CTL
Lmna +/+ mcm Sun1 -/- + TMX
Lmna flx/flx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

Fig. 29C

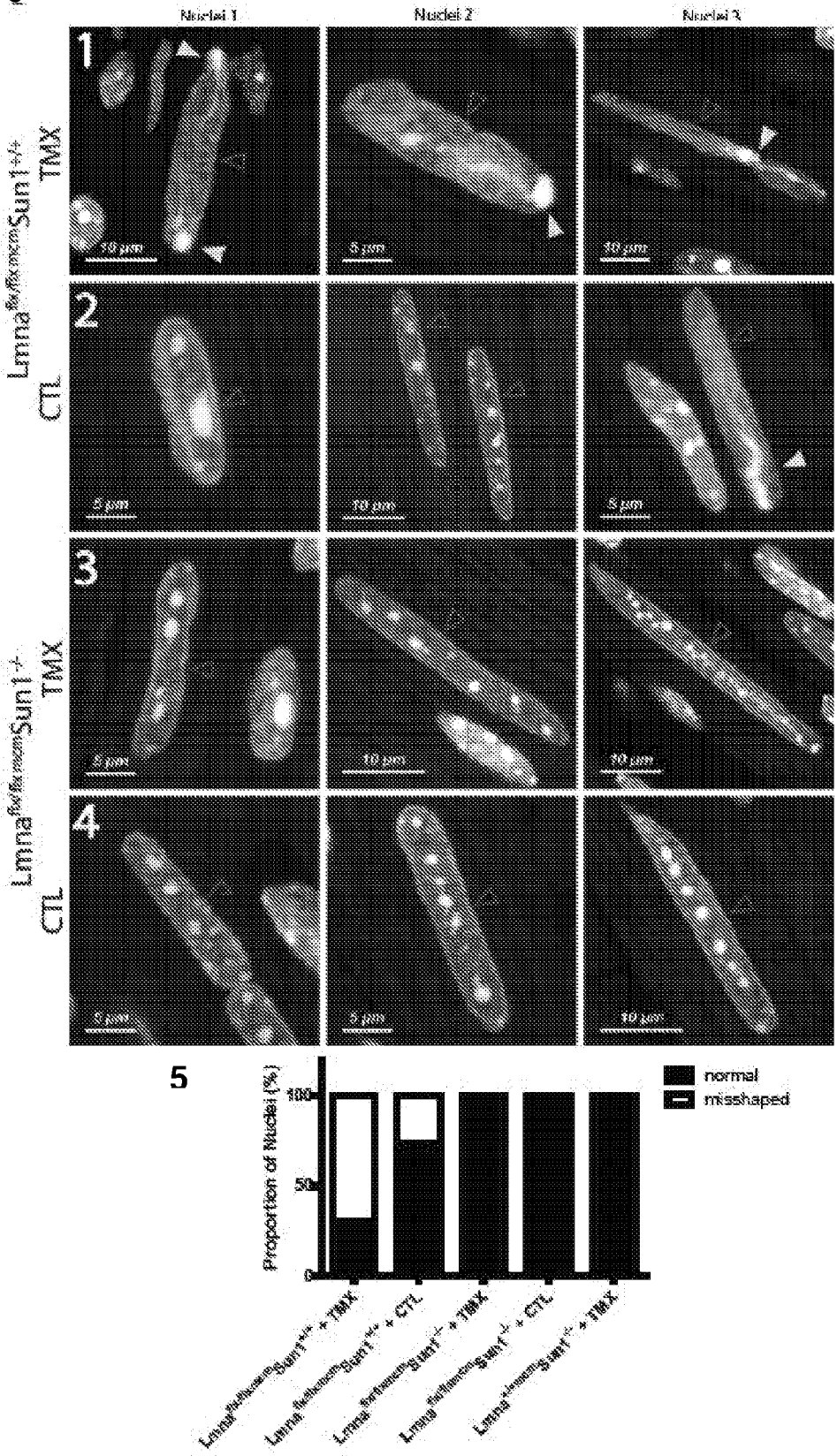

Nuclei 1
Nuclei 2
Nuclei 3
1
2
3
4
5
TMX
CTL
TMX
CTL
50 µm
5 µm
10 µm
5 µm
5 µm
10 µm
5 µm
10 µm
5 µm
5 µm
10 µm
normal
misshaped
Proportion of Nuclei (%)
100
50
0

Fig. 29D

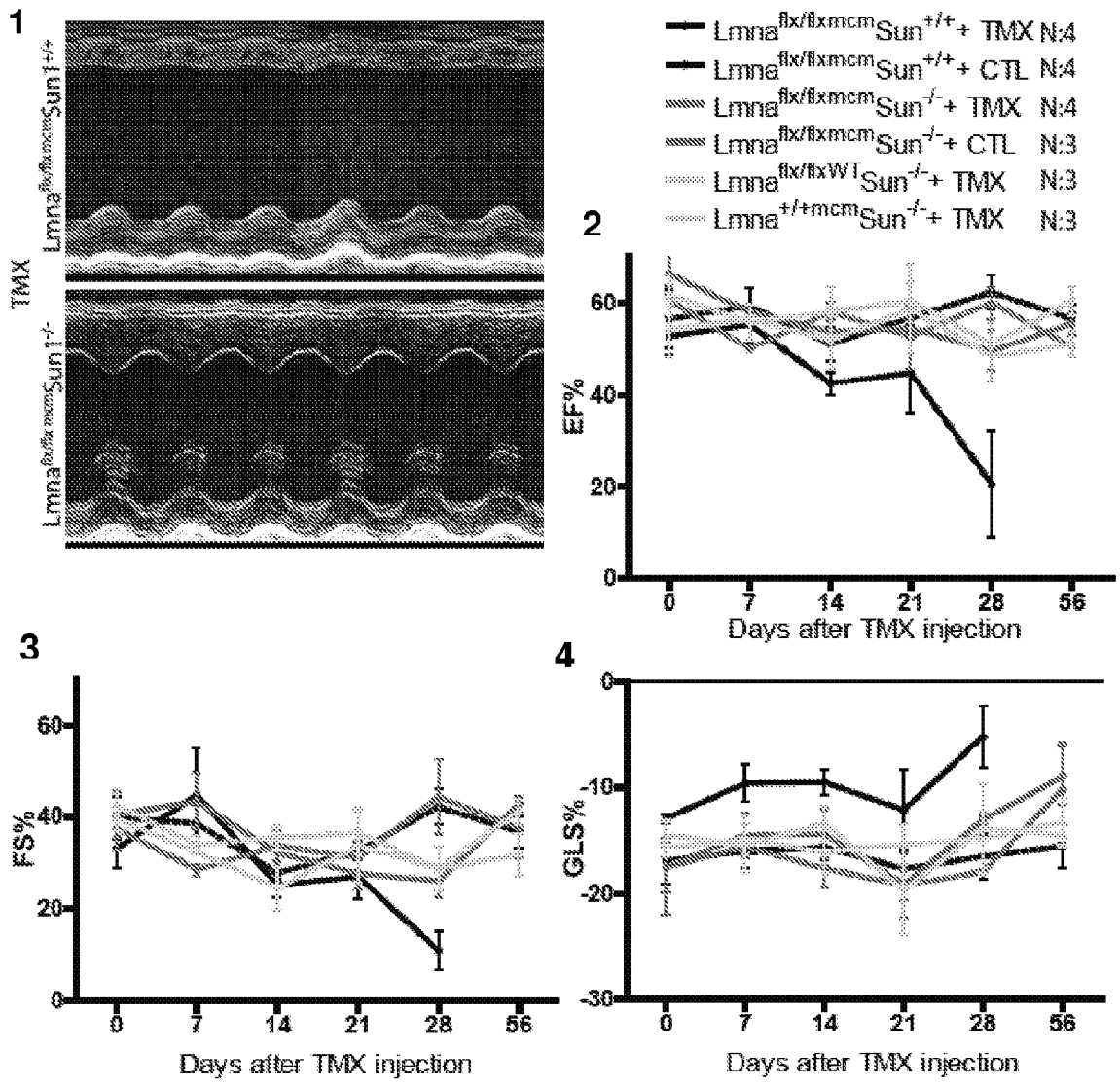

1
TMX
Lmna flx/flxmcm Sun1 +/+
Lmna flx/flxmcm Sun1 -/-
2
Lmna flx/flxmcm Sun +/+ + TMX N:4
Lmna flx/flxmcm Sun +/+ + CTL N:4
Lmna flx/flxmcm Sun -/- + TMX N:4
Lmna flx/flxmcm Sun -/- + CTL N:3
Lmna flx/flxWT Sun -/- + TMX N:3
Lmna +/+mcm Sun -/- + TMX N:3
EF%
Days after TMX injection
3
FS%
Days after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

