In previous lesson

In HGPS:

- the mutation leads to alternative splicing in exon 11 and to the loss of 50 amino acids in prelamin A
- ZMPSTE24 enzyme not cleave the farnesylated C-terminus of this protein.
- This mutant protein, called progerin, remains permanently farnesylated
- Alteration of lamin A processing induce nuclear shape and protein complexes dysorganization.

Hutchinson-Gilford progeria syndrome (HGPS)
PROGERIA

is caused by a **point mutation in the LMNA gene** that activates a cryptic donor splice site and yields a truncated form of prelamin A called progerin

LAMINA ALTERATIONS INDUCE DISEASE

Progeria, or Hutchinson–Gilford progeria syndrome (HGPS), is a rare, fatal genetic disease characterized by an **appearance of accelerated aging in children**.

This syndrome is typically caused by mutations **in codon 1824, cryptic splincing site (p.G608G, no change aminoacid)** of the LMNA, leading to the production of a mutated form of lamin A precursor called **progerin**.

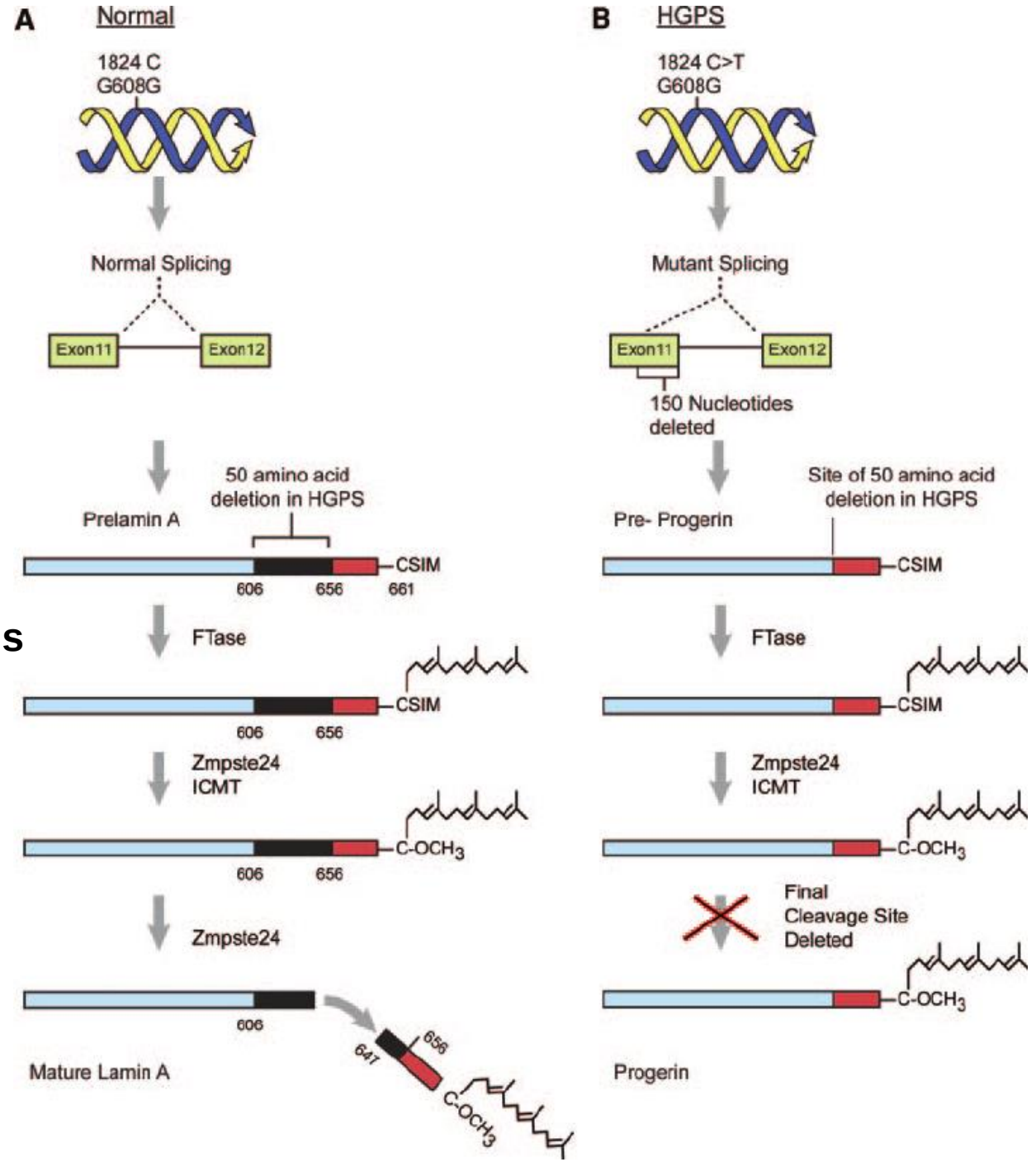
Progerin accumulates in cells causing progressive molecular defects, including nuclear shape abnormalities, chromatin disorganization, damage to DNA and delays in cell proliferation.

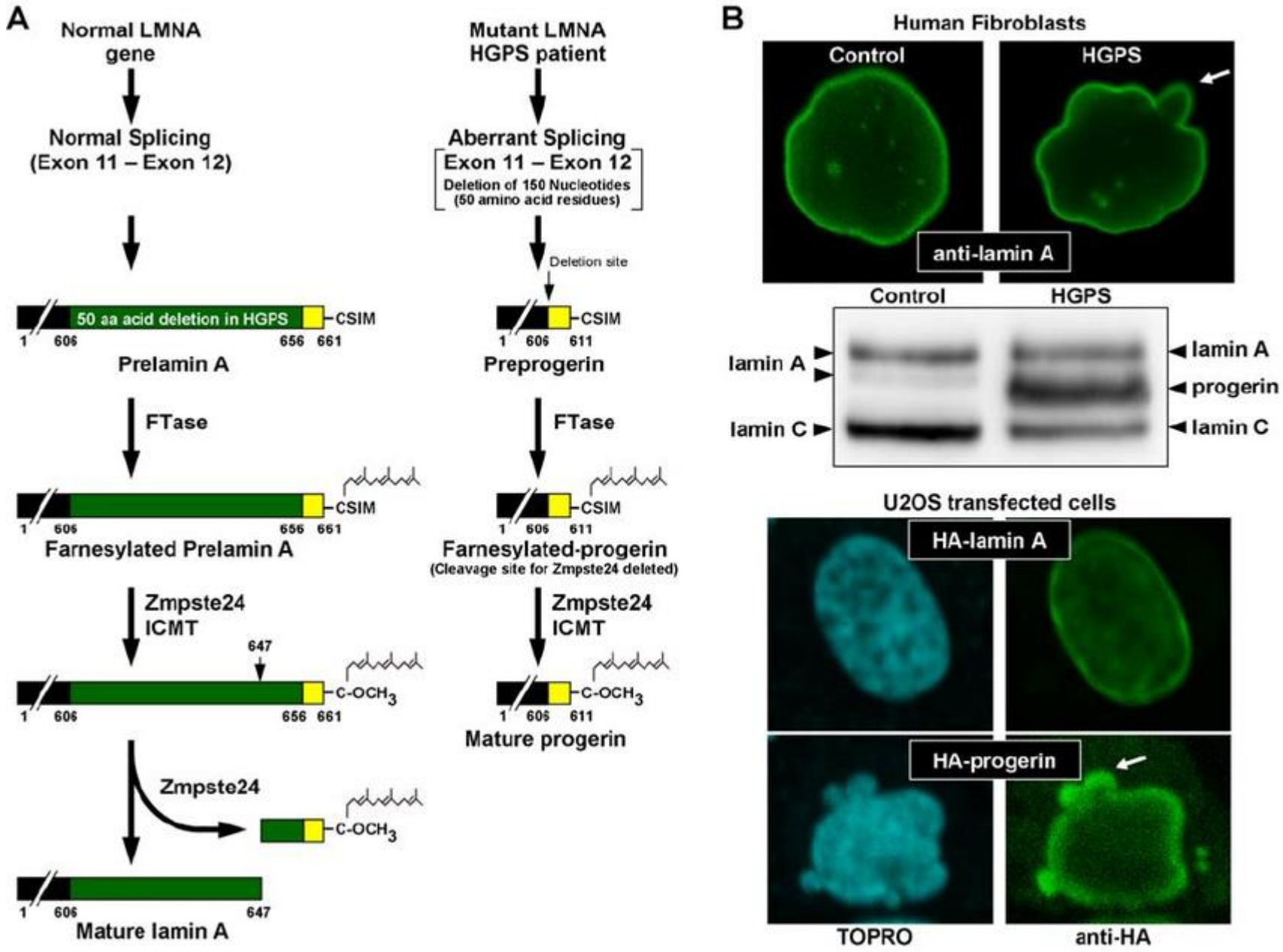
PRELAMININ A

farnesylated C-terminus

ZMPSTE24 enzyme cleave the farnesylated C-terminus

LAMININ A

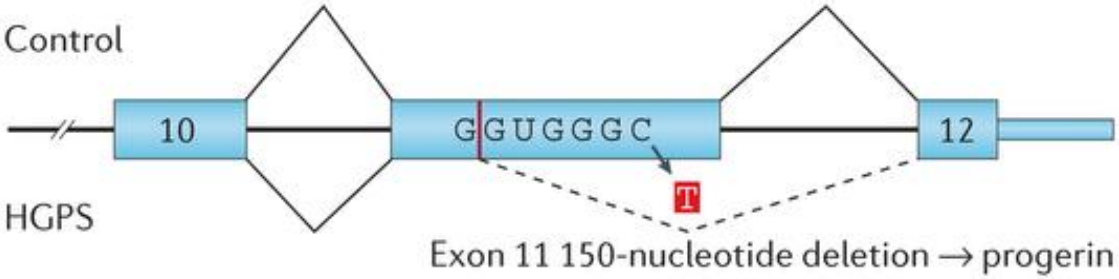


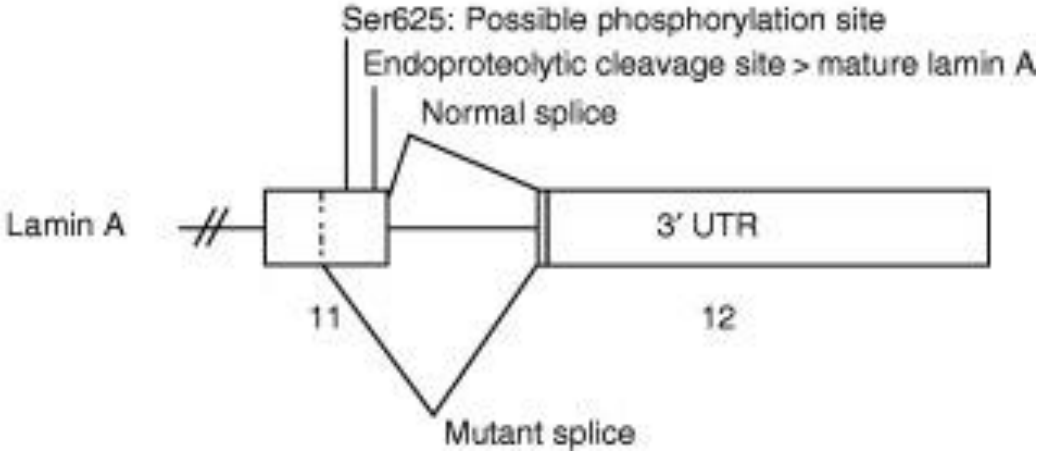

A
Normal LMNA gene
Normal Splicing (Exon 11 – Exon 12)
50 aa acid deletion in HGPS
CSIM
1 606 656 661
Prelamin A
FTase
CSIM
1 606 656 661
Farnesylated Prelamin A
Zmpste24 ICMT
647
C-OCH3
1 606 656 661
Zmpste24
C-OCH3
1 606 647
Mature lamin A
Mutant LMNA HGPS patient
Aberrant Splicing
Exon 11 – Exon 12
Deletion of 150 Nucleotides (50 amino acid residues)
Deletion site
CSIM
1 606 611
Preprogerin
FTase
CSIM
1 606 611
Farnesylated-progerin
(Cleavage site for Zmpste24 deleted)
Zmpste24 ICMT
C-OCH3
1 606 611
Mature progerin
B
Human Fibroblasts
Control
HGPS
anti-lamin A
Control
HGPS
lamin A
lamin C
lamin A
progerin
lamin C
U2OS transfected cells
HA-lamin A
HA-progerin
TOPRO
anti-HA

A splicing **defect in exon 11 of the LMNA** gene results in the **150 nucleotides** deletion in exon11 of Lamin A, creating protein lacking 50 amino acids of the carboxy-terminal globular domain.

Hutchinson-Gilford progeria syndrome (HGPS)

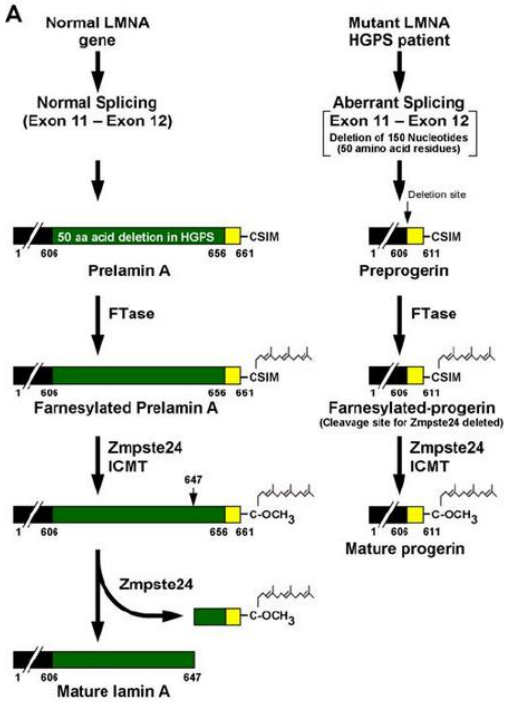
Alternative 5′ss (c. 1824C>T)





Loss of cleavage site for protease

THERAPHY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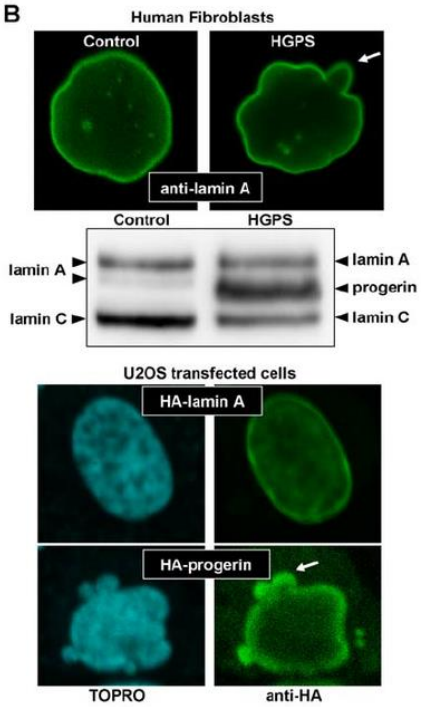
Deletion of 150 Nucleotides (50 amino acid residues)
Deletion site
Preprogerin
Farnesylated-progerin
(Cleavage site for Zmpste24 deleted)
Mature progerin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Human Fibroblasts
Control
HGPS
anti-lamin A
lamin A
lamin C
progerin
U2OS transfected cells
HA-lamin A
HA-progerin
TOPRO
anti-HA

Splicing-Directed Therapy in a New Mouse Model of Human Accelerated Aging

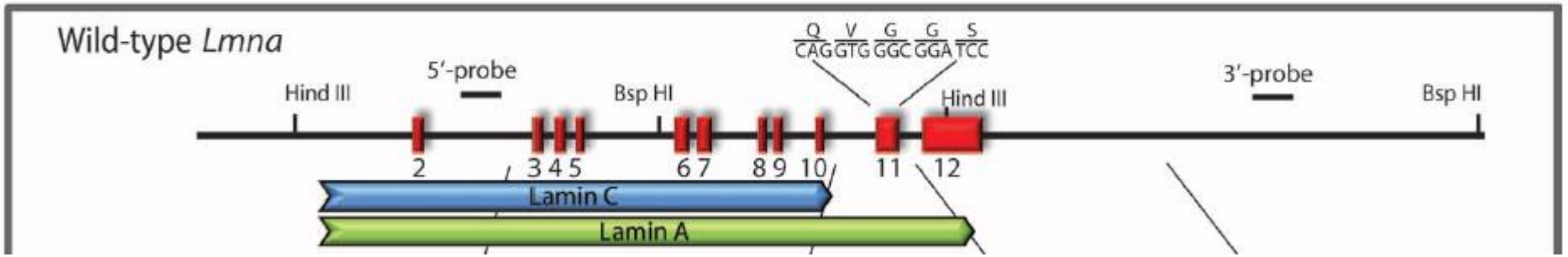
Fernando G. Osorio,[1] Claire L. Navarro,[2] Juan Cadiñanos,[1]* Isabel C. López-Mejía,[3] Pedro M. Quirós,[1] Catherine Bartoli,[2] José Rivera,[4] Jamal Tazi,[3] Gabriela Guzmán,[5] Ignacio Varela,[1] Danielle Depetris,[2] Félix de Carlos,[6] Juan Cobo,[6] Vicente Andrés,[4] Annachiara De Sandre-Giovannoli,[2,7] José M. P. Freije,[1] Nicolas Lévy,[2,7] Carlos López-Otín[1†]

Hutchinson-Gilford progeria syndrome (HGPS) is caused by a point mutation in the *LMNA* gene that activates a cryptic donor splice site and yields a truncated form of prelamin A called progerin. Small amounts of progerin are also produced during normal aging. Studies with mouse models of HGPS have allowed the recent development of the first therapeutic approaches for this disease. However, none of these earlier works have addressed the aberrant and pathogenic *LMNA* splicing observed in HGPS patients because of the lack of an appropriate mouse model. Here, we report a genetically modified mouse strain that carries the HGPS mutation. These mice accumulate progerin, present histological and transcriptional alterations characteristic of progeroid models, and phenocopy the main clinical manifestations of human HGPS, including shortened life span and bone and cardiovascular aberrations. Using this animal model, we have developed an antisense morpholino–based therapy that prevents the pathogenic *Lmna* splicing, markedly reducing the accumulation of progerin and its associated nuclear defects. Treatment of mutant mice with these morpholinos led to a marked amelioration of their progeroid phenotype and substantially extended their life span, supporting the effectiveness of antisense oligonucleotide–based therapies for treating human diseases of accelerated aging.

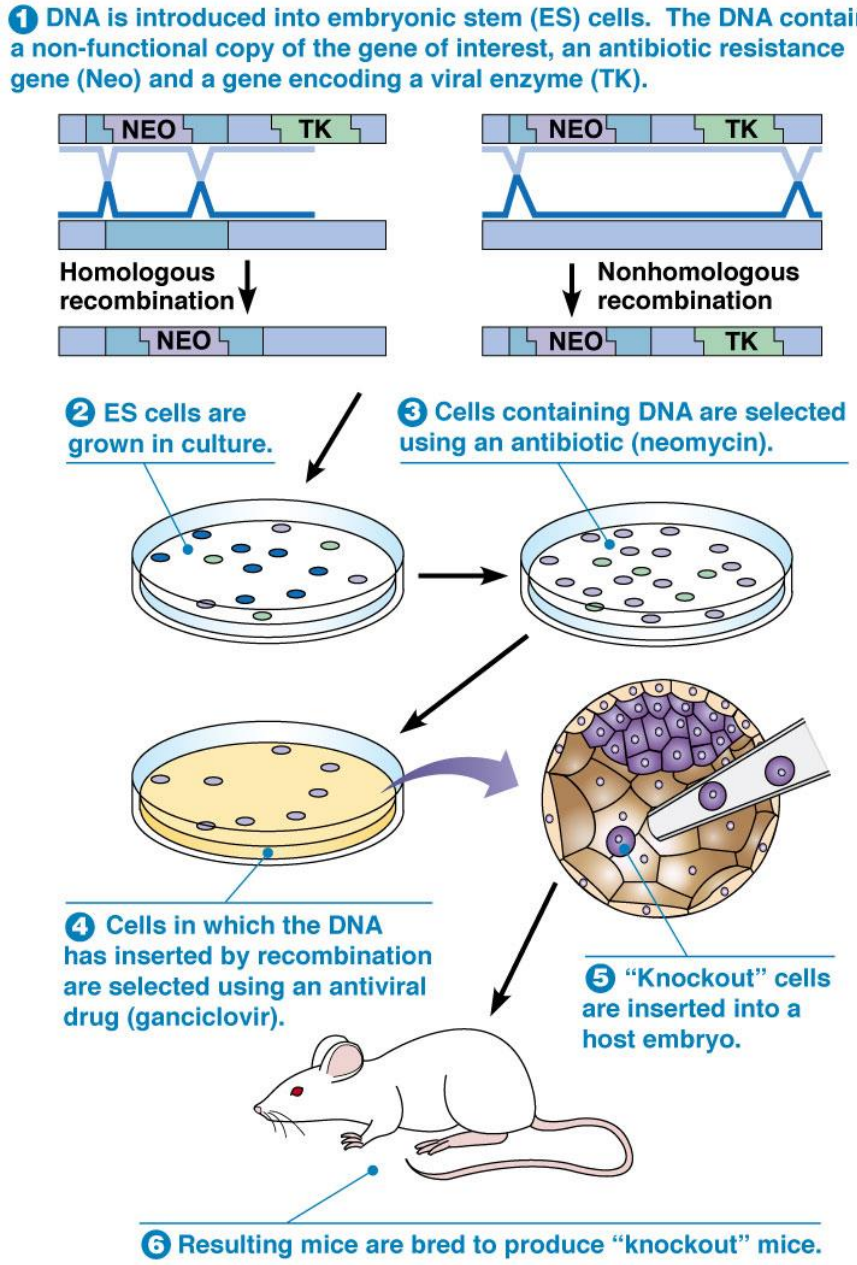
CONDITIONAL TRANSGENIC MICE

PROGERIN MODEL

Wild type locus Lmna that encodes for lamin C and lamin A



Enrichment for homologous recombinants

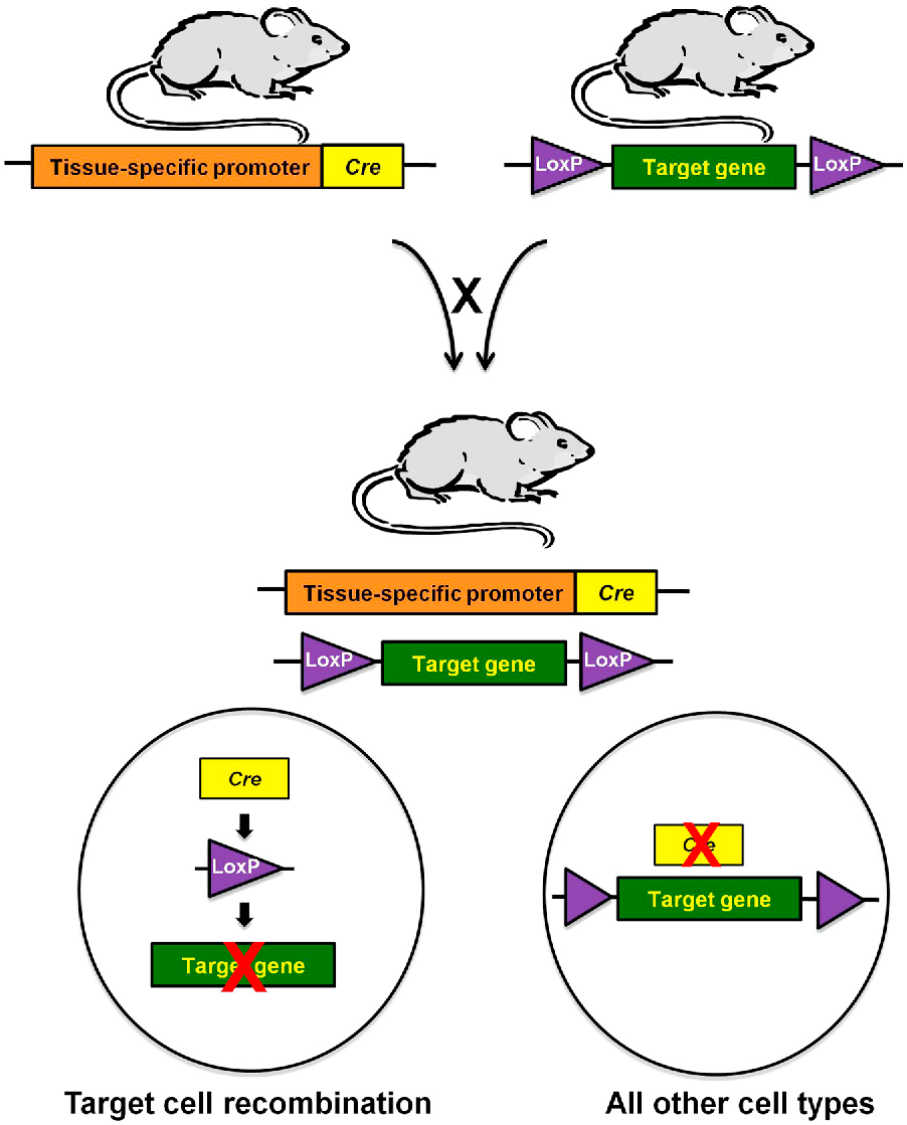


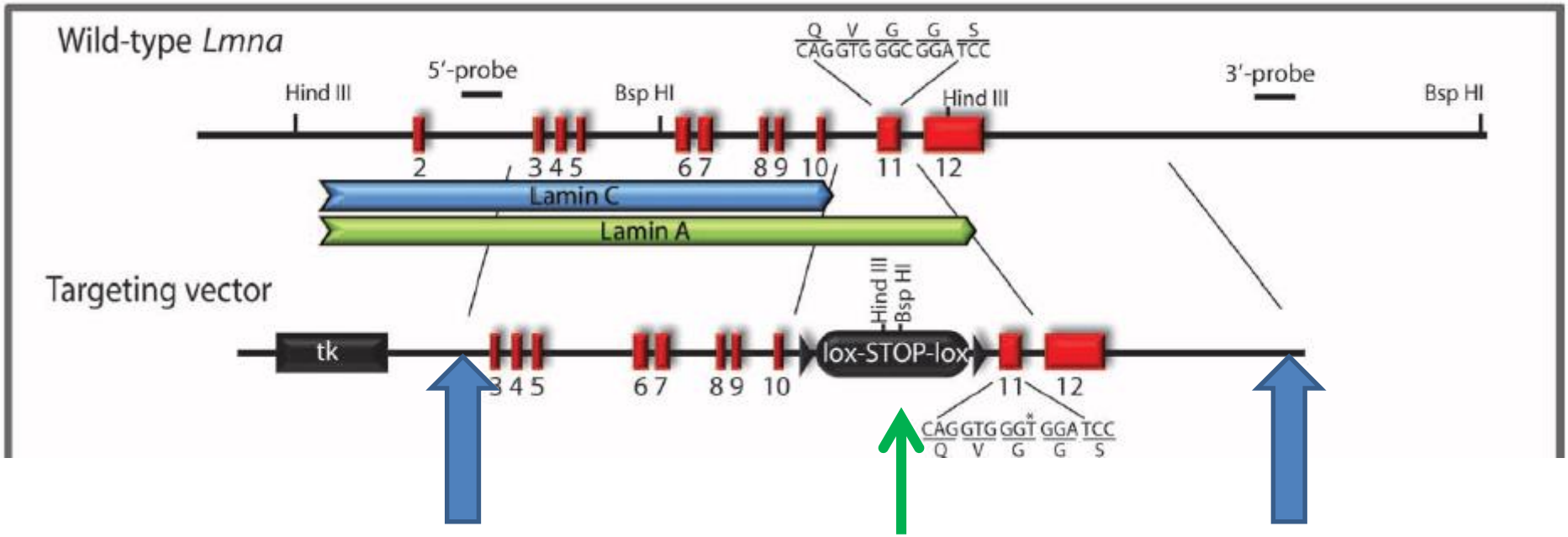
TK: thymidine kinase
Converts ganciclovir in a toxic product

Cre/LoxP system

1. mouse with a targeted "floxed" allele
2. mouse with a cre recombinase enzyme expressed under the control of a transgenic promoter

- Breed together to generate a line that carries both
- Tissue specific deletion of floxed allele in tissues where cre recombinase is expressed