Fig. 30A

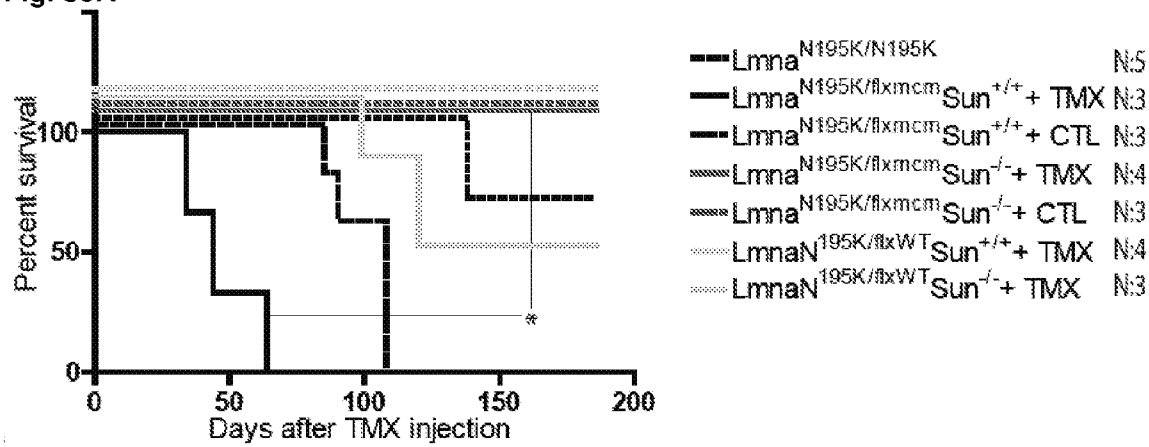


Fig. 30B

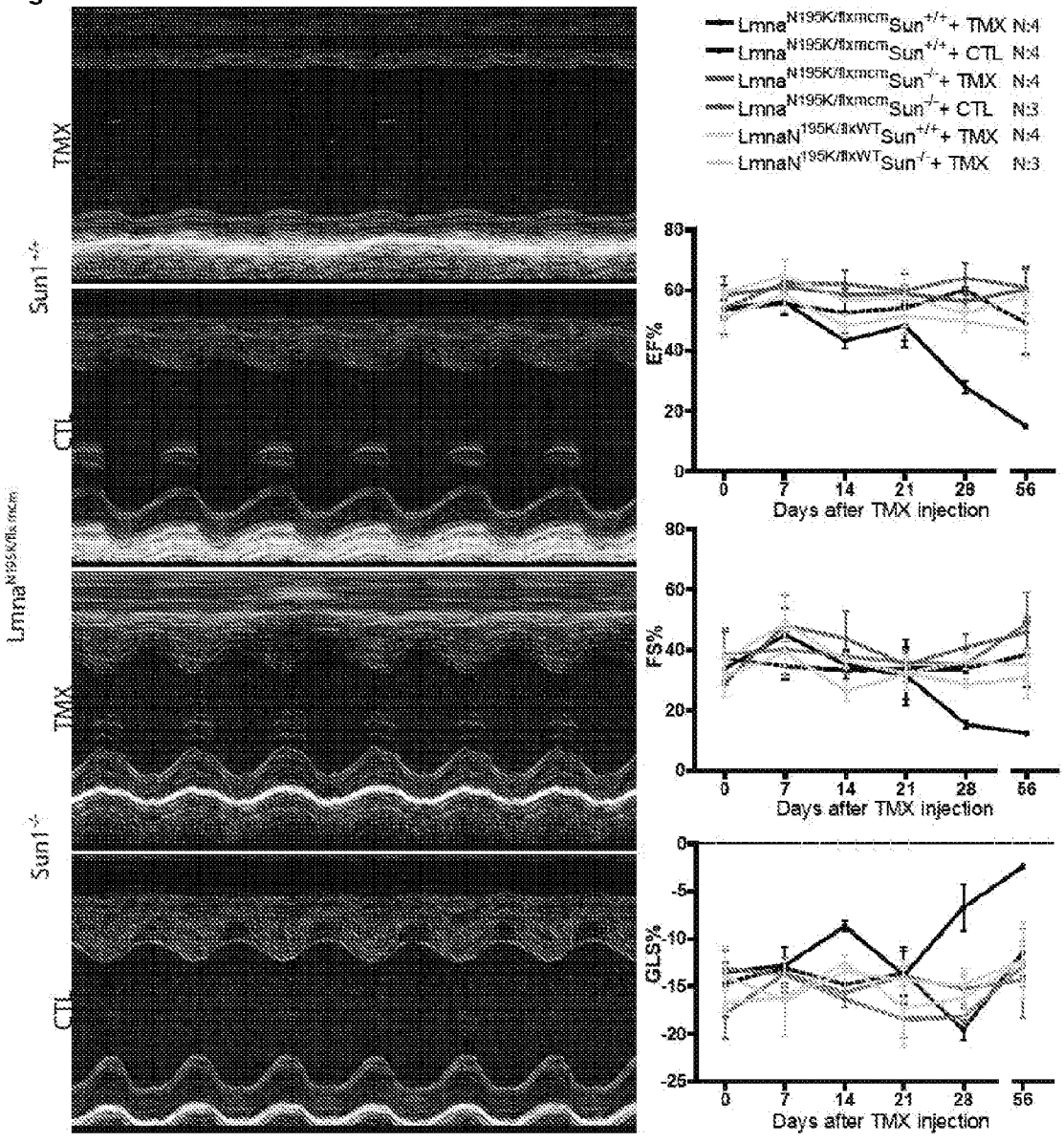


Fig. 31A

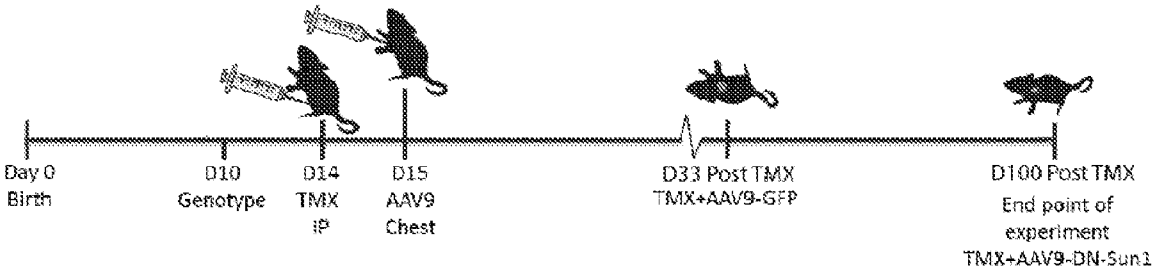


Fig. 31B

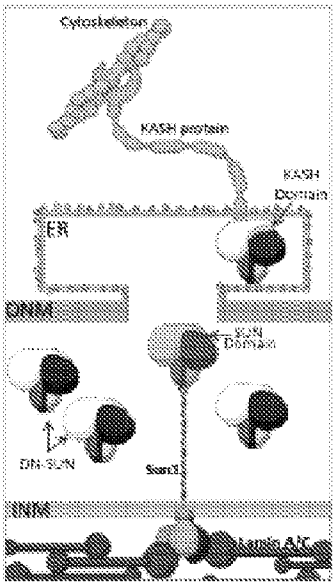


Fig. 31C

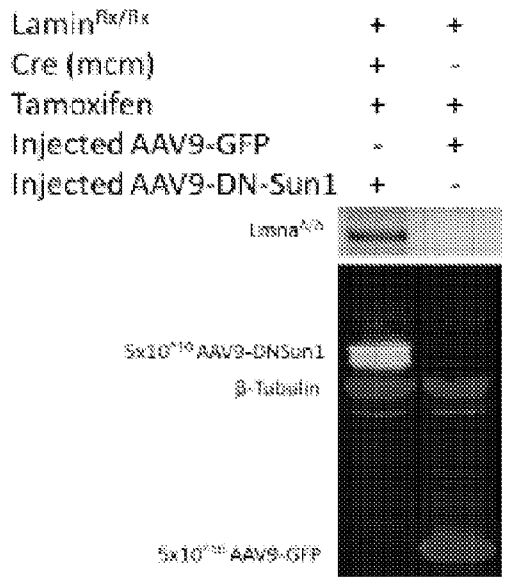


Fig. 31D

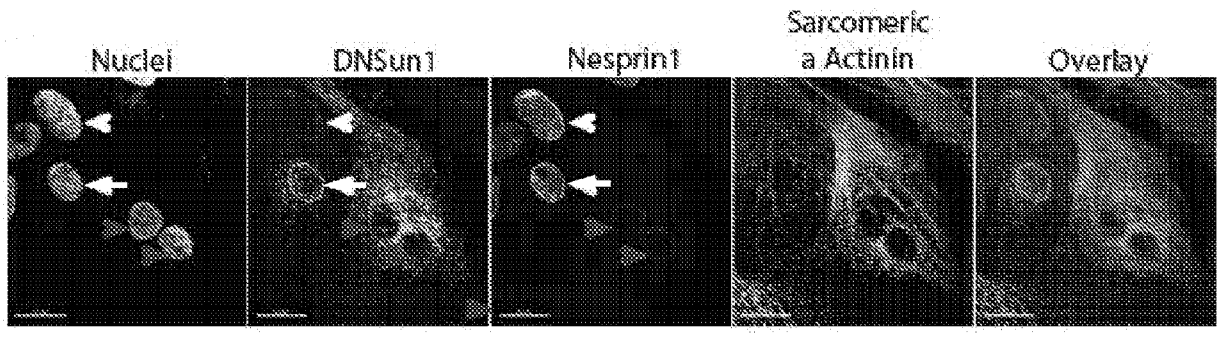


Fig. 31E (i)

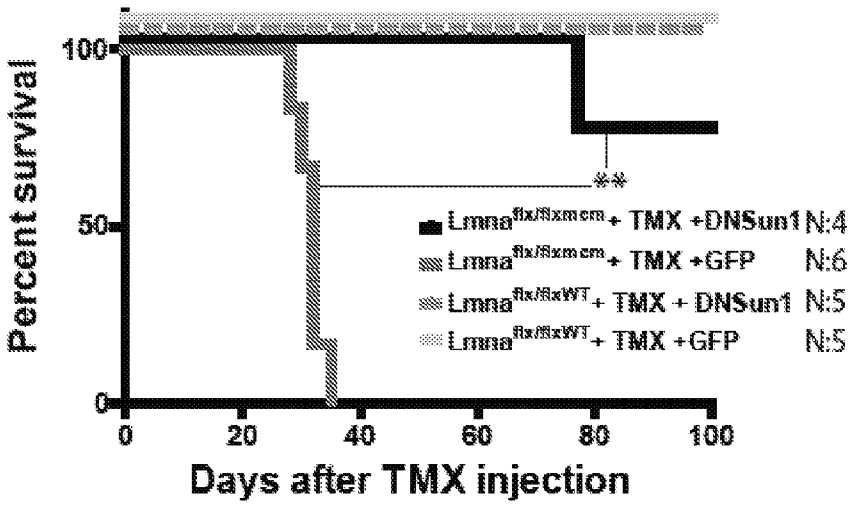


Fig. 31E (ii)