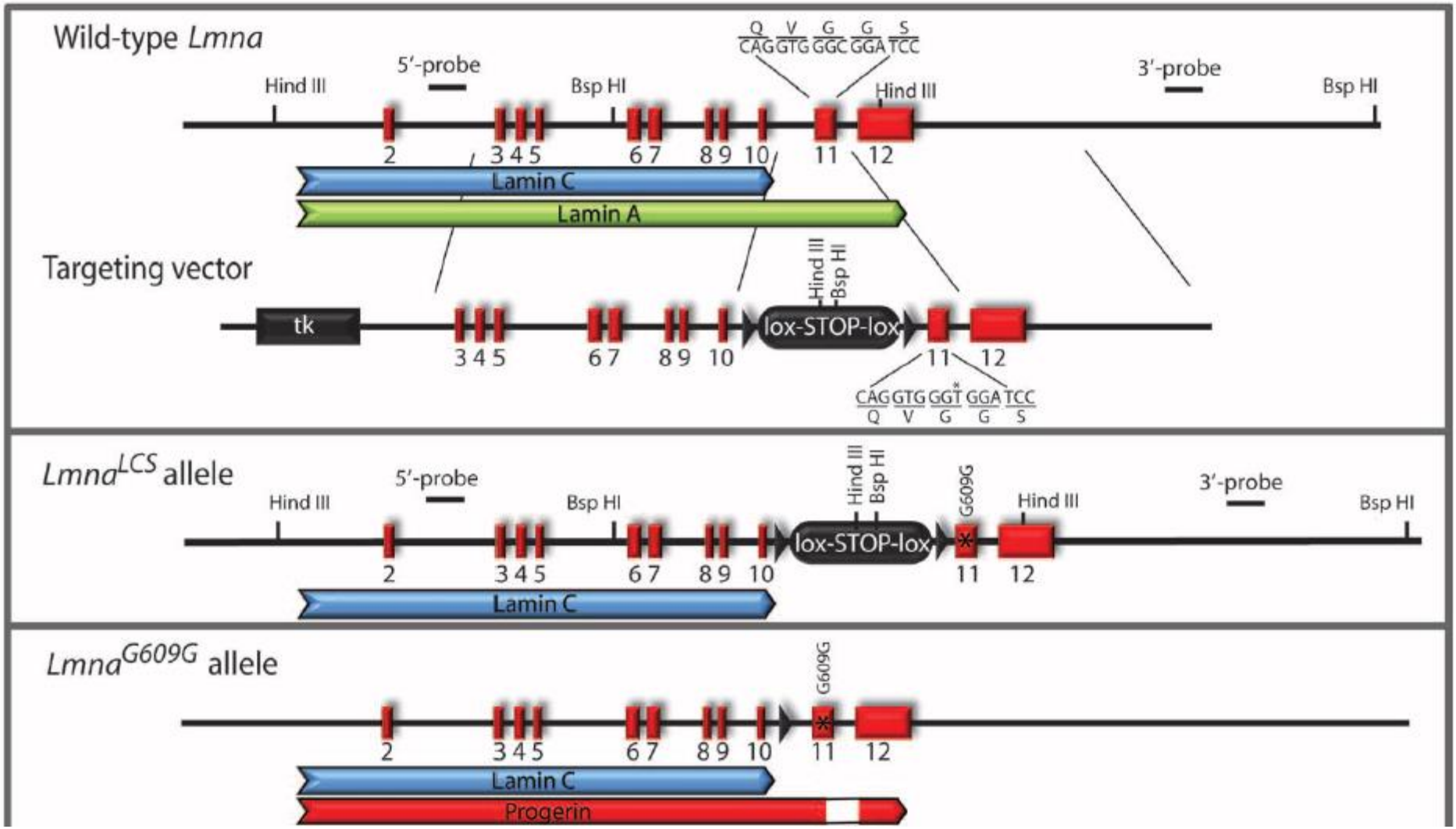




Sites for homologous recombination

neomycin

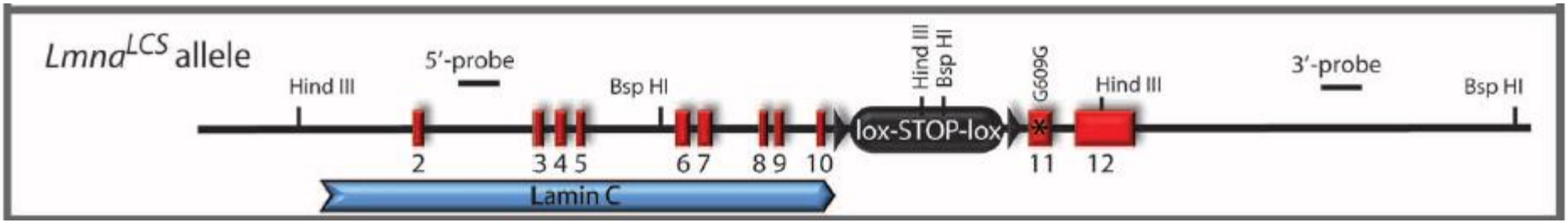
A conditional mutant allele with a neomycin resistance gene flanked by two loxP sites close to Lmna intron 10. This cassette was able to prevent the formation of prelamin A transcripts by blocking lamin A–specific splicing and is obtained the transcript for lamin C.



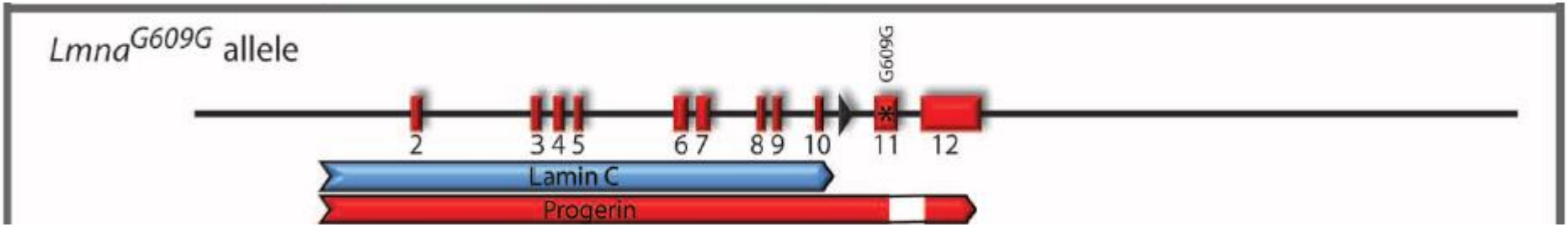
LmnaLCS/+mice and crossed them with a **Cre-deleter mouse strain** to obtain germline removal of the neomycin resistance cassette. LmnaG609G knock-in allele, which expressed lamin C, lamin A, and progerin.

Plan to create Progerin model:

- LmnaLCS (Lamin C–Stop), directs only the expression of lamin C and allows study of the potential effects of lamin A deficiency.

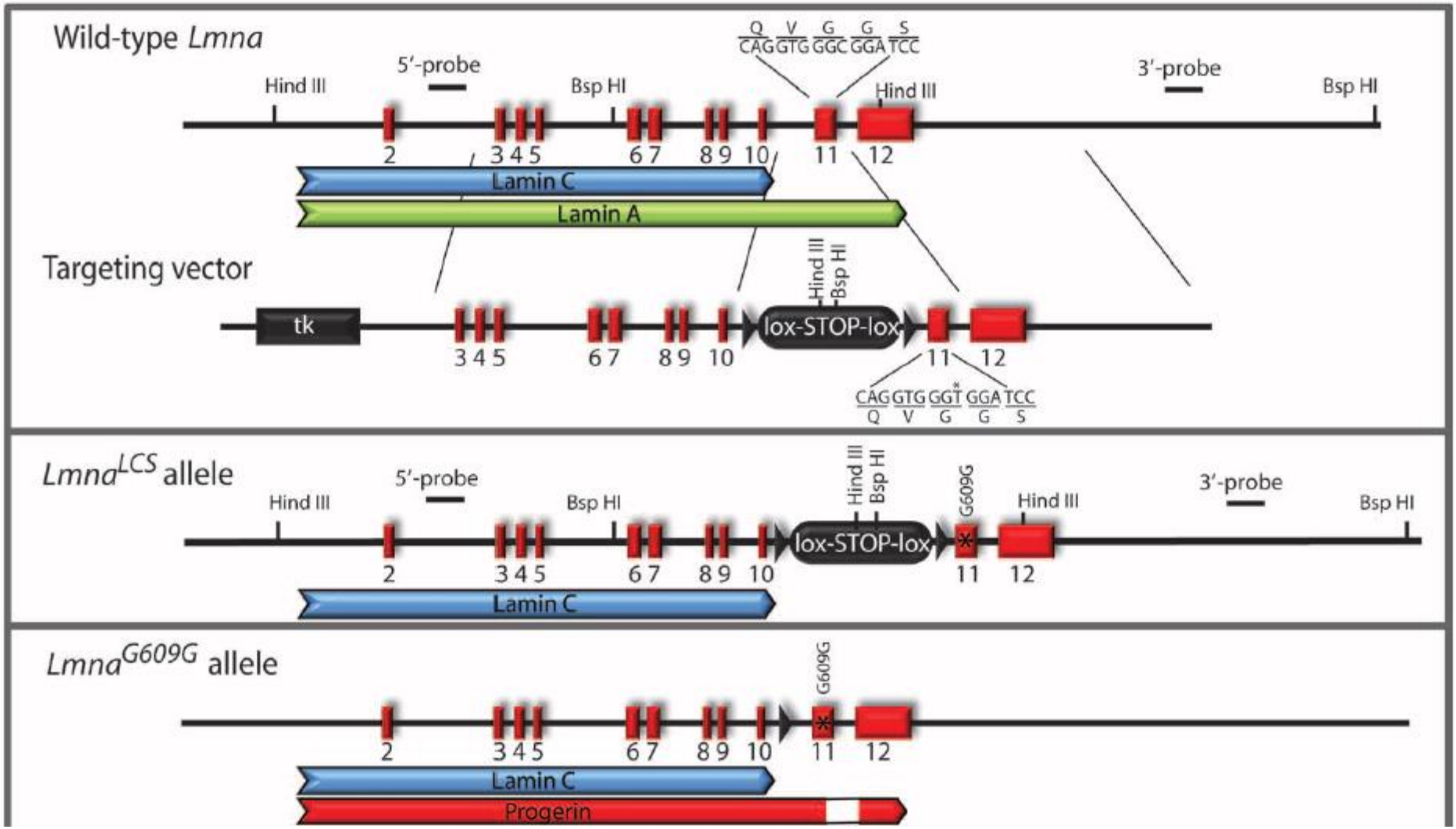


- LmnaG609G knock-in allele, which expressed lamin C, lamin A, and progerin



CONDITIONAL TRANSGENIC MICE

GENOTYPING
AND
CHARACTERIZATION



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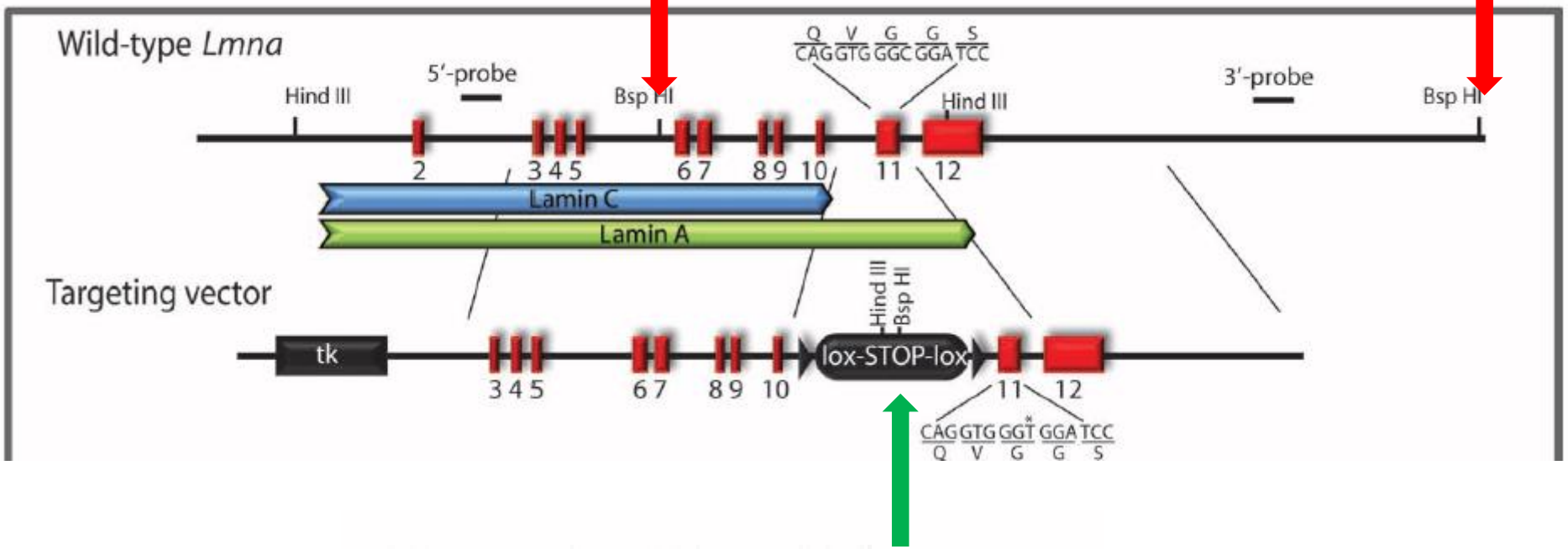
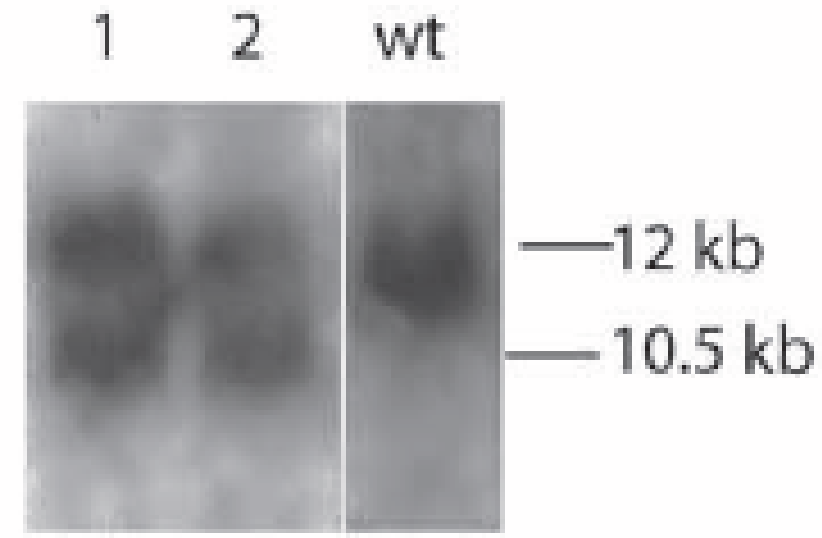
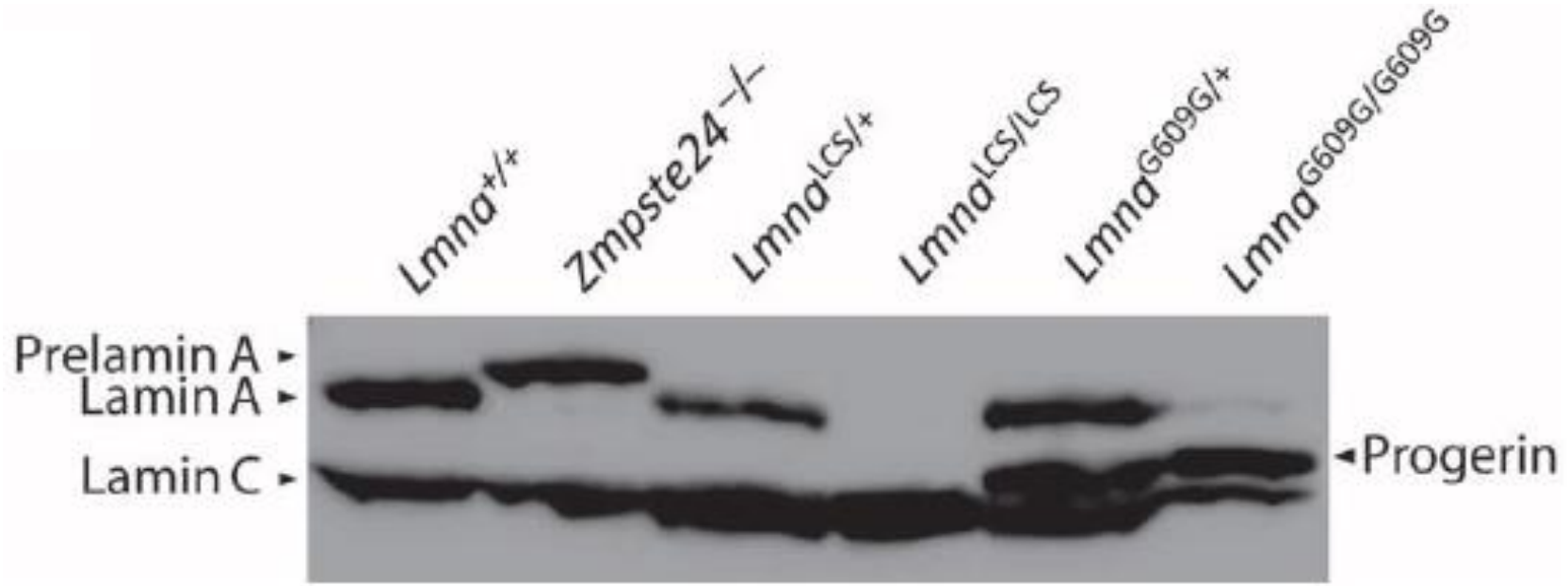