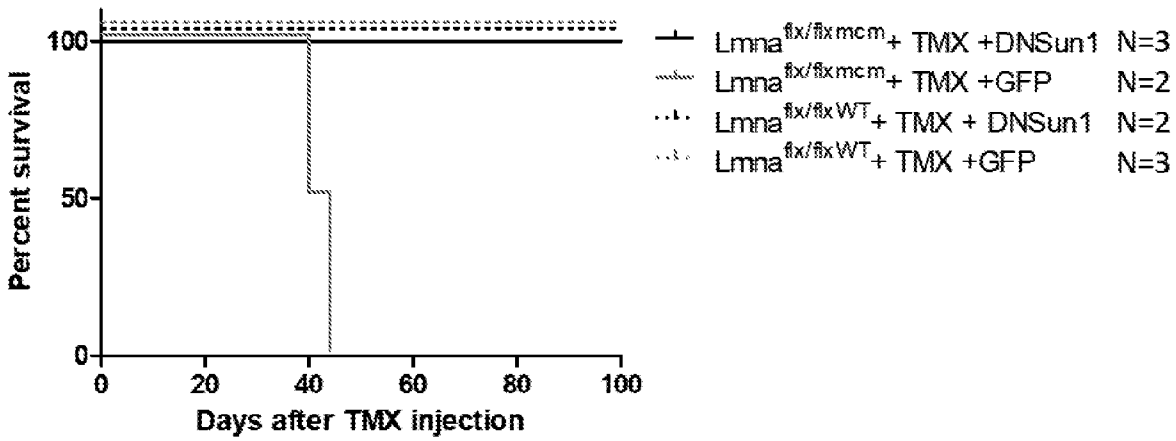


Fig. 31F

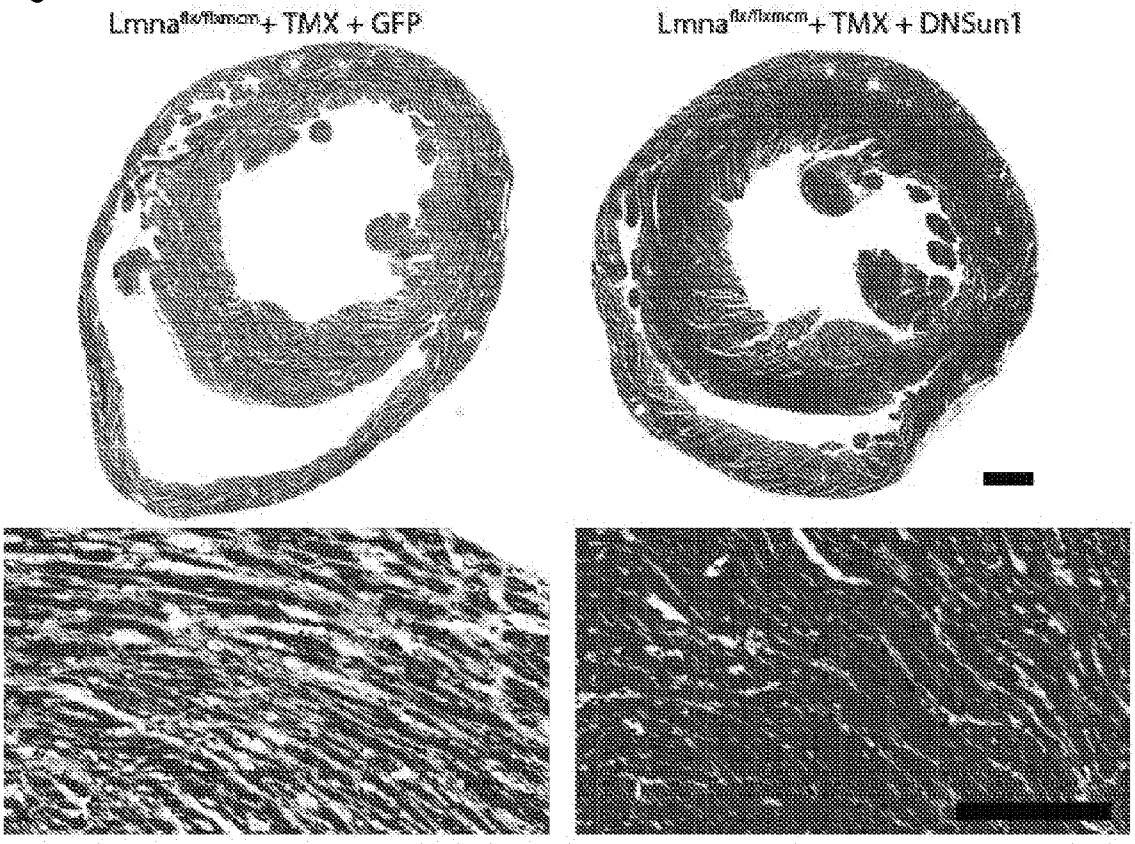


Fig. 31G

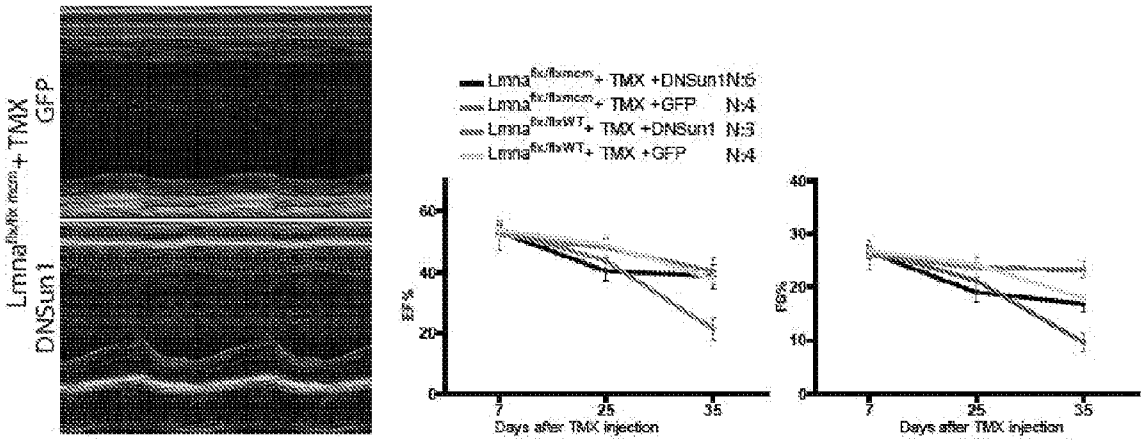


Fig. 32A

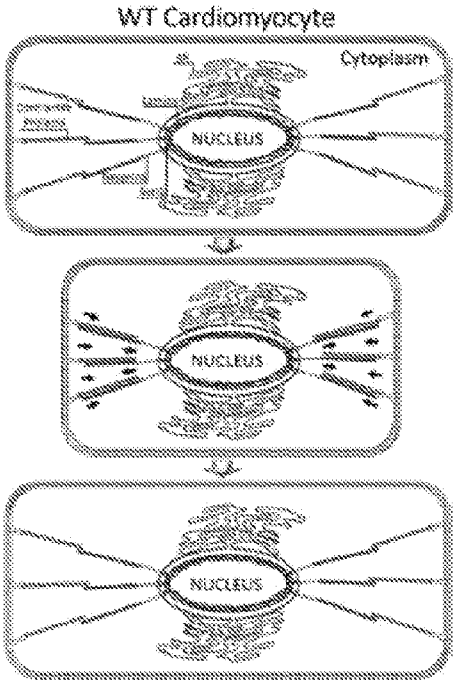


Fig. 32B

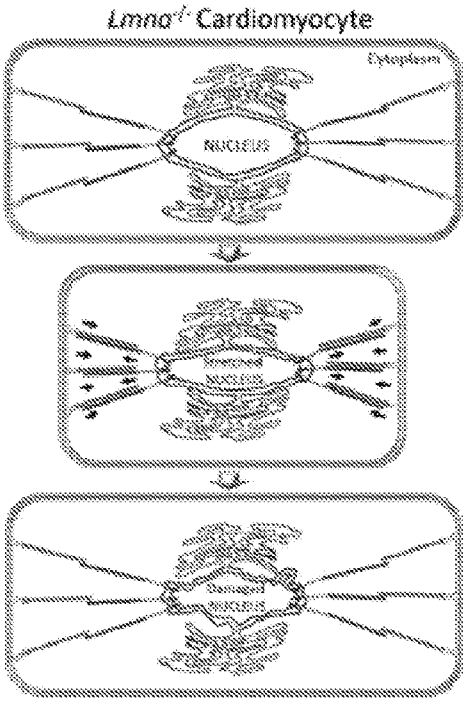


Fig. 32C

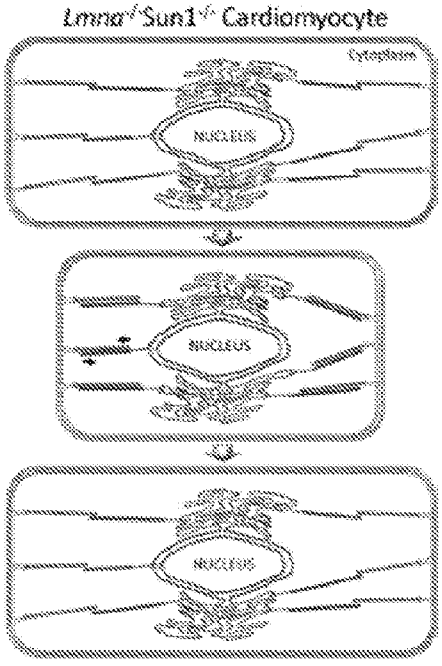


Fig. 32D

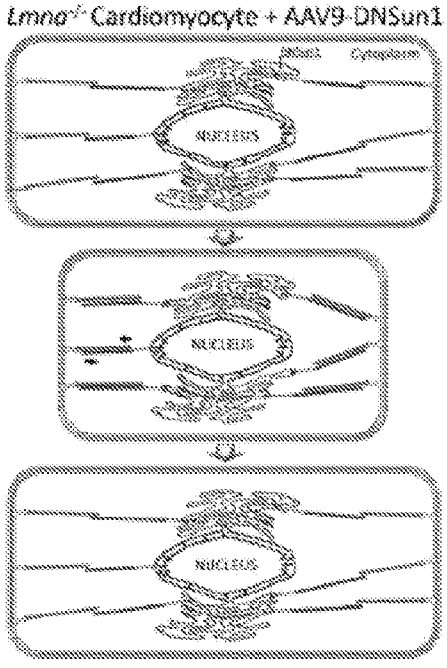


Fig. 33