Wild-type Lmna
Hind III
5′-probe
Bsp HI
Q V G G S
CAG GTG GGC GGA TCC
Hind III
3′-probe
Bsp HI
2
3 4 5
6 7
8 9 10
11
12
Lamin C
Lamin A
Targeting vector
Hind III
Bsp HI
tk
lox-STOP-lox
3 4 5
6 7
8 9 10
11
12
CAG GTG GGT GGA TCC
Q V G G S

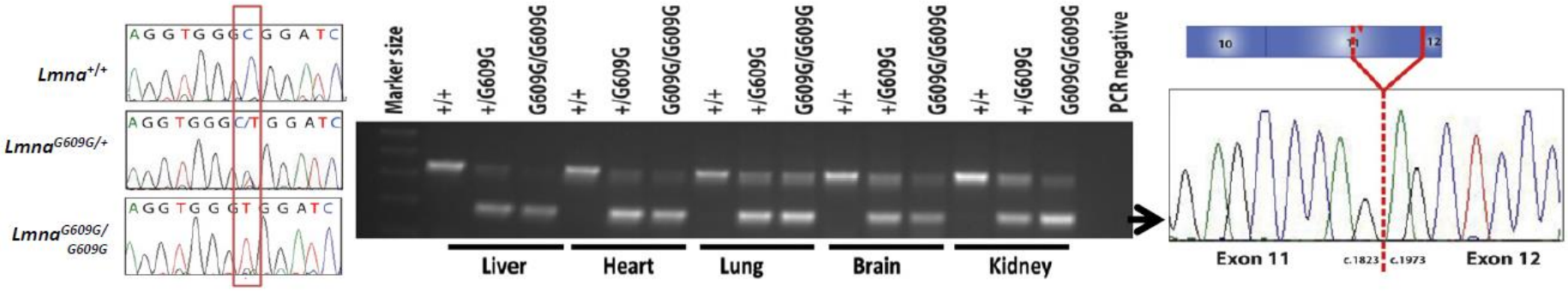

3′-probe (Bsp HI)
1
2
wt
12 kb
10.5 kb

PROGERIN AND LAMININ TYPES PROTEIN EXPRESSION

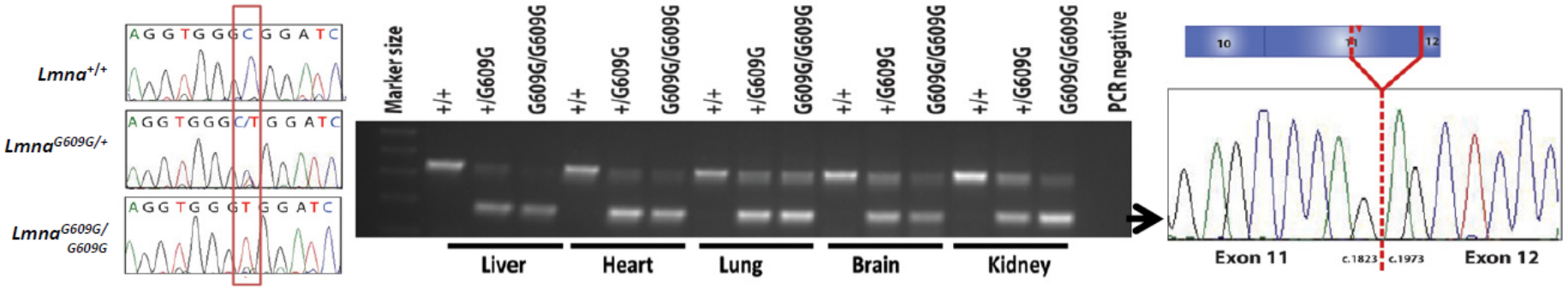


Western (immuno) blot analysis of mouse adult fibroblasts obtained from the mice with the various genotypes used in the study. Lamin A, lamin C, prelamin A, and progerin were detected with a monoclonal antibody against lamin A/C (Manlac-1).

GENOTYPING OF SEVERAL MICE TISSUES



GENOTYPING OF SEVERAL MICE TISSUES

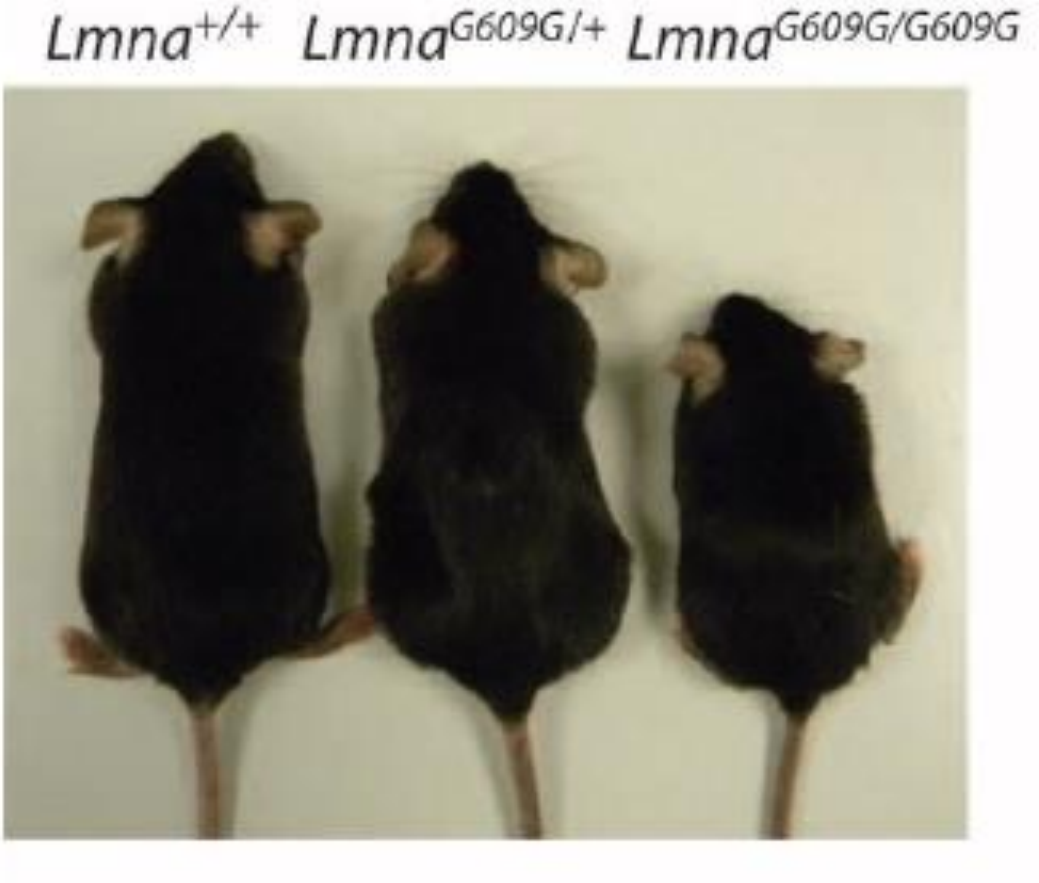


Genomic sequencing of *Lmna* exon 11 showing the wild-type sequence or heterozygous/homozygous mutations respectively in $Lmna^{+/+}$, $Lmna^{G609G/+}$and $Lmna^{G609G/G609G}$ mice (left panel). Semi-quantitative RT-PCR transcriptional analysis of mouse tissues. Primers were located in exons 10 (Fw) and 12 (Rv), yielding 334 bp fragments for wild-type lamin A-encoding transcripts and 184 bp fragments for progerin-encoding transcripts (middle panel). The smaller transcript from liver was sequenced, confirming the internal 150 nt deletion and the aberrant junction between exons 11 and 12 (right panel), as indicated on the representation of the terminal part of the lamin A transcript.

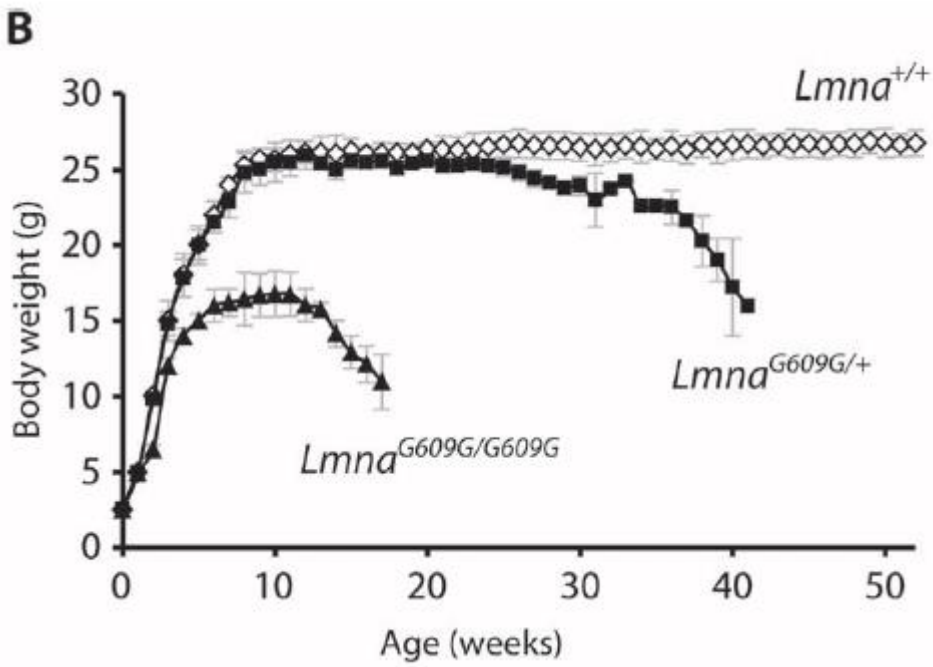
CONDITIONAL TRANSGENIC MICE
PROGERIN MODEL