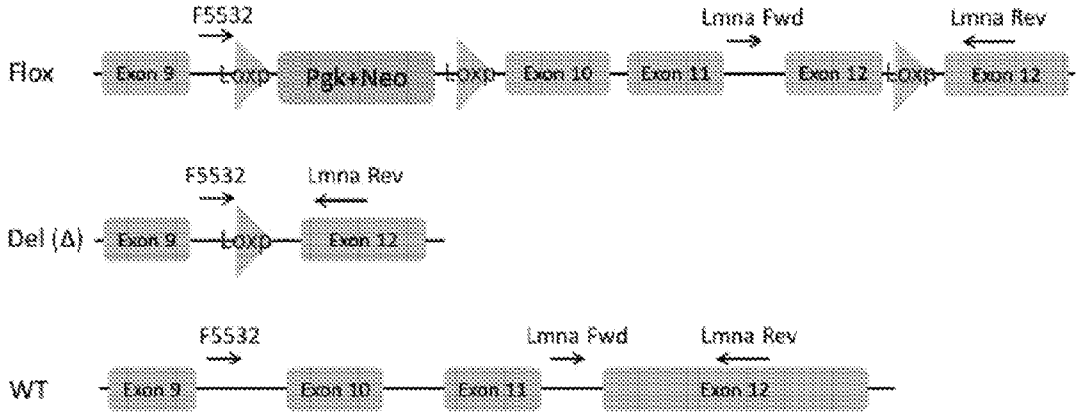


Fig. 34

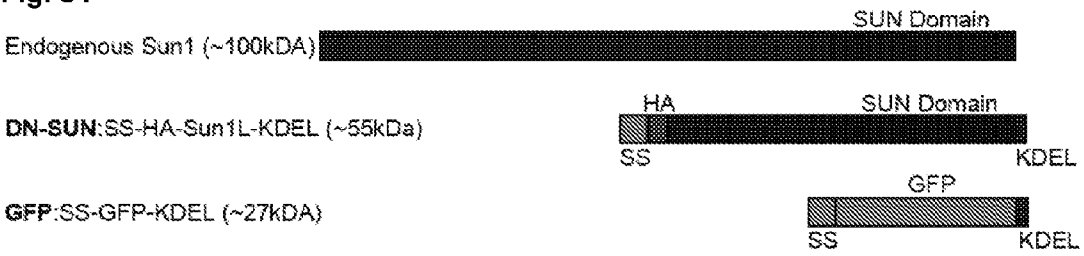


Fig. 35

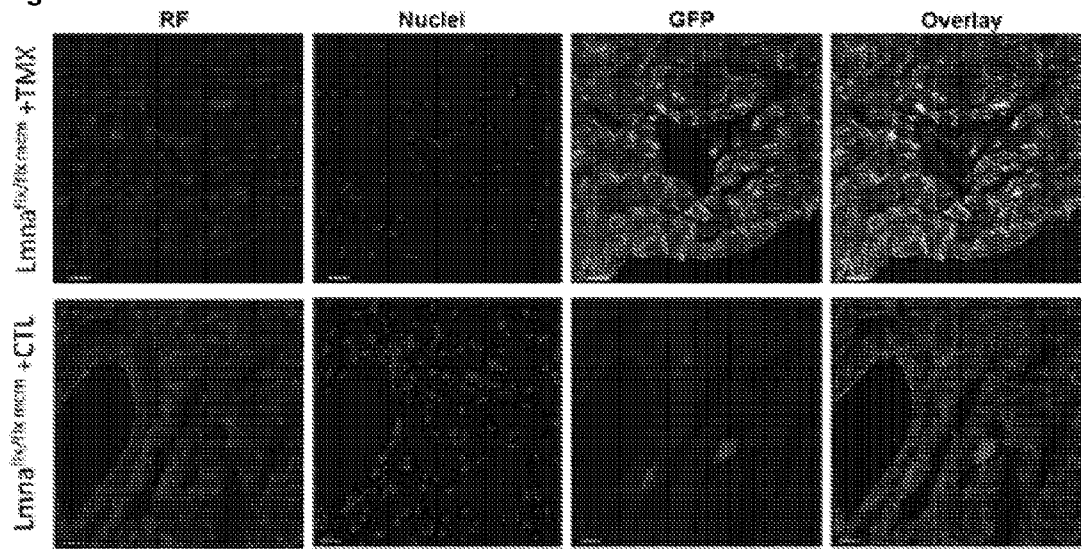
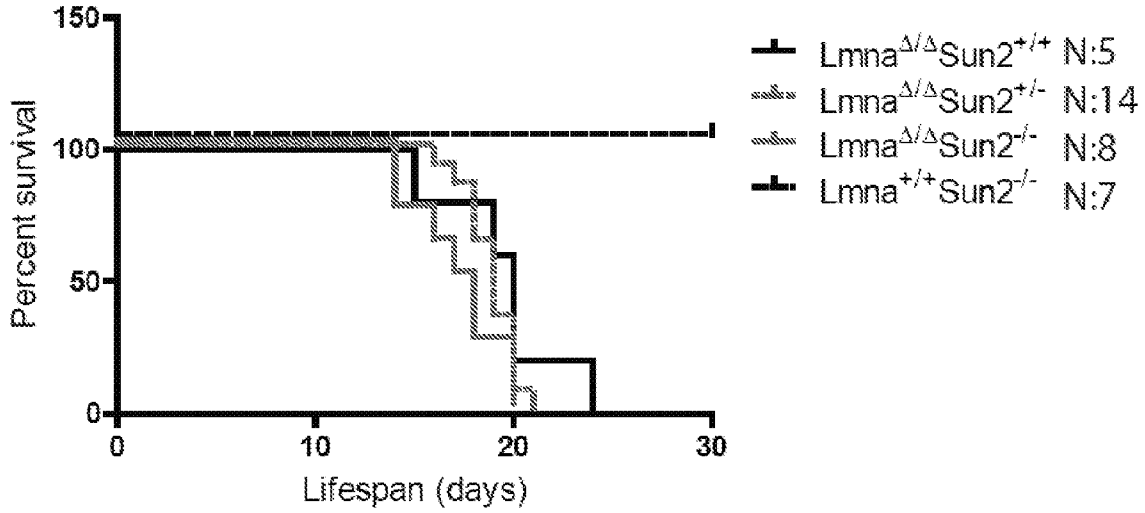


Fig. 36


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

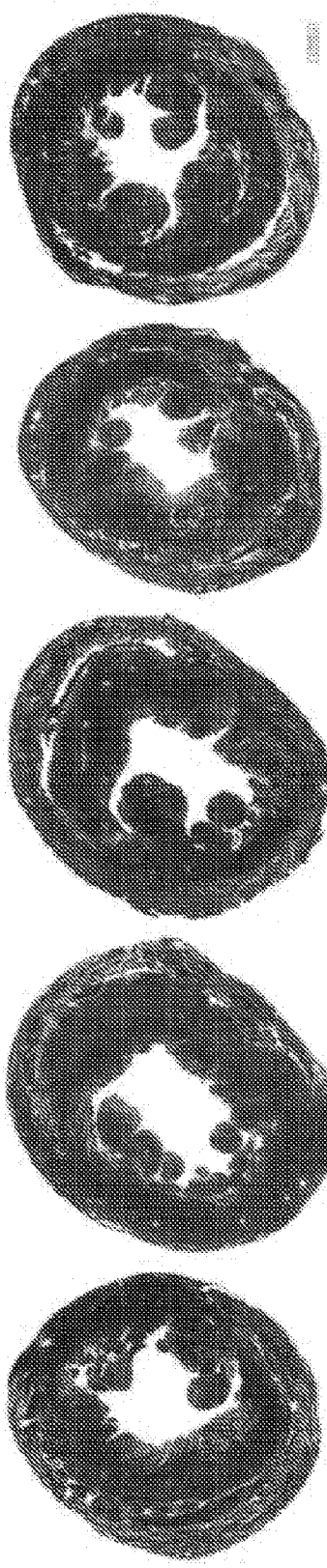

Lmna+/+ nscmSun1-/- + TMX
Lmnaflx/flx WTSun1-/- + TMX
Lmnaflx/flx nscmSun1-/- + CTL
Lmnaflx/flx nscmSun1-/- + TMX