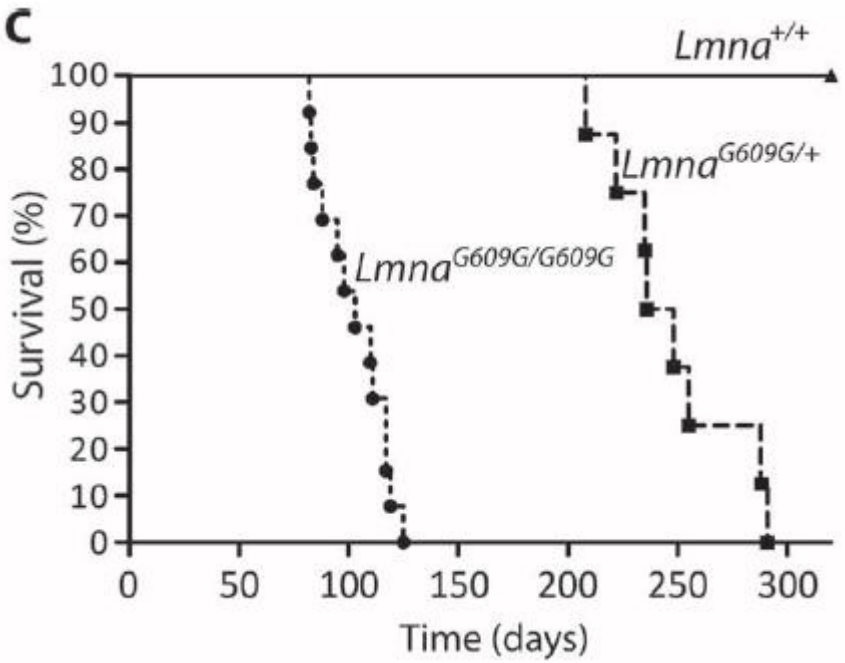
PHENOTYPE

Lmna G609G has reduction in growth rates

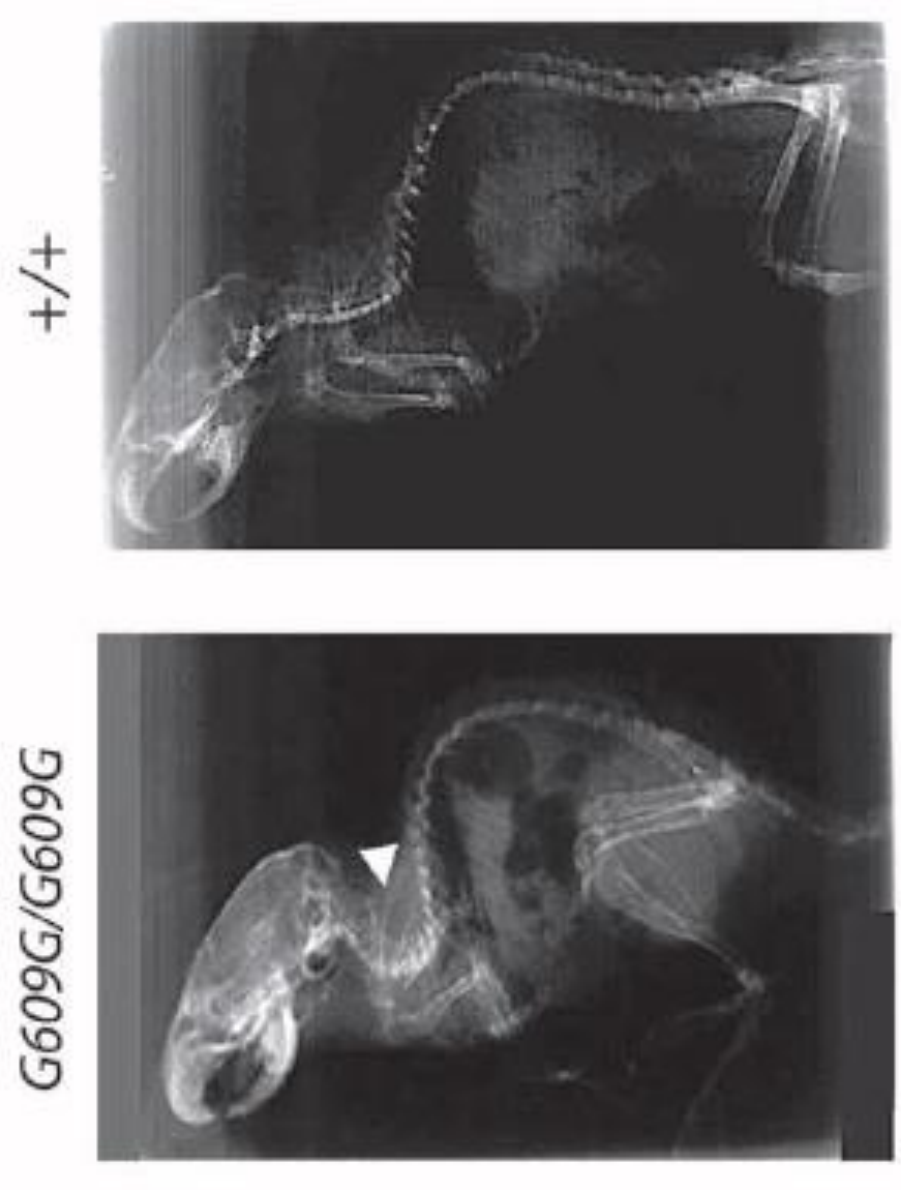


Lmna G609G has loss of weight and short life

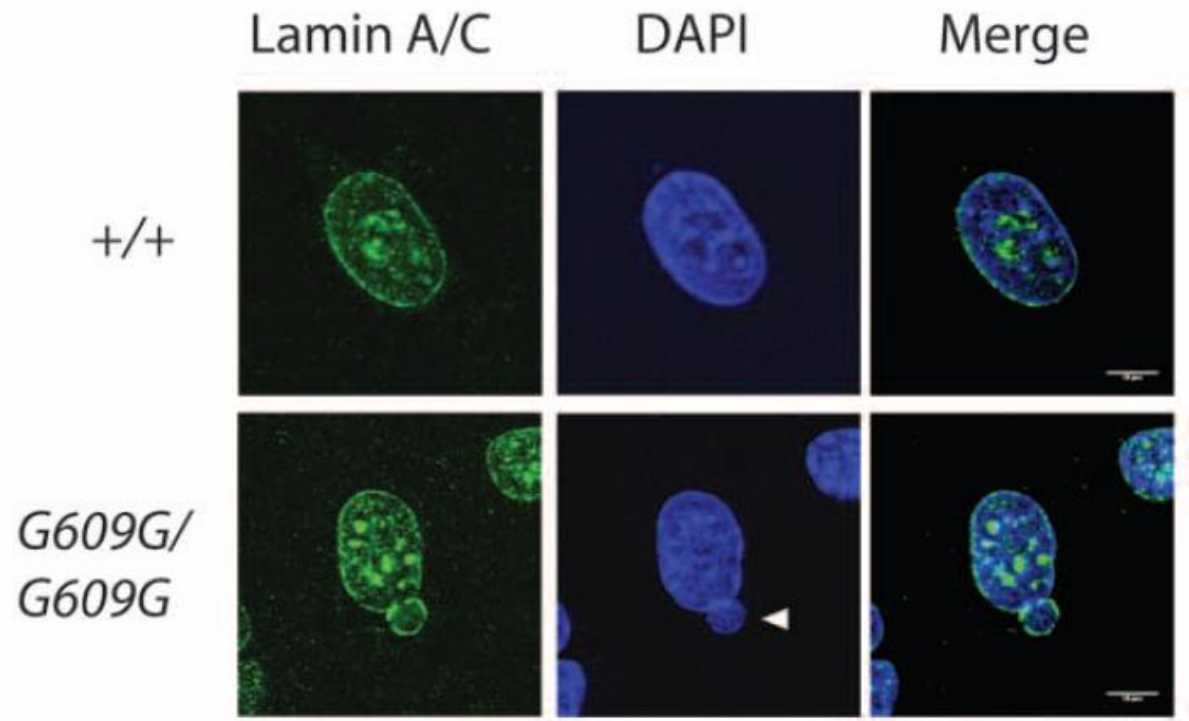


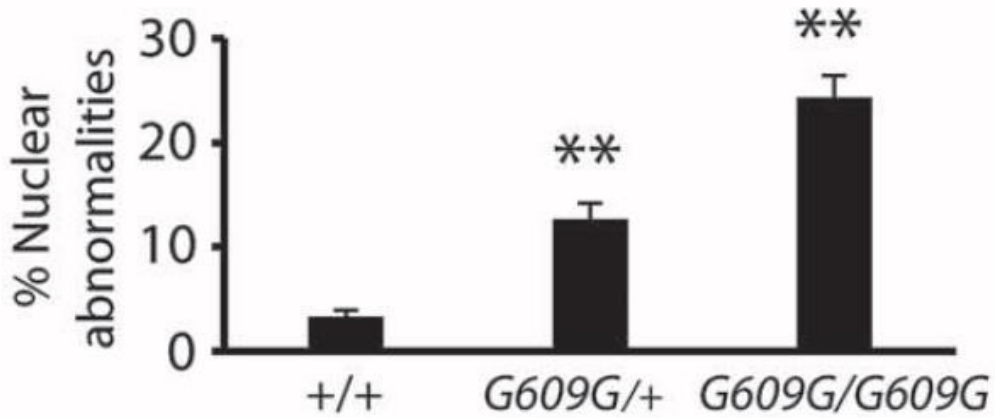


Lmna G609G has a marked curvature of the spine (cervicothoracic lordokyphosis)

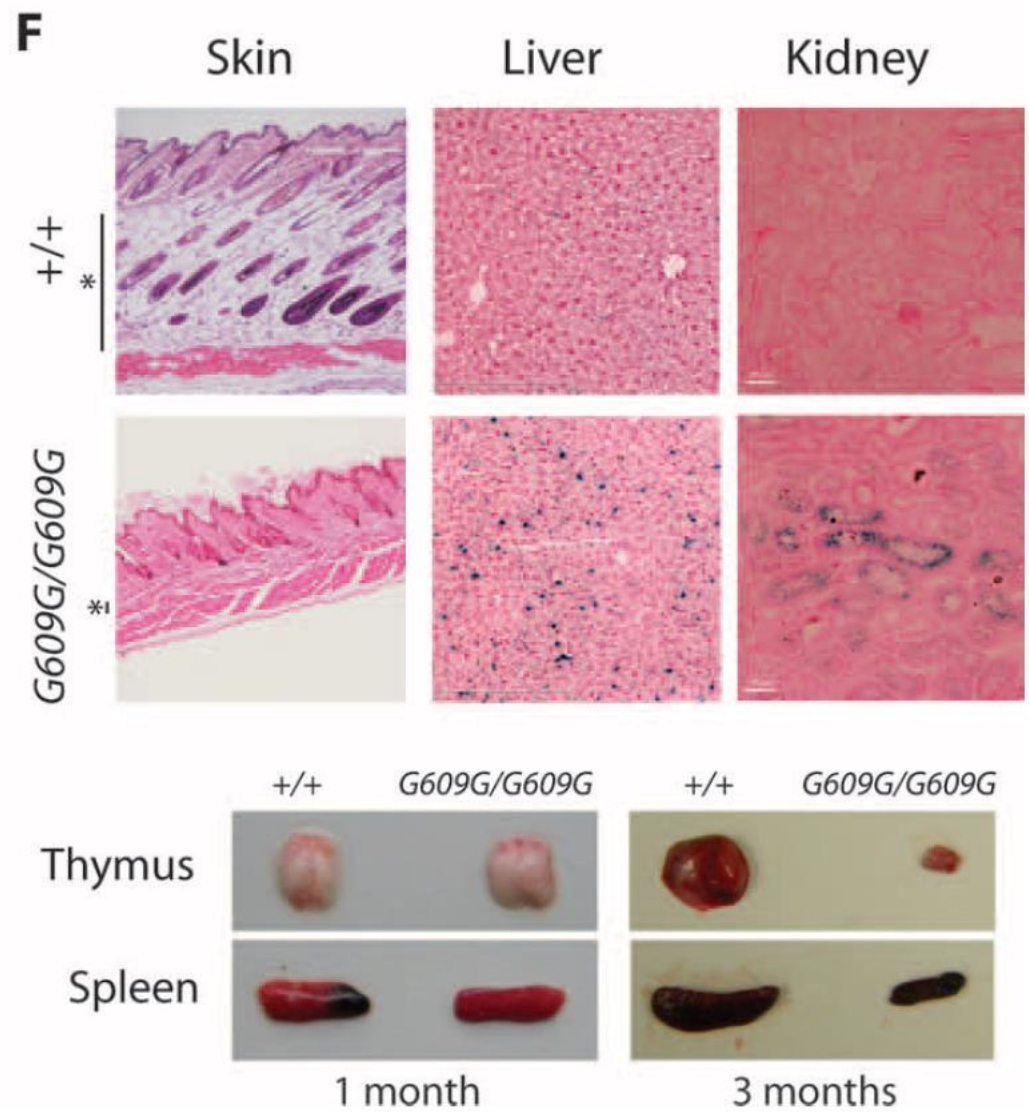


Progerin distribution is altered in mutant mice
Using immunofluorescence assay by confocal microscopy

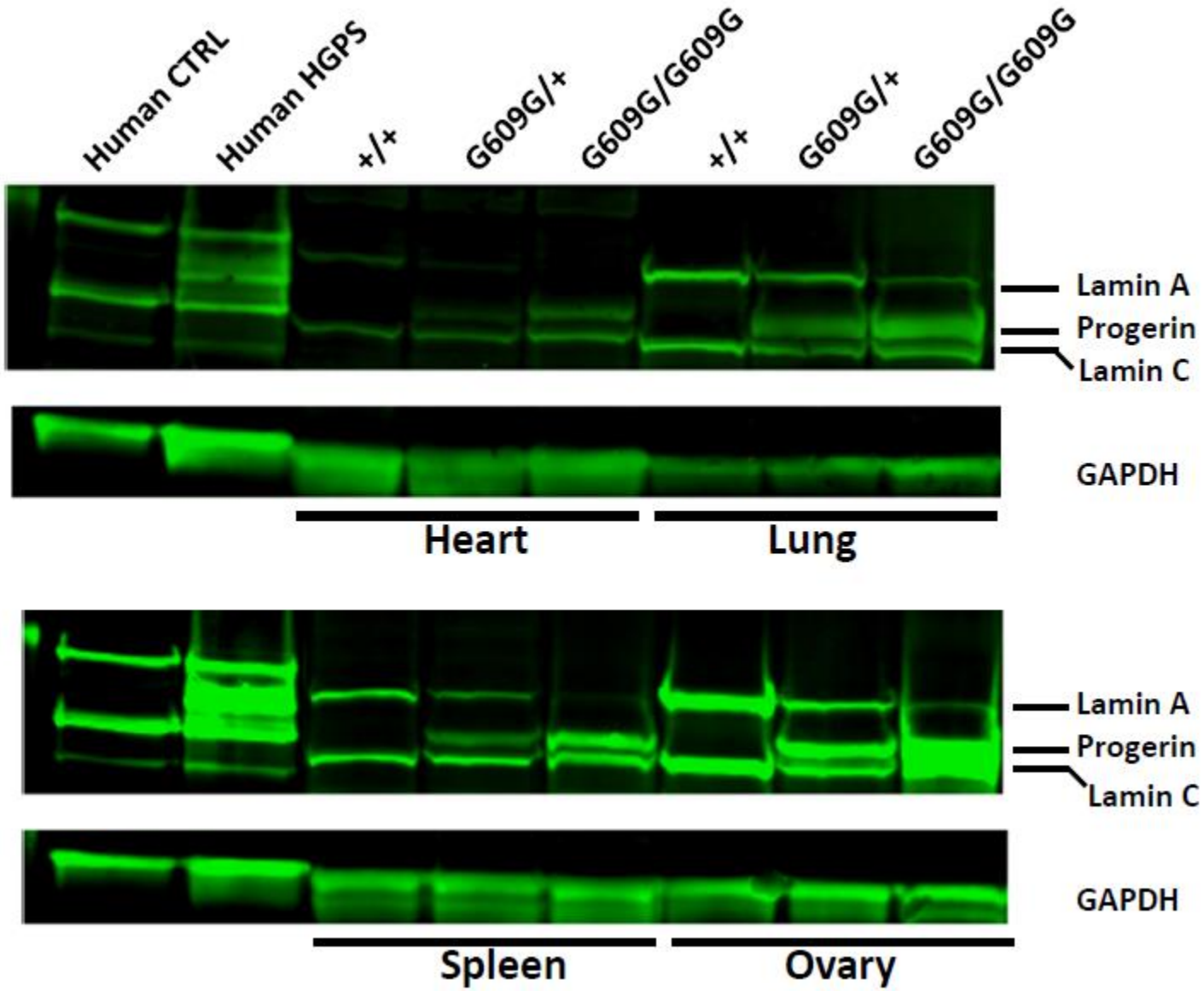




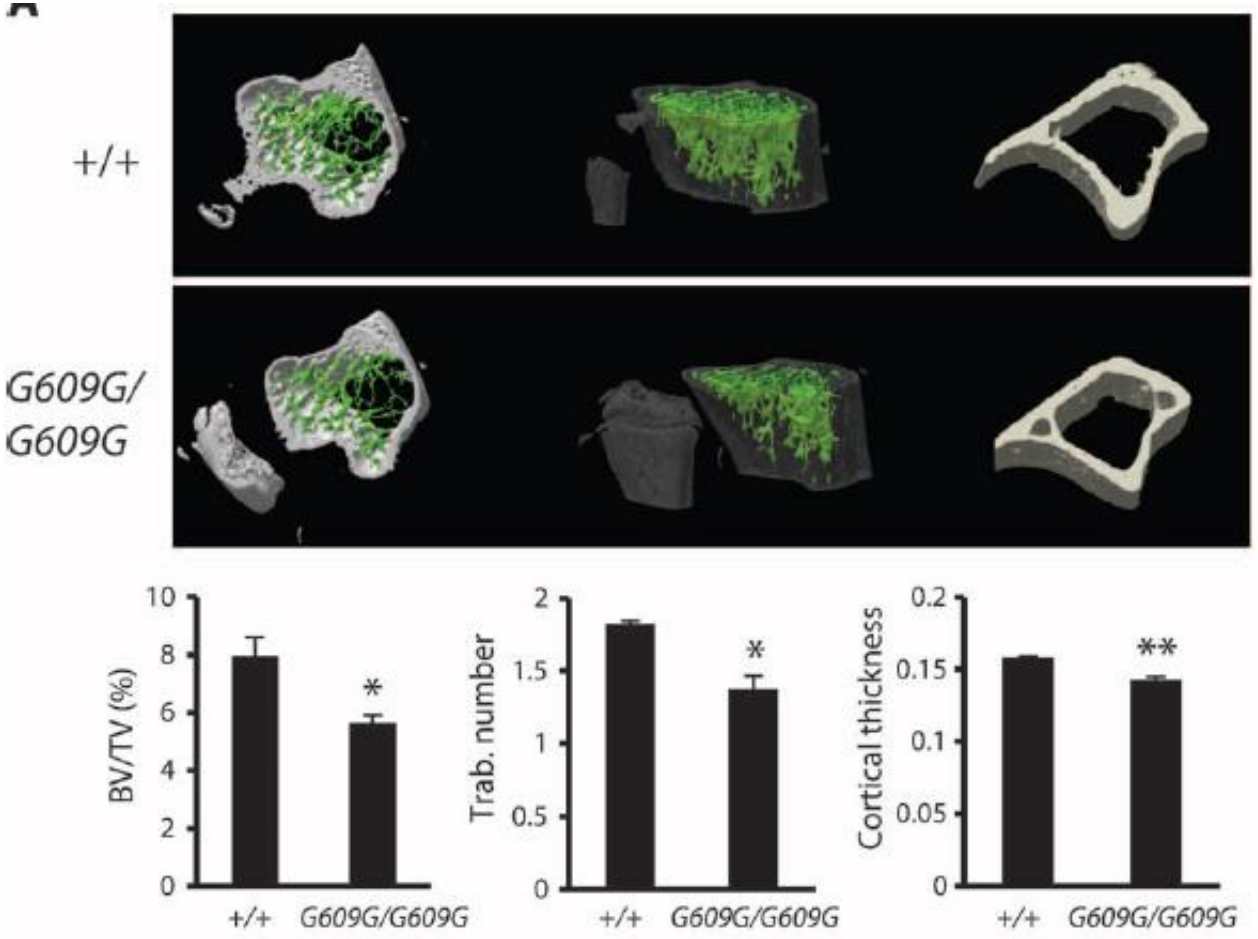
Tissue Senescence are tested using b-galattosidase



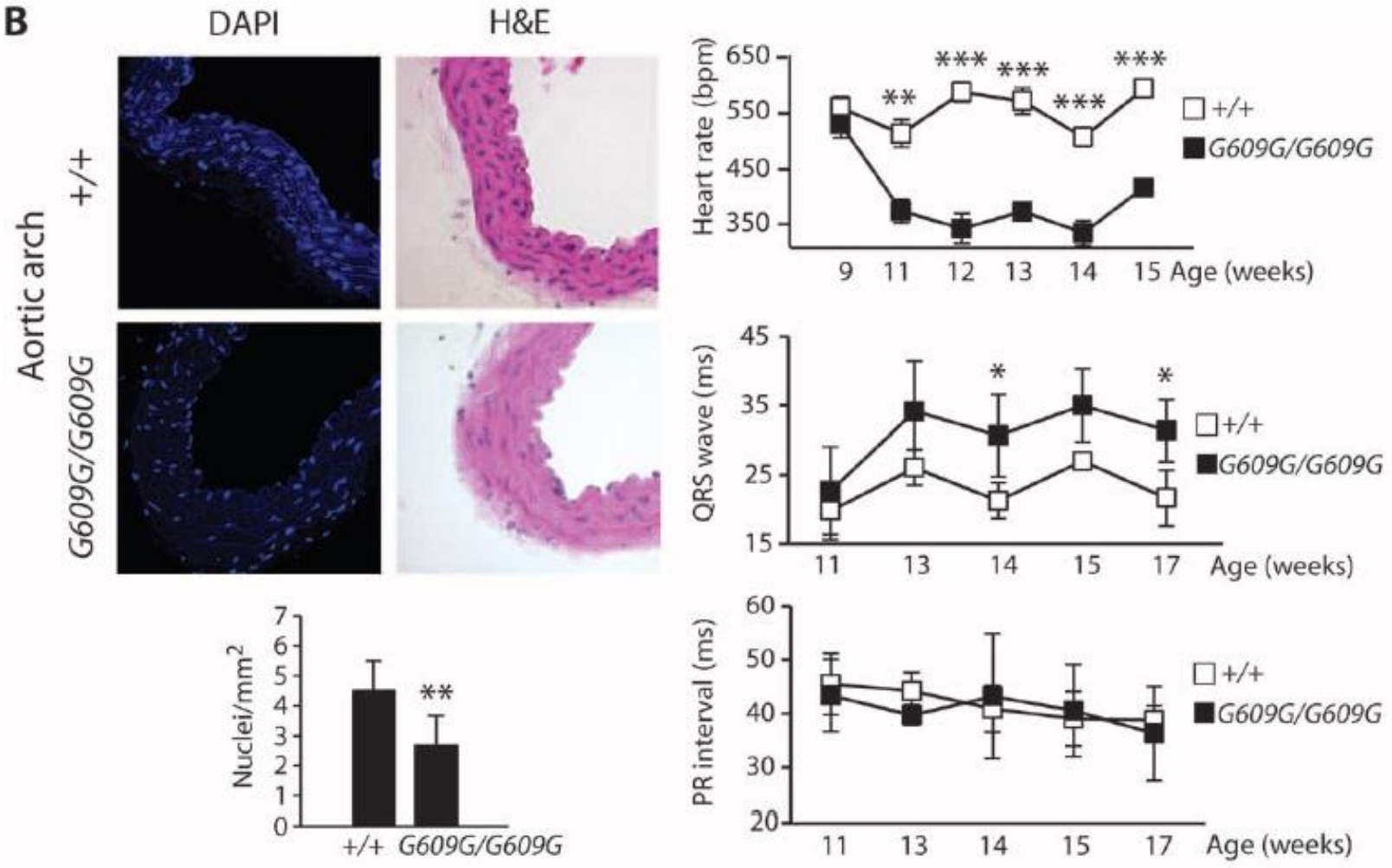
Progerin and laminin types are tested in several mice tissues



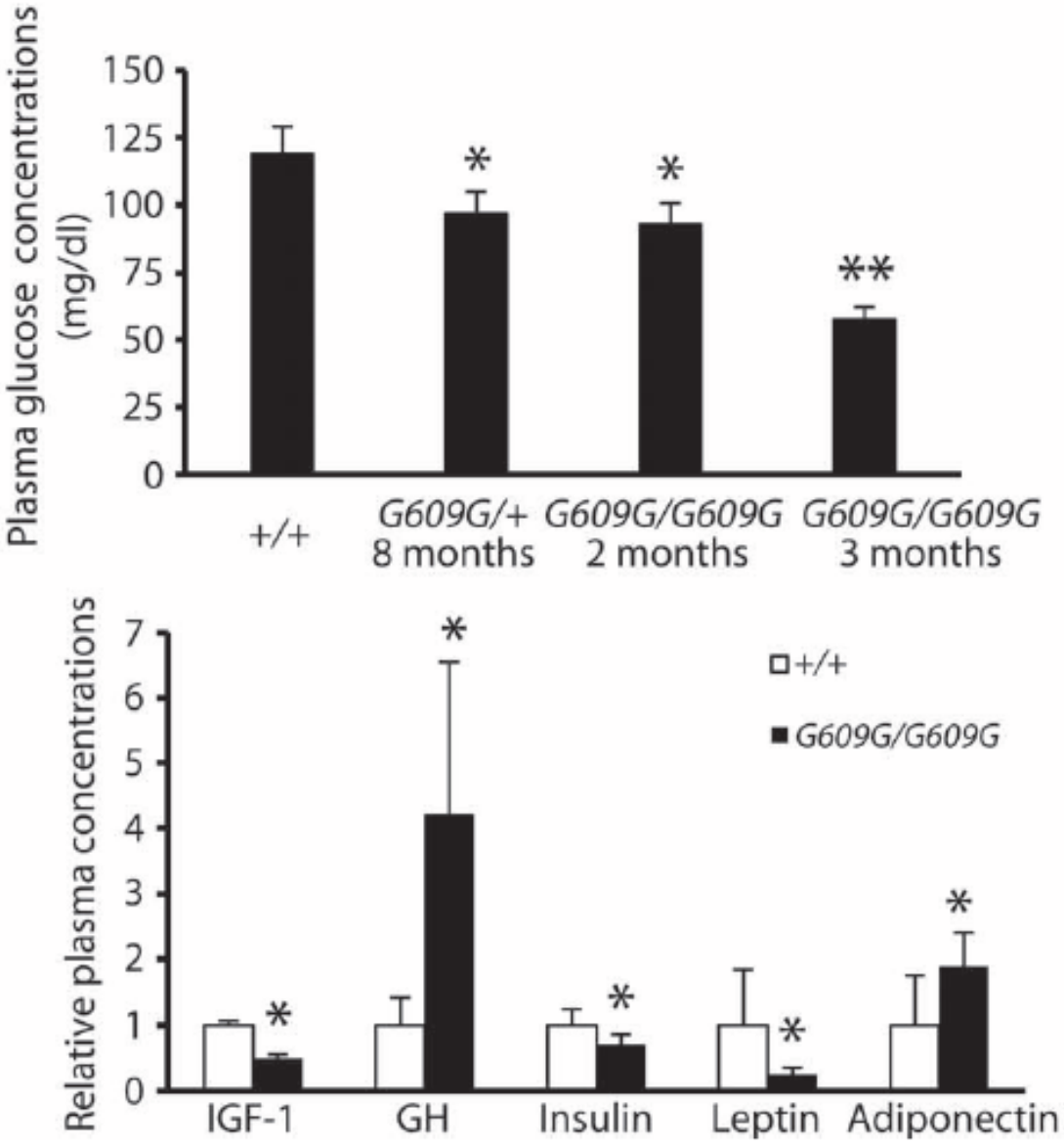
Tibias of mutant mice showed a reduction in bone density and cortical thickness as well as an increased porosity



Alteration of heat parameters functionality

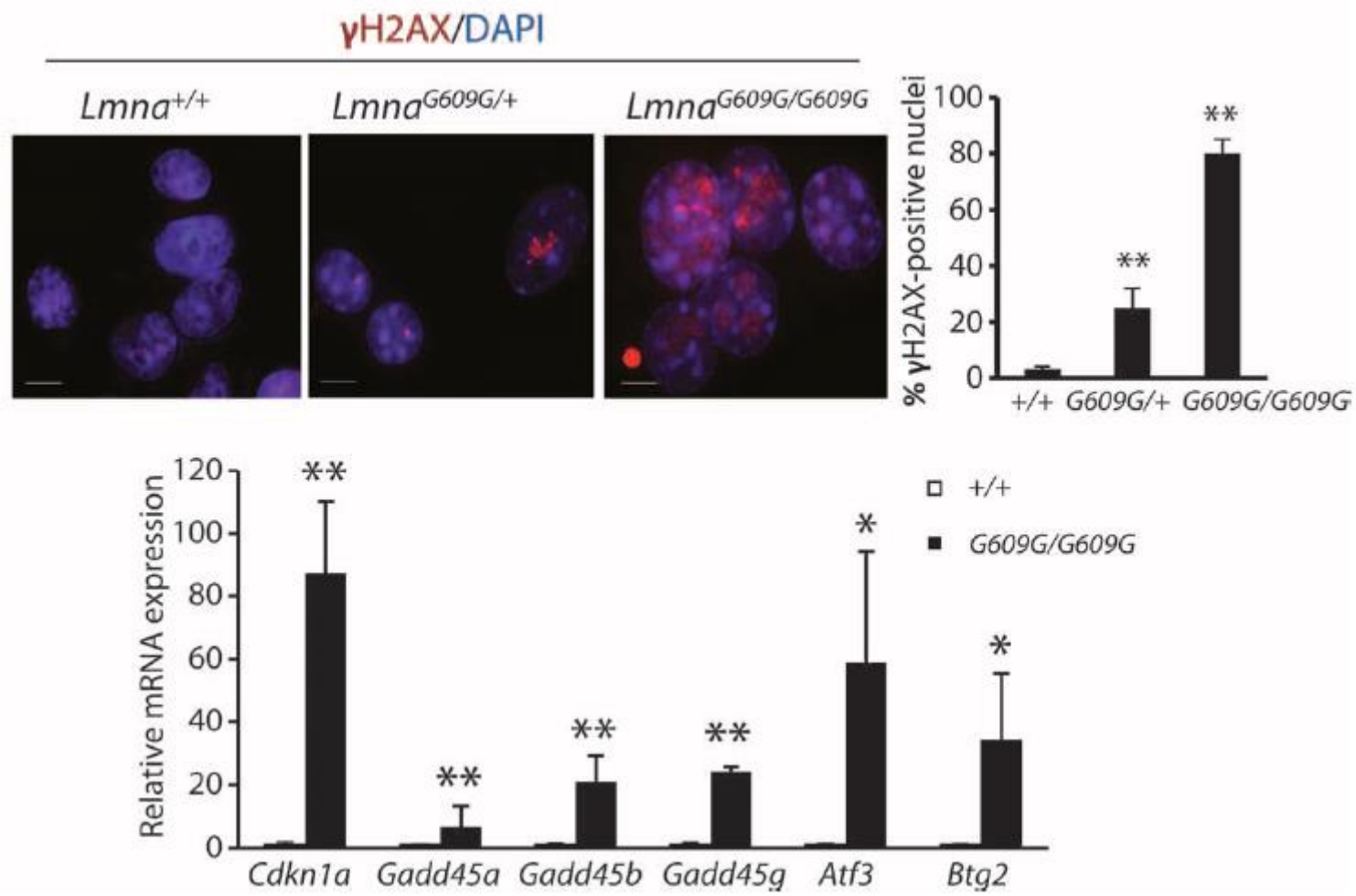


Alteration of glucose concentration and hormones associated with metabolism



DNA damage and gene validation of microarray

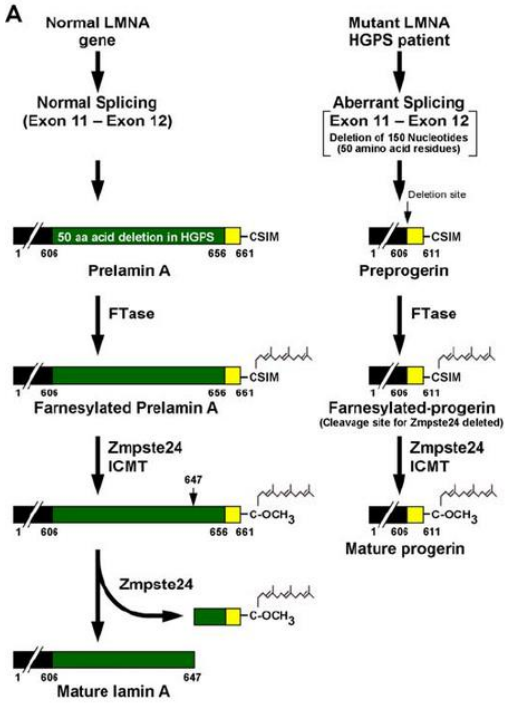
H2AX, marker for the amount of nuclear DNA double-strand breaks