Fig. 37A

Fig. 37C

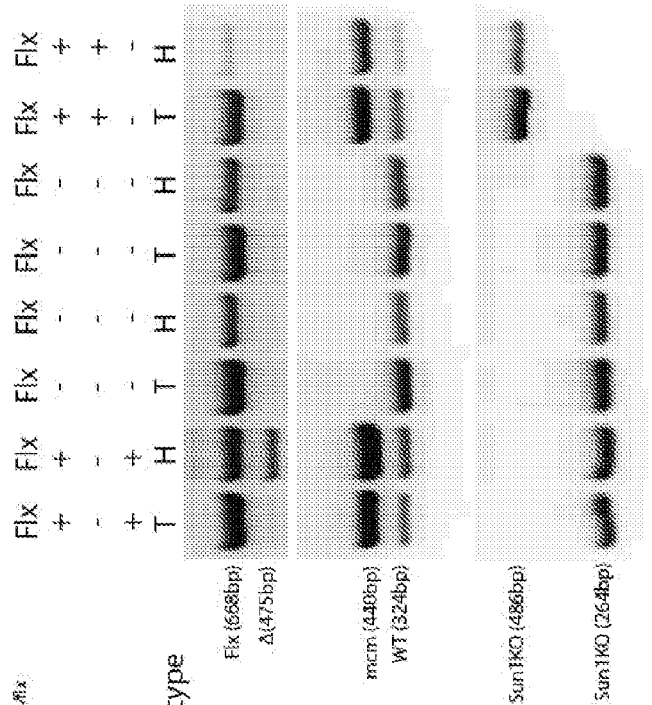


Fig. 37B

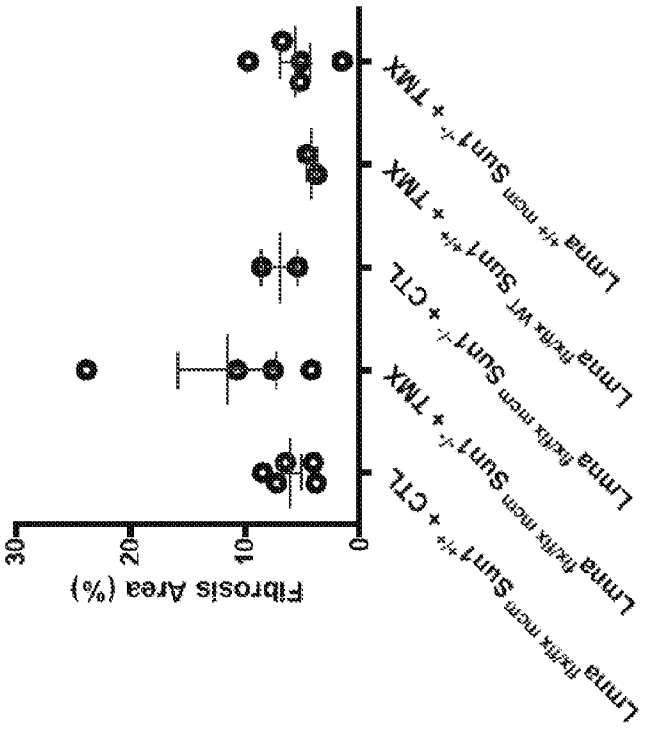


Fig. 37D

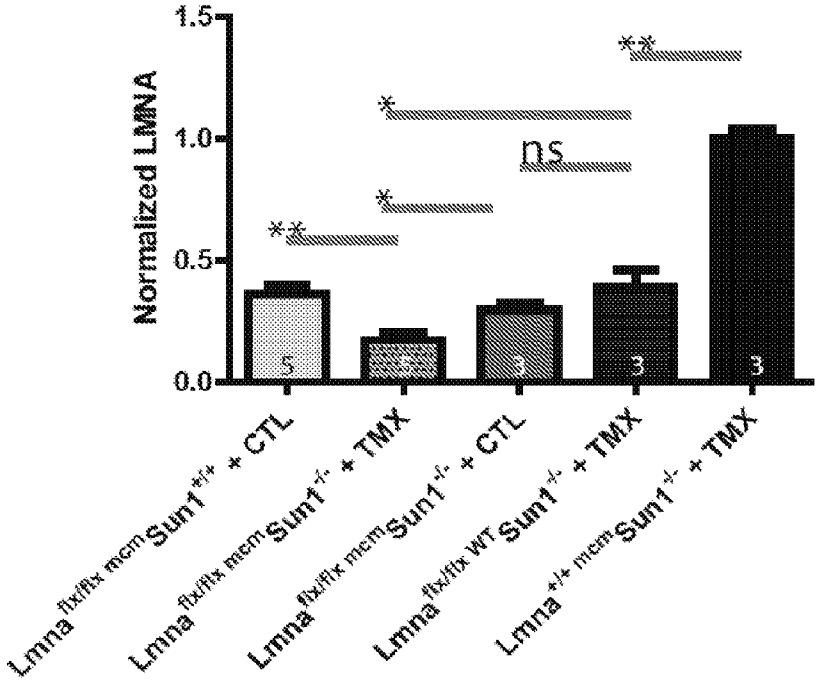

Normalized LMNA
1.5
1.0
0.5
0.0
**
*
ns
*
**
5
5
3
3
3
Lmna fl/fl mcm Sun1 +/+ + CTL
Lmna fl/fl mcm Sun1 -/- + TMX
Lmna fl/fl mcm Sun1 -/- + CTL
Lmna fl/fl WT Sun1 -/- + TMX
Lmna +/+ mcm Sun1 -/- + TMX

Fig. 37E

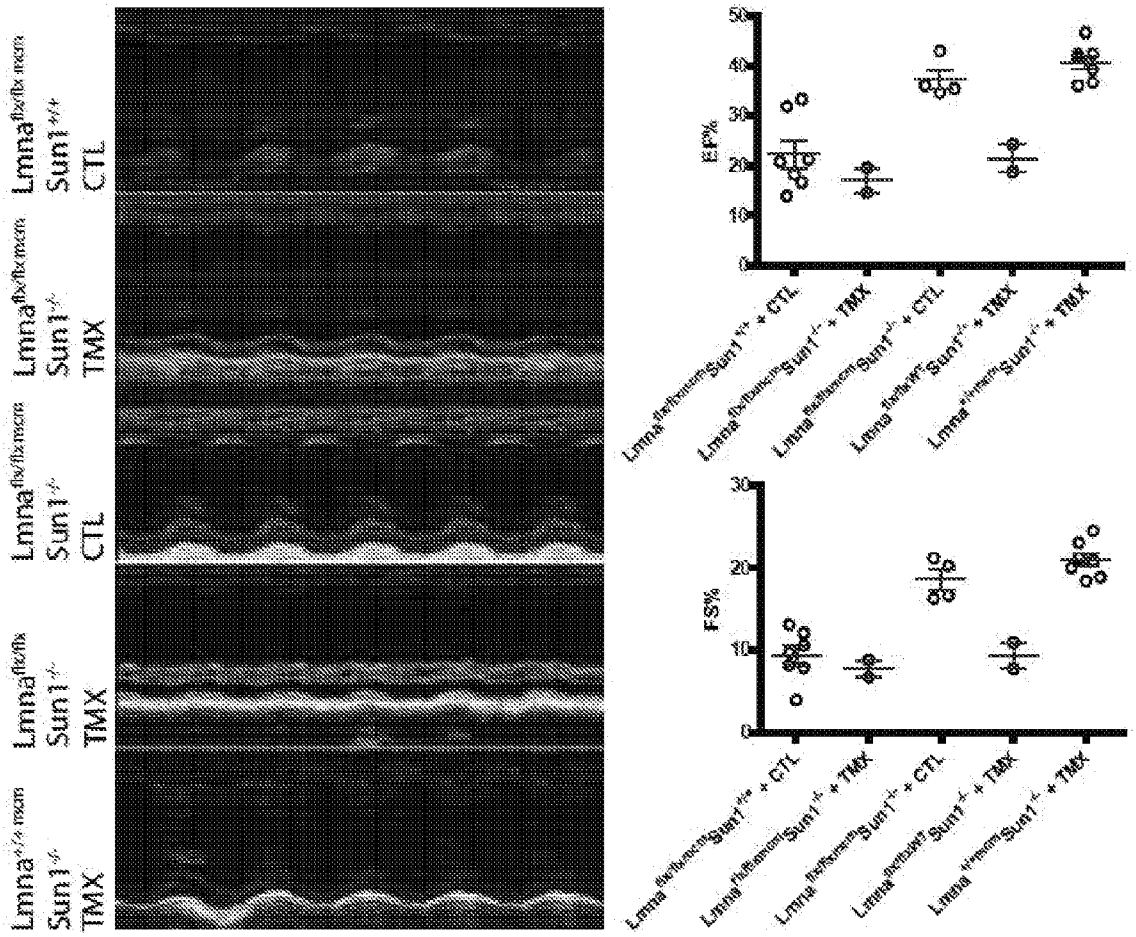

Lmna flx/flx mcm Sun1 +/+ CTL
Lmna flx/flx mcm Sun1 -/- TMX
Lmna flx/flx mcm Sun1 -/- CTL
Lmna flx/flx Sun1 -/- TMX
Lmna -/- mcm Sun1 -/- TMX
EF%
FS%
Lmna flx/flx mcm Sun1 +/+ + CTL
Lmna flx/flx mcm Sun1 -/- + TMX
Lmna flx/flx mcm Sun1 -/- + CTL
Lmna flx/flx Sun1 -/- + TMX
Lmna -/- mcm Sun1 -/- + TMX

Fig. 38

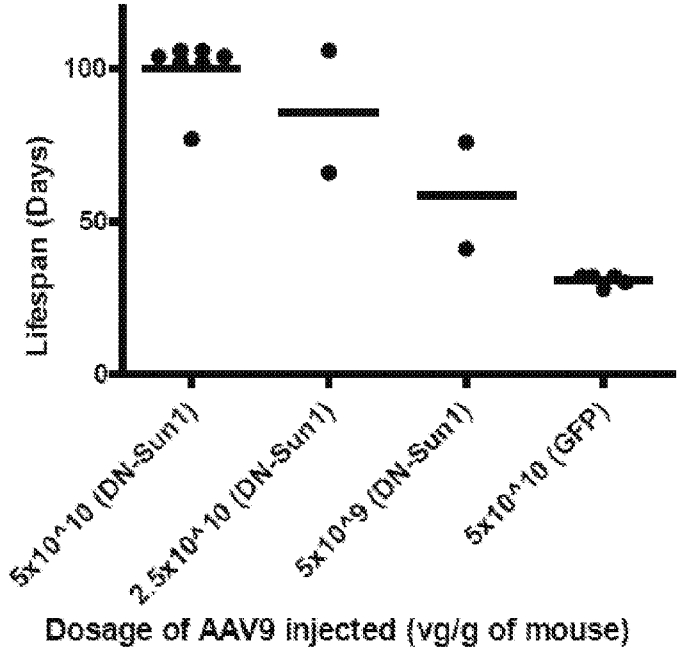

100
50
0
Lifespan (Days)
5x10^10 (DN-Sun1)
2.5x10^10 (DN-Sun1)
5x10^9 (DN-Sun1)
5x10^10 (GFP)
Dosage of AAV9 injected (vg/g of mouse)

Fig. 39A

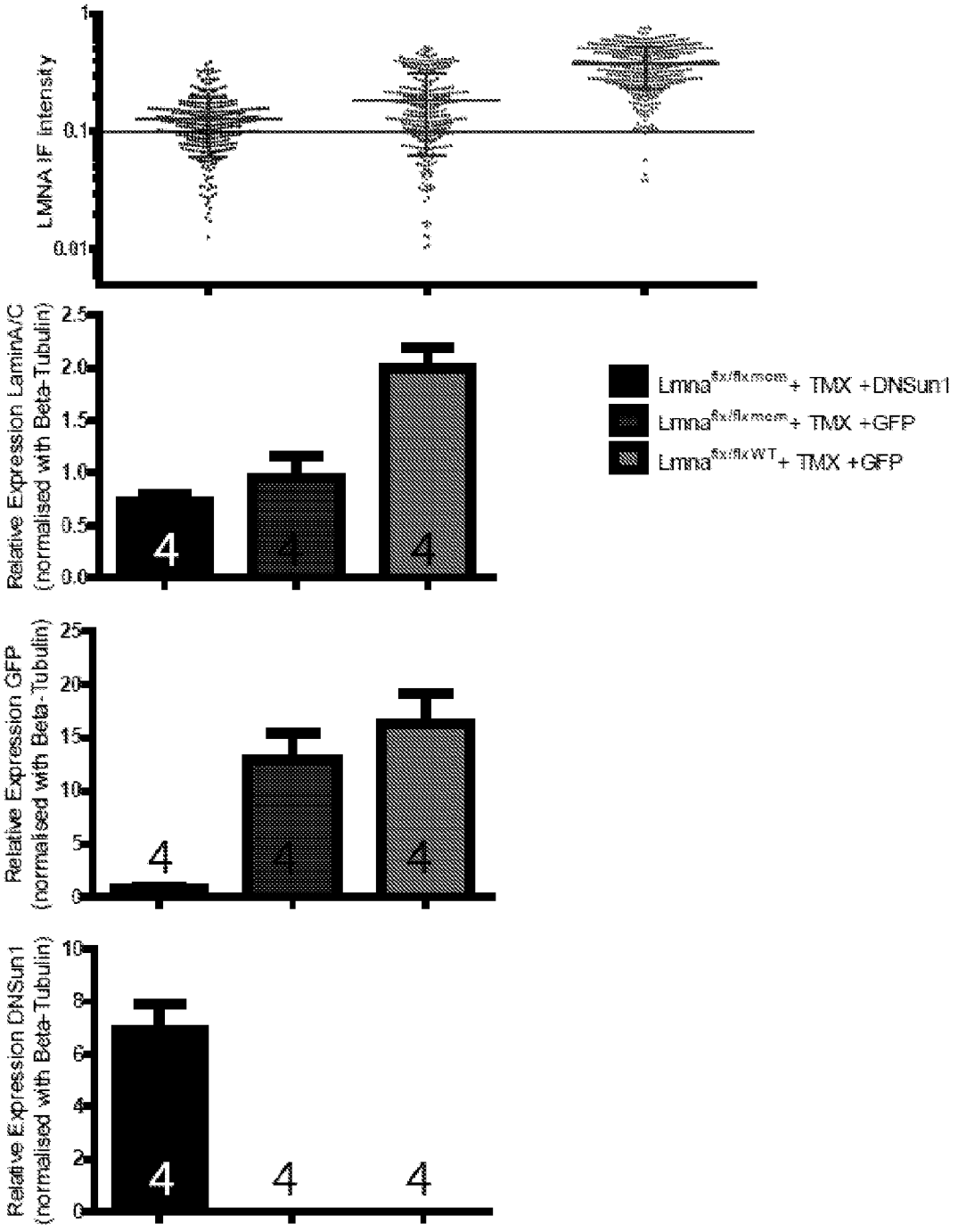

LMNA IF Intensity
1
0.1
0.01
Relative Expression LaminA/C (normalised with Beta-Tubulin)
2.5
2.0
1.5
1.0
0.5
0.0
4
4
4
Lmna$^{fx/fxmcm}$ + TMX +DNSun1
Lmna$^{fx/fxmcm}$ + TMX +GFP
Lmna$^{fx/fxWT}$ + TMX +GFP
Relative Expression GFP (normalised with Beta-Tubulin)
25
20
15
10
5
0
4
4
4
Relative Expression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

Fig. 39B

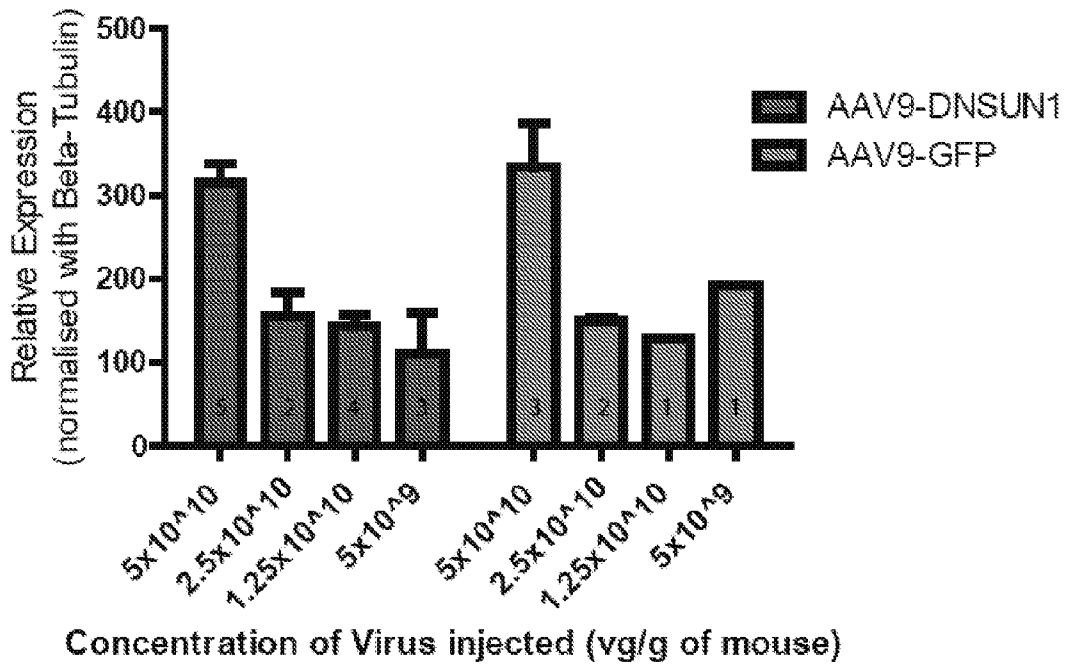


Fig. 39C

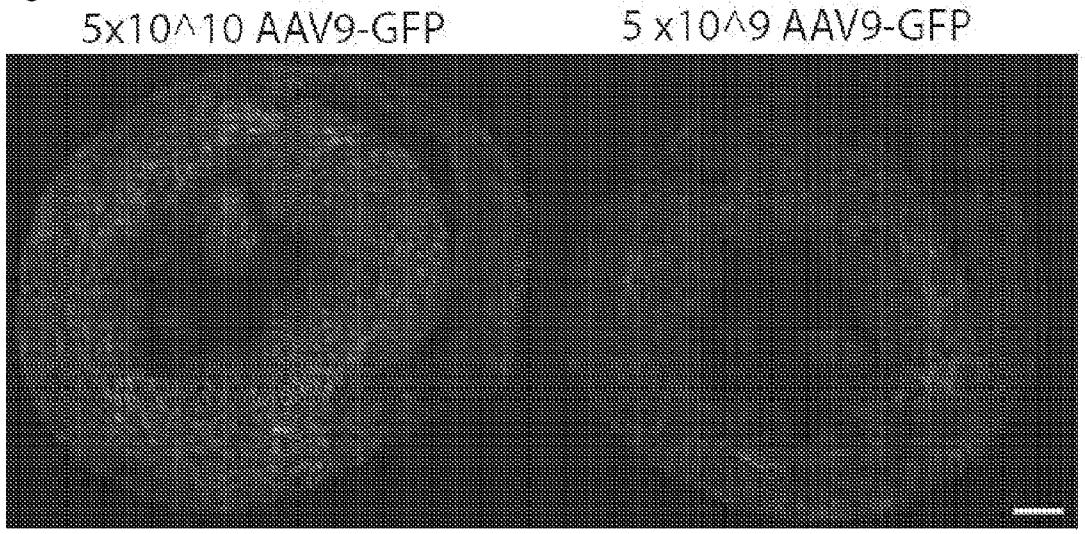


Fig. 40A

```
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     CACAGAAGCGcaagcacatatcattgtgaaacaatgctctgaagctgtactcccaaagacaa  2216
SEQ ID NO:70   Sun1_plus4   CACAGAAGCGcaagcacatatcattgtgaacaatgctctgaagctgtactcccaaagacaa  2220
SEQ ID NO:71   Sun1_del7    CACAGAAGCGcaagcacatatcattgtgaacaatgctctgaagctgtactcccaaagacaa  2209
                             ************************************************************
```

Fig. 40B

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

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

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

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

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

Fig. 40C

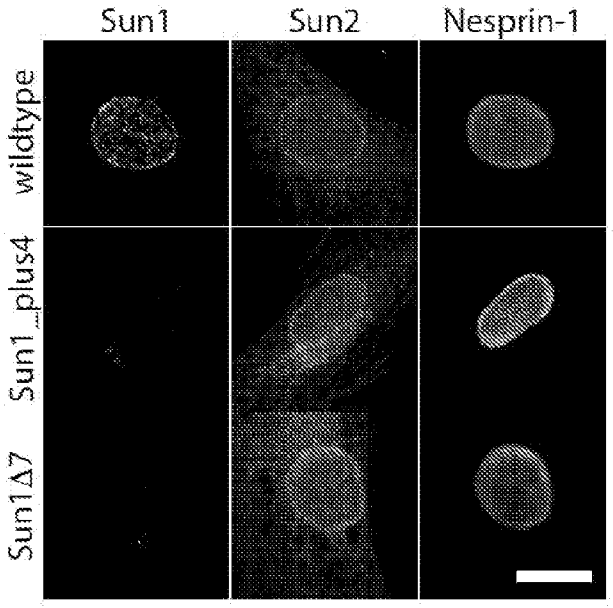


Fig. 41

A

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

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

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

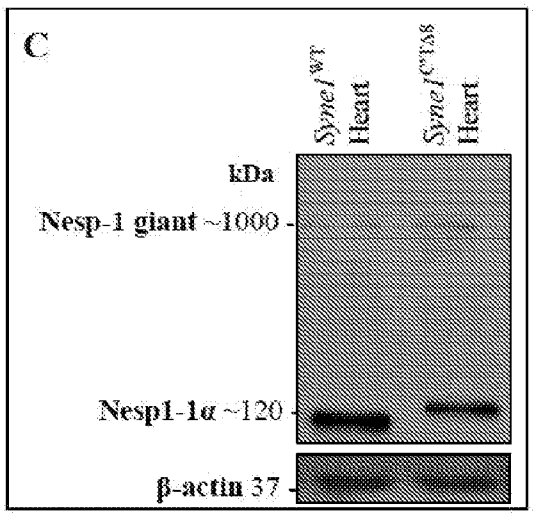
B

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

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

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

Fig. 41 (continued)