PROGERIA MODEL
CONDITIONAL TRANSGENIC MICE

- Lmna G609G has reduction in growth rates and body weight
- Lmna G609G changes in cytoskeleton structure
- Lmna G609G is associated with blebbing membrane (bleb is a protrusion of cell membrane) and DNA damage
- Lmna G609G has tissues senescence and changes in organs size

THERAPHY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Preprogerin
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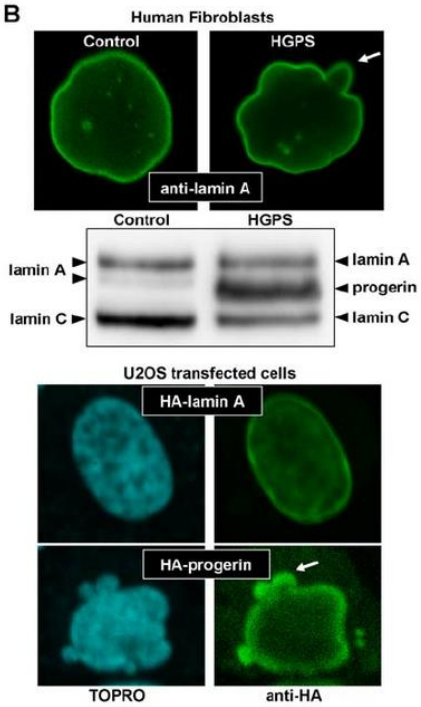
Mature progerin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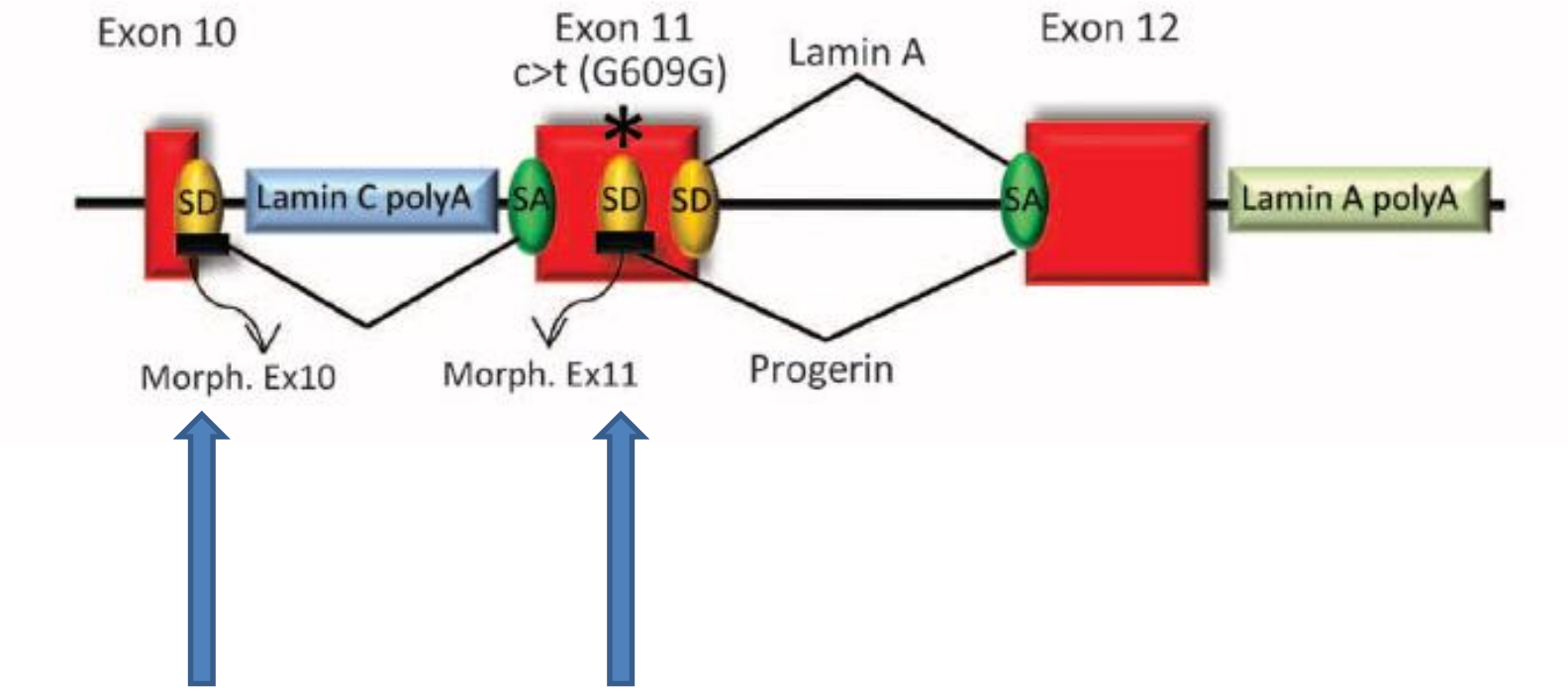
Human Fibroblasts
Control
HGPS
anti-lamin A
lamin A
lamin C
progerin
U2OS transfected cells
HA-lamin A
HA-progerin
TOPRO
anti-HA

Schematic representation of the morpholino-based strategy for Lmna splicing modulation



Morpholino target regions

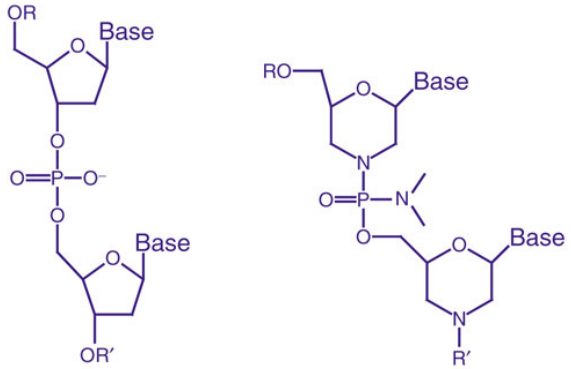
MORFOLINO

Morfolino are molecules similar to **RNA or DNA** with nitrogenous bases,

morpholine rings are linked through **uncharged phosphorodiamidate groups,**

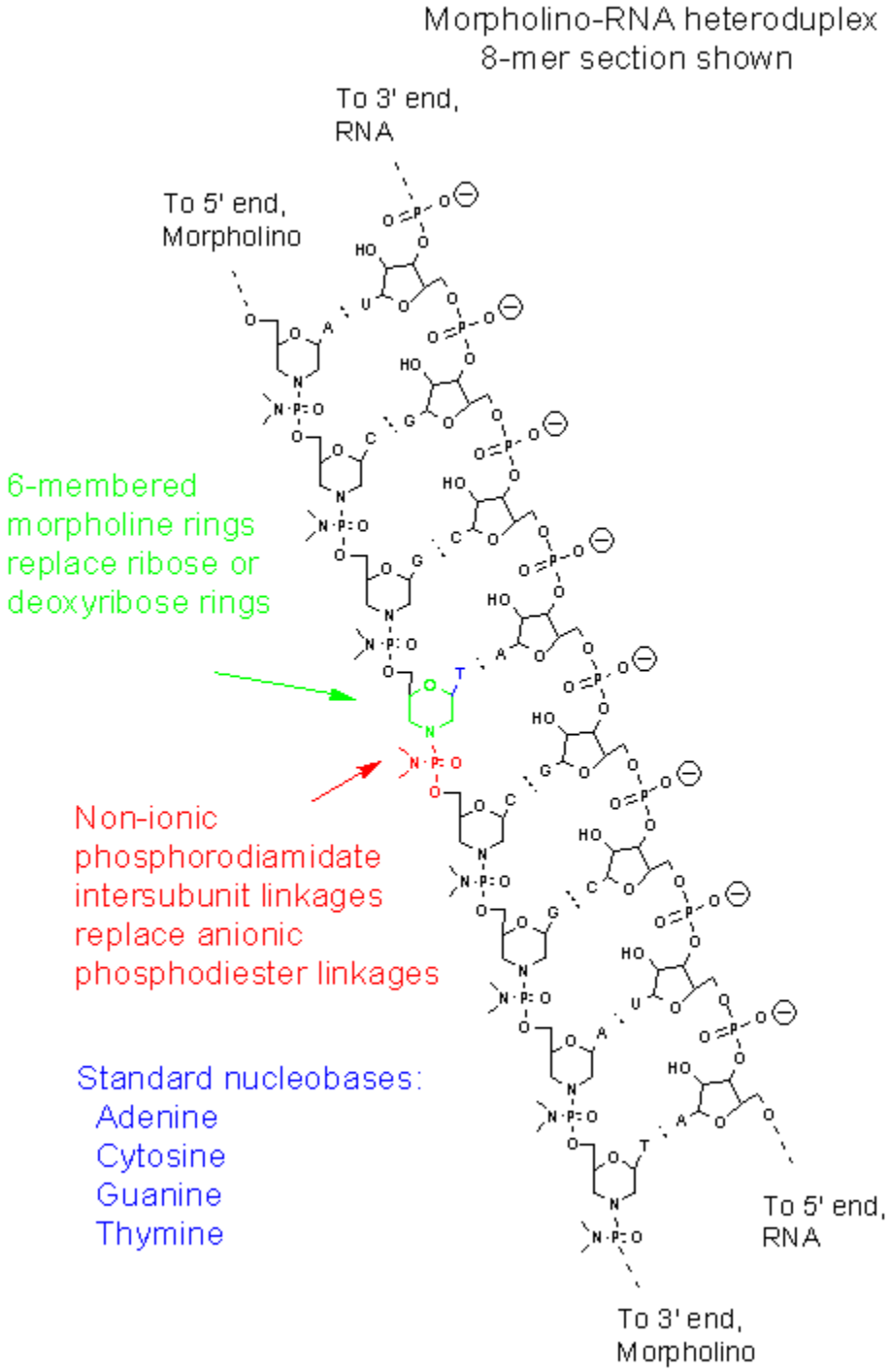
Morfolinos are 18–30 bases in length and **bind to targeted RNA sequences by base pairing**,

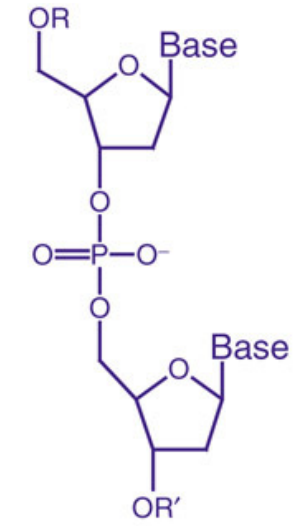
phosphorodiamidate morpholino oligomers (PMOs or Morpholinos) do not result in degradation of their target RNA: not recognized by cellular nucleases.



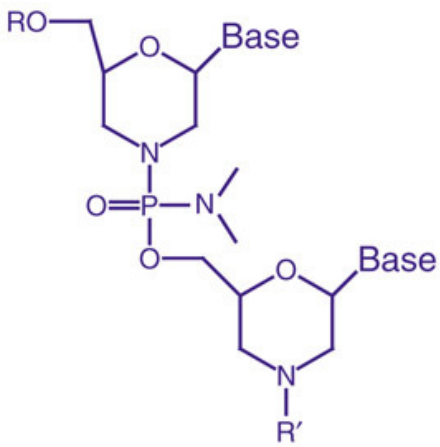
Phosphodiester DNA

Morpholino





Phosphodiester
DNA



Morpholino

Phosphorodiamidate group

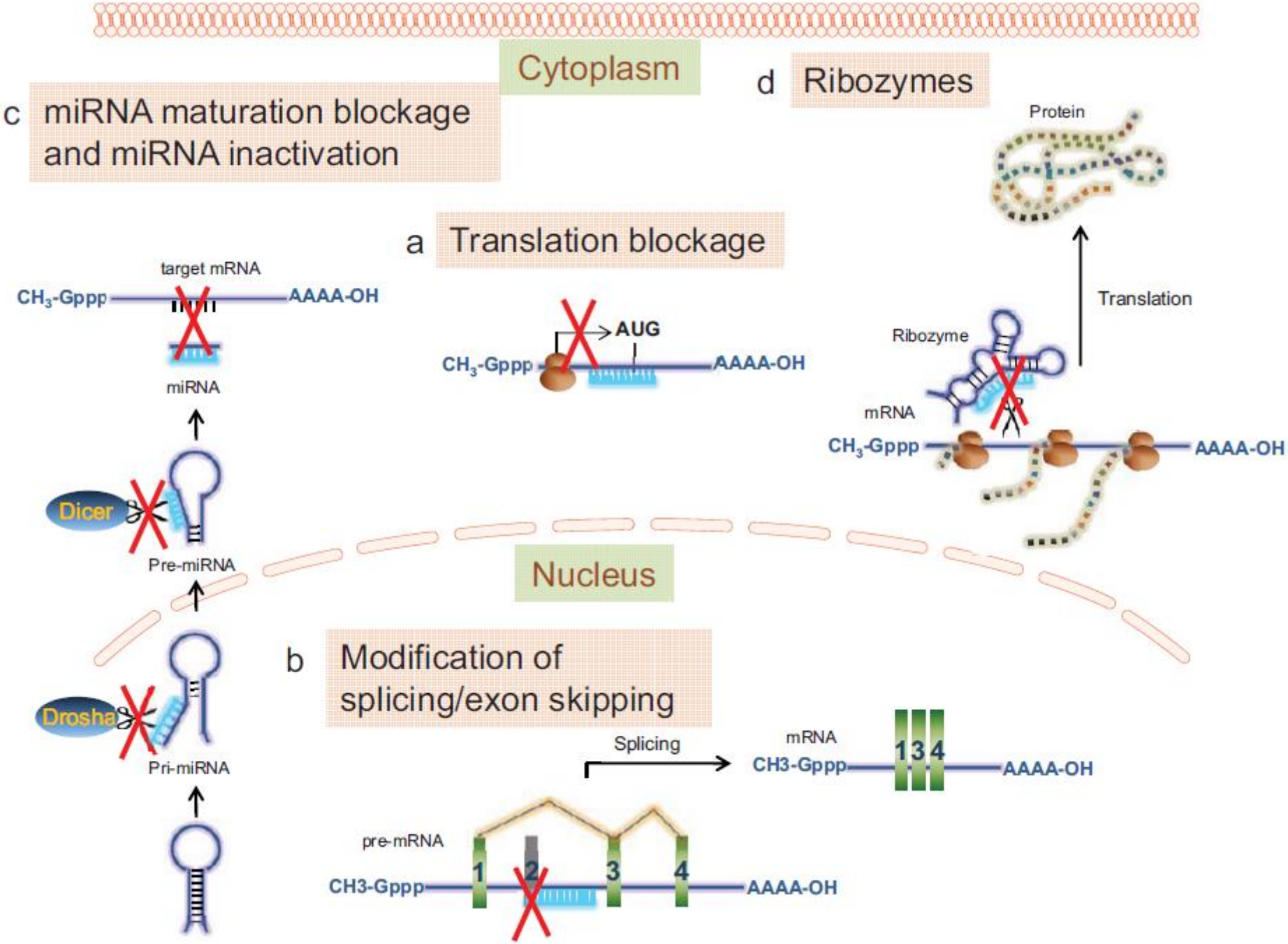
Mechanisms of action of antisense morpholino oligomers.