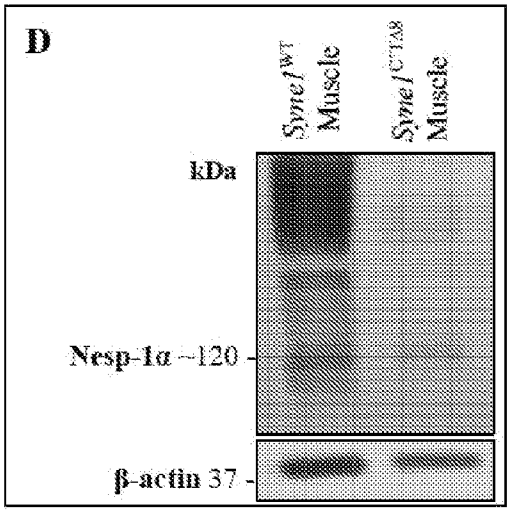


Fig. 42

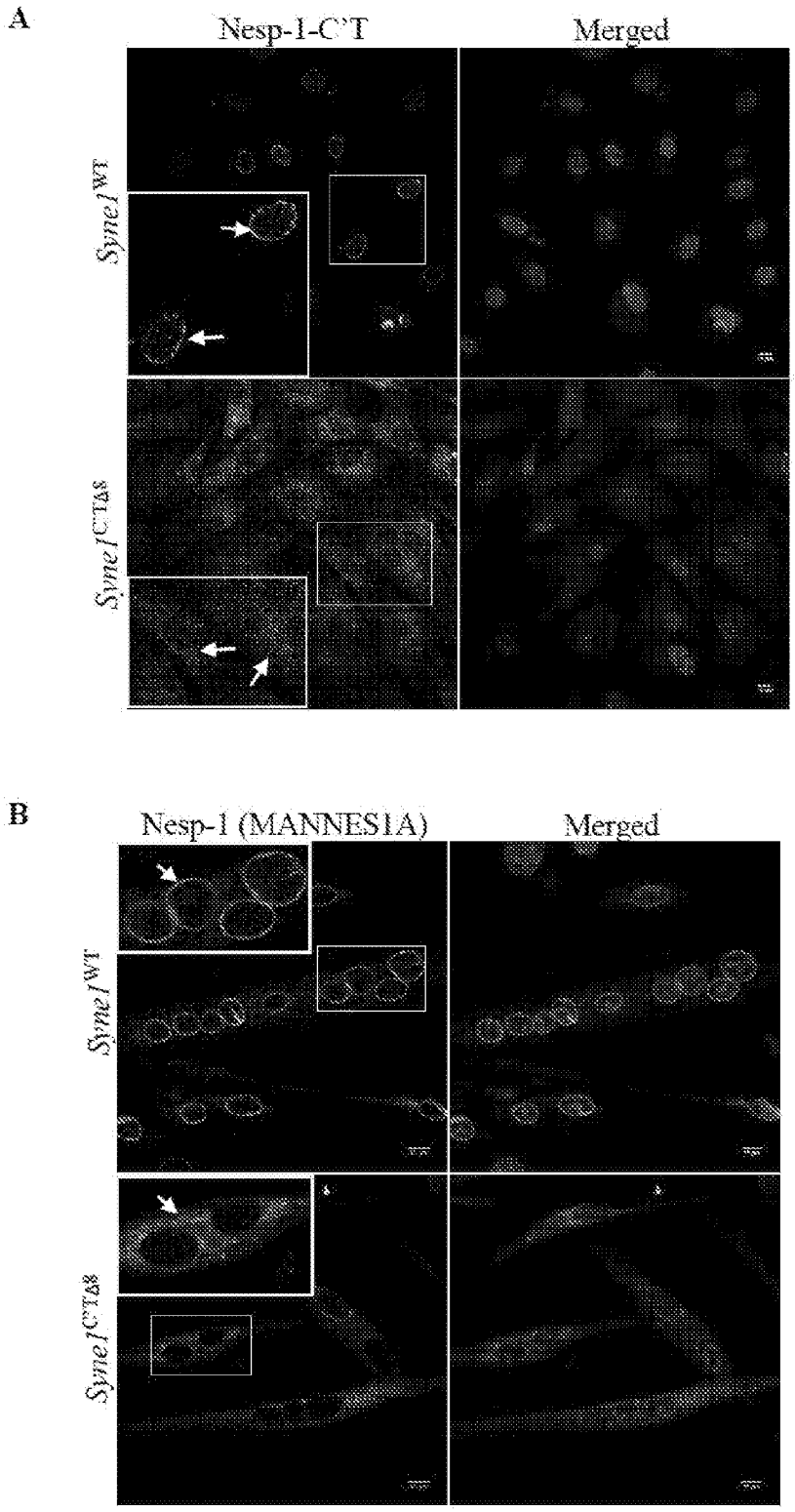

A
Nesp-1-C'T
Merged
Syne1WT
Syne1CTΔs
B
Nesp-1 (MANNES1A)
Merged
Syne1WT
Syne1CTΔs

Fig. 43

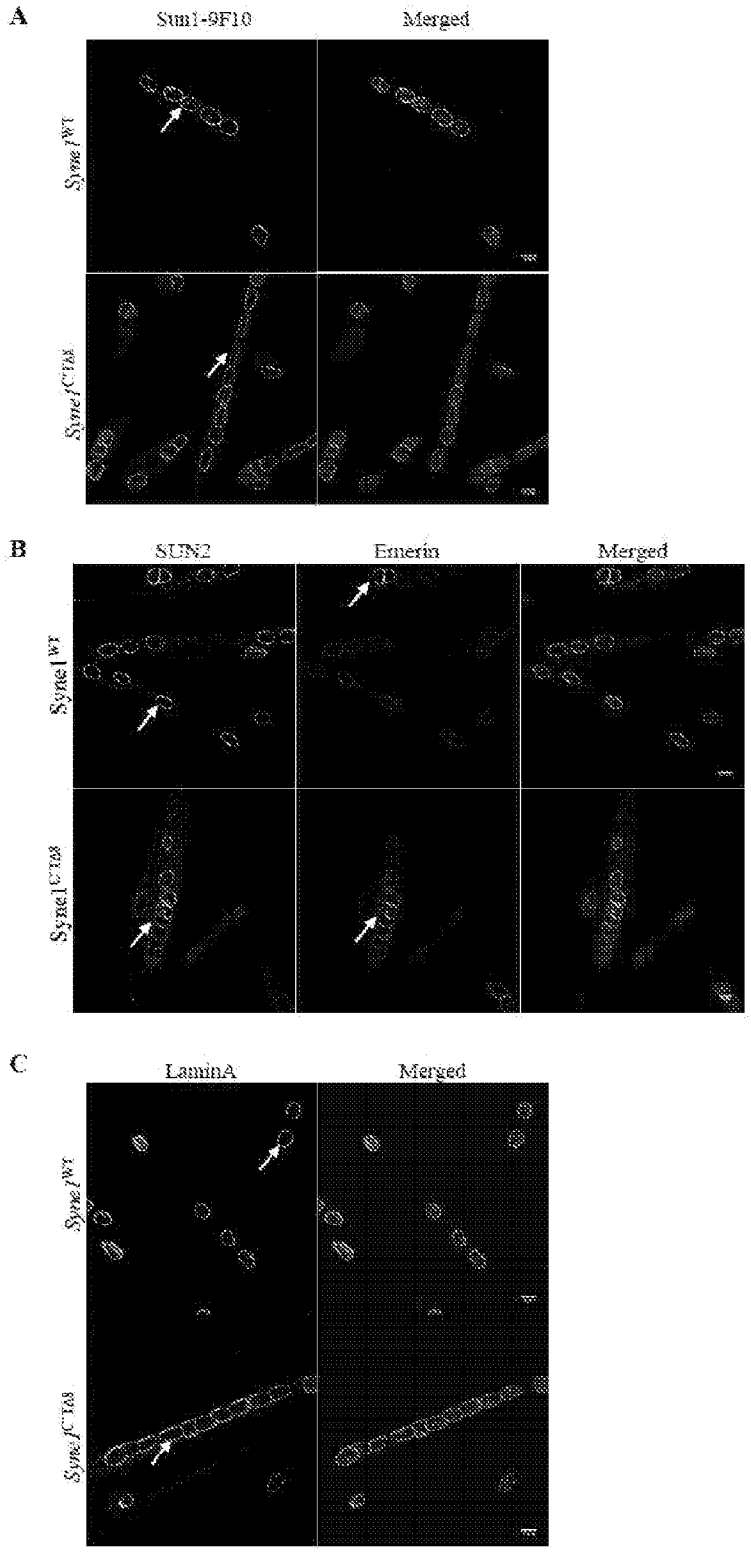

A
Sun1-9F10
Merged
Syne1wt
Syne1CTA
B
SUN2
Emerin
Merged
Syne1wt
Syne1CTA
C
LaminA
Merged
Syne1wt
Syne1CTA

Fig. 44

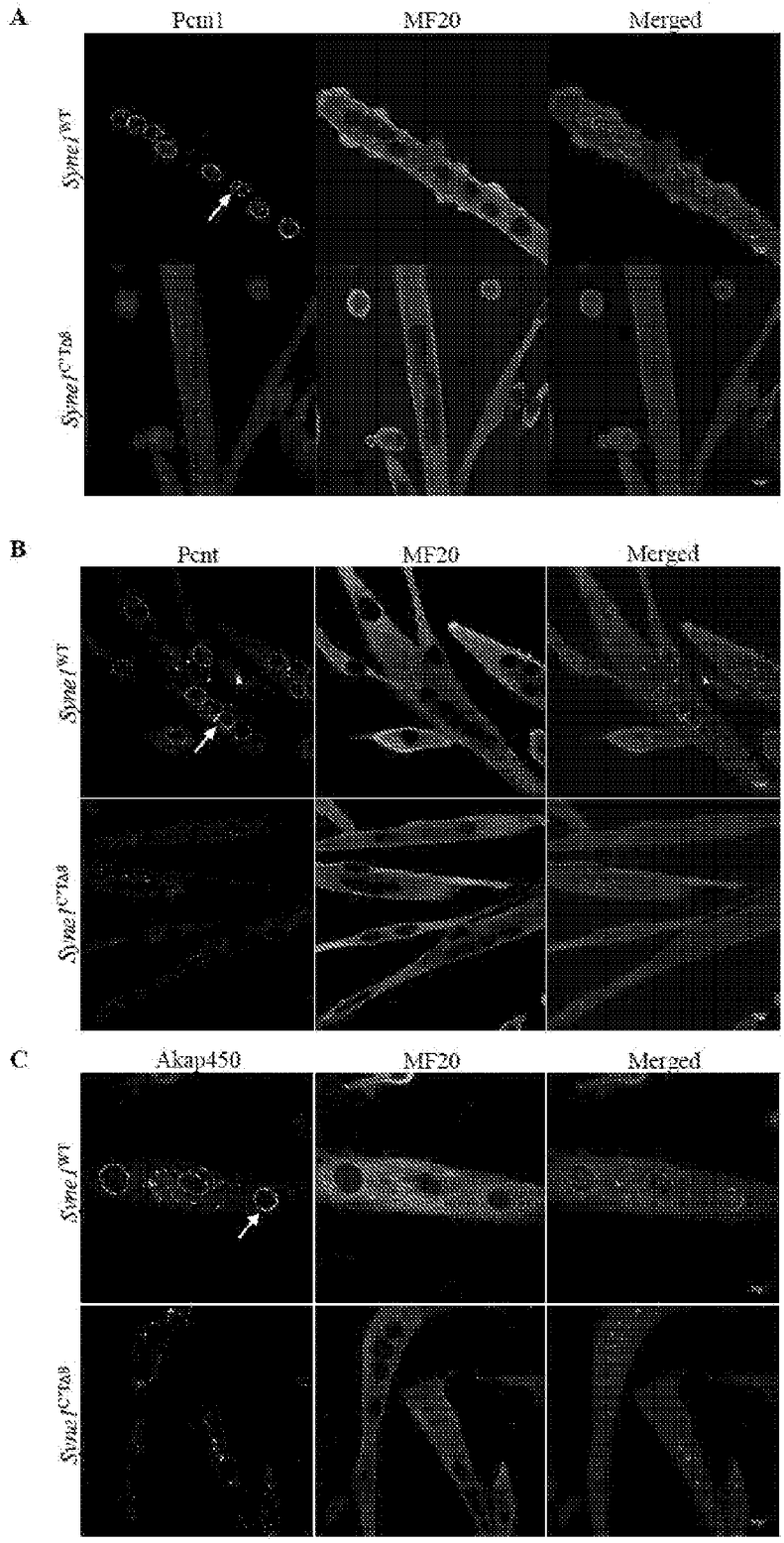
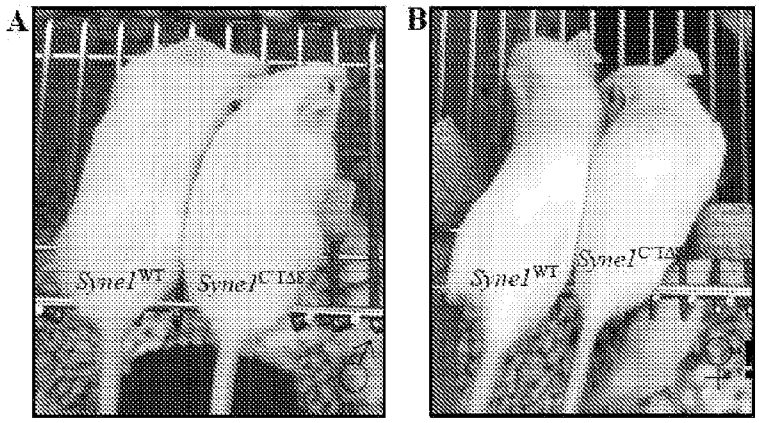

A
Pcm1
MF20
Merged
Syne1WT
Syne1CTa8
B
Pcnt
MF20
Merged
Syne1WT
Syne1CTa8
C
Akap450
MF20
Merged
Syne1WT
Syne1CTa8

Fig. 45


A
B
Syne1WT
Syne1CTΔ8
Syne1WT
Syne1CTΔ8

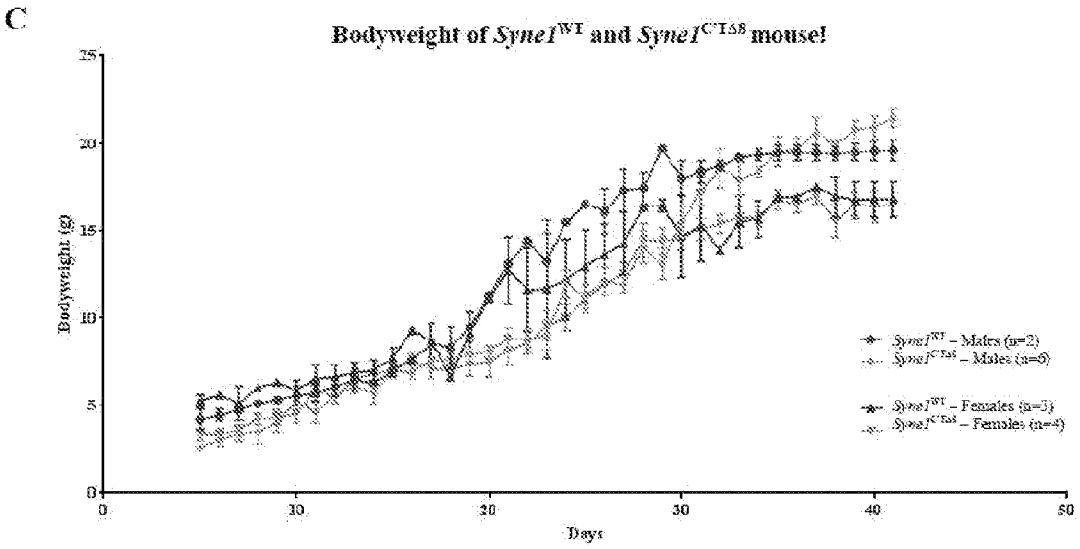

C
Bodyweight of Syne1WT and Syne1CTΔ8 mouse!
Bodyweight (g)
Days
0
5
10
15
20
25
0
10
20
30
40
50
Syne1WT – Males (n=2)
Syne1CTΔ8 – Males (n=6)
Syne1WT – Females (n=3)
Syne1CTΔ8 – Females (n=4)

Fig. 46A

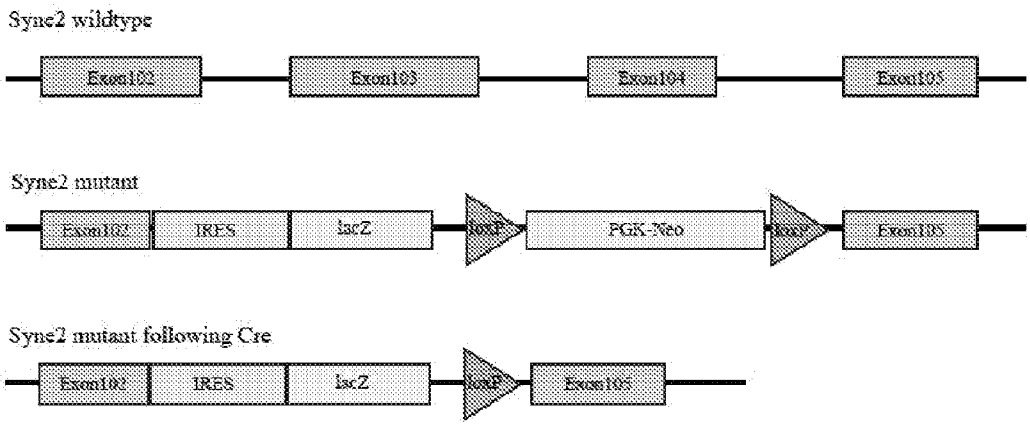


Fig. 46B

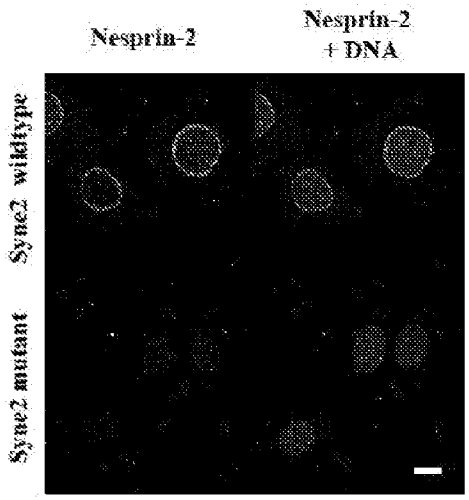


Fig. 46C

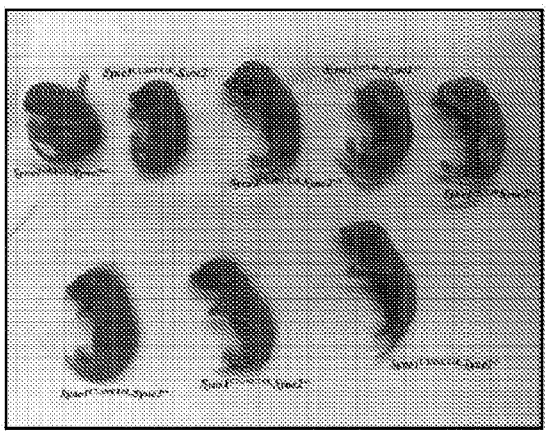
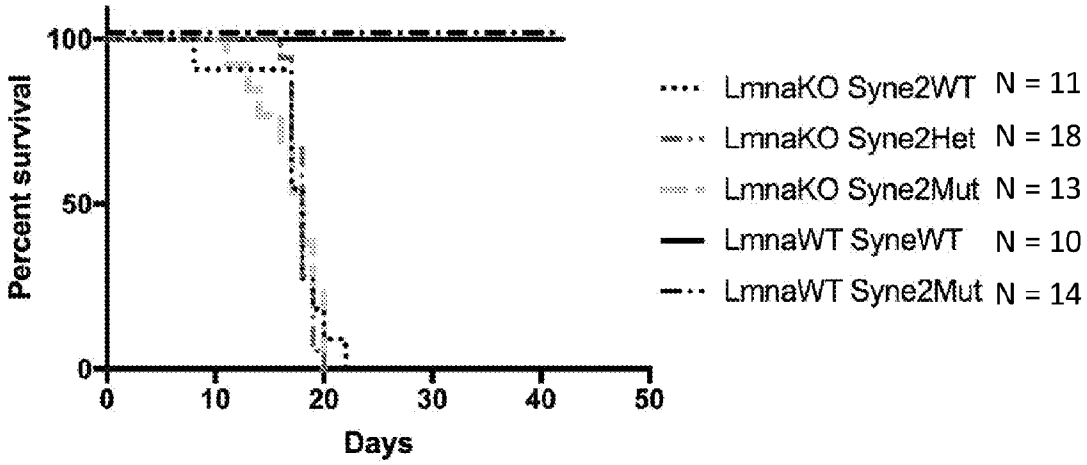


Fig. 47


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