(a) **Translational blockage**. PMOs block the translation initiation complex binding sites on mRNA and prevent translation from occurring.

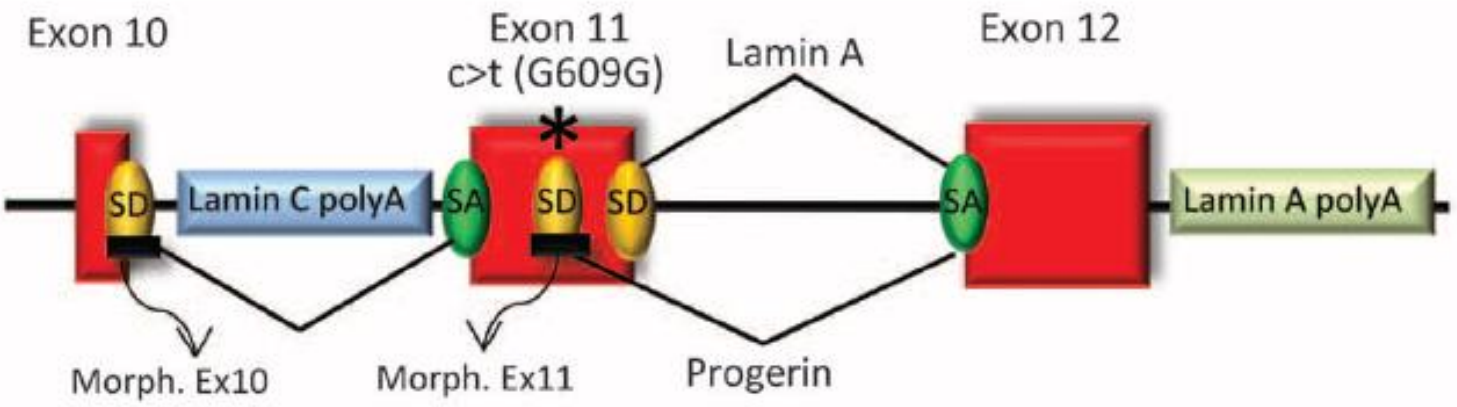
(b) **Modification of splicing/exon skipping**. PMOs block splice sites on pre-mRNA, prevent recognition of these sites by the spliceosome that in turn causes exon skipping.

(c) **miRNA maturation blockage and miRNA inactivation**. PMOs may block maturation enzyme cleavage sites (i.e., Drosha, Dicer) on pri- or pre-miRNA to prevent its maturation. PMOs may complementarily bind to mature miRNA and prevent it from binding to target mRNA.

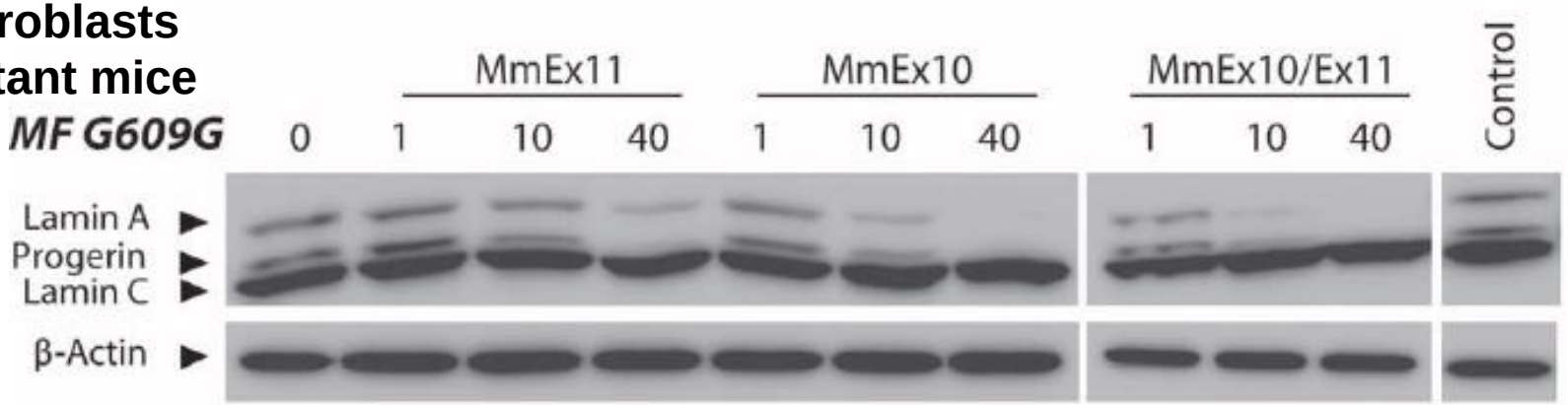
(d) **Ribozymes.** PMOs may bind to enzymatically active RNAs (ribozymes), blocking their active sites and preventing them from cleaving their target mRNAs.


Cytoplasm
c miRNA maturation blockage and miRNA inactivation
d Ribozymes
Protein
a Translation blockage
target mRNA
CH3-Gppp
AAAA-OH
AUG
Translation
Ribozyme
mRNA
miRNA
Dicer
Pre-miRNA
Nucleus
b Modification of splicing/exon skipping
Drosha
Pri-miRNA
Splicing
mRNA
CH3-Gppp
1 3 4
AAAA-OH
pre-mRNA
1 2 3 4

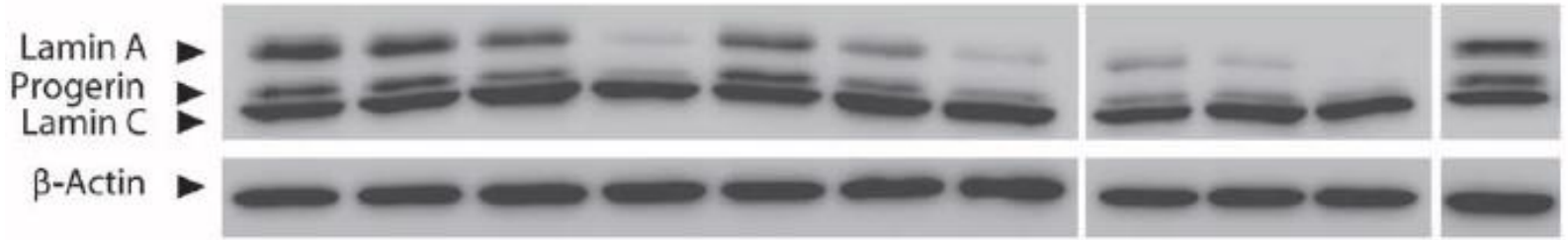
Both MmEx10 and MmEx11 morpholinos each reduced progerin amount in a dose-dependent manner



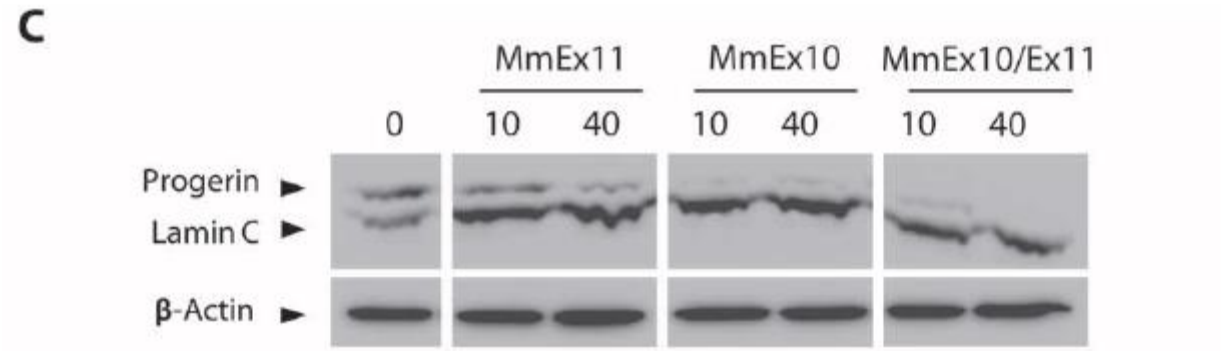
Fibroblasts mutant mice

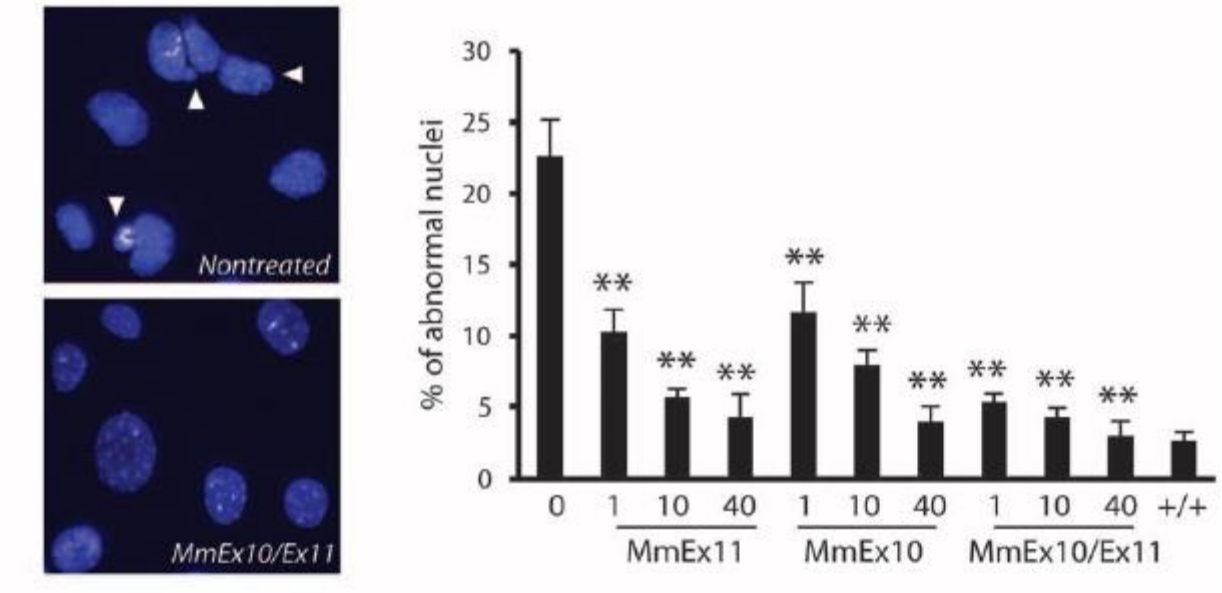


HF G608G **Fibroblasts from patients**



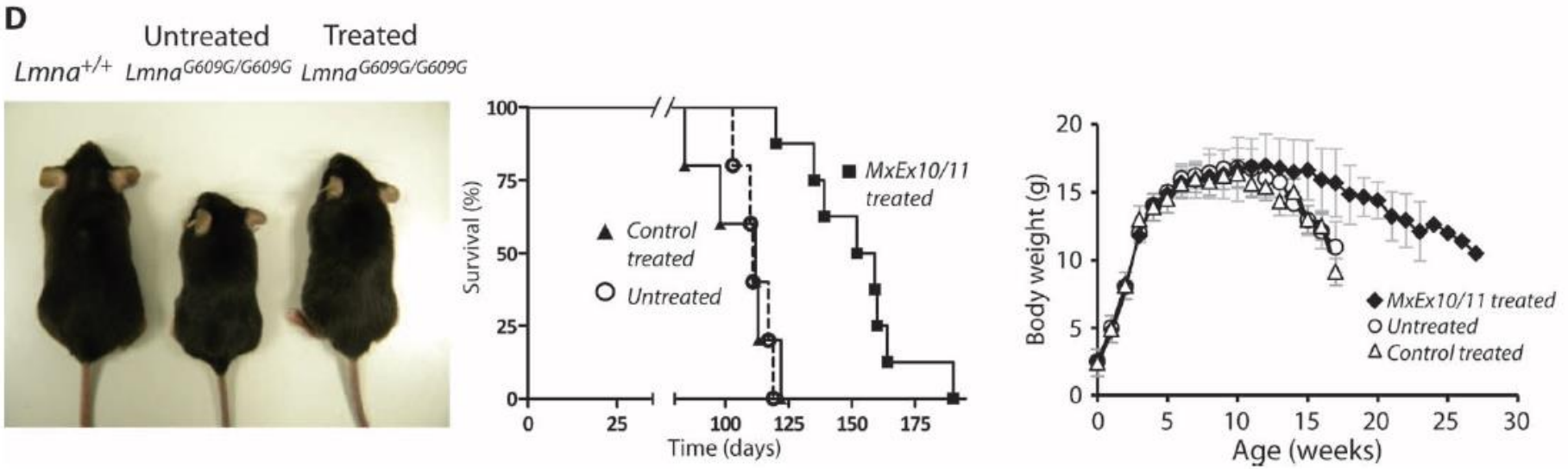
MmEx10 and MmEx11 inhibited progerin production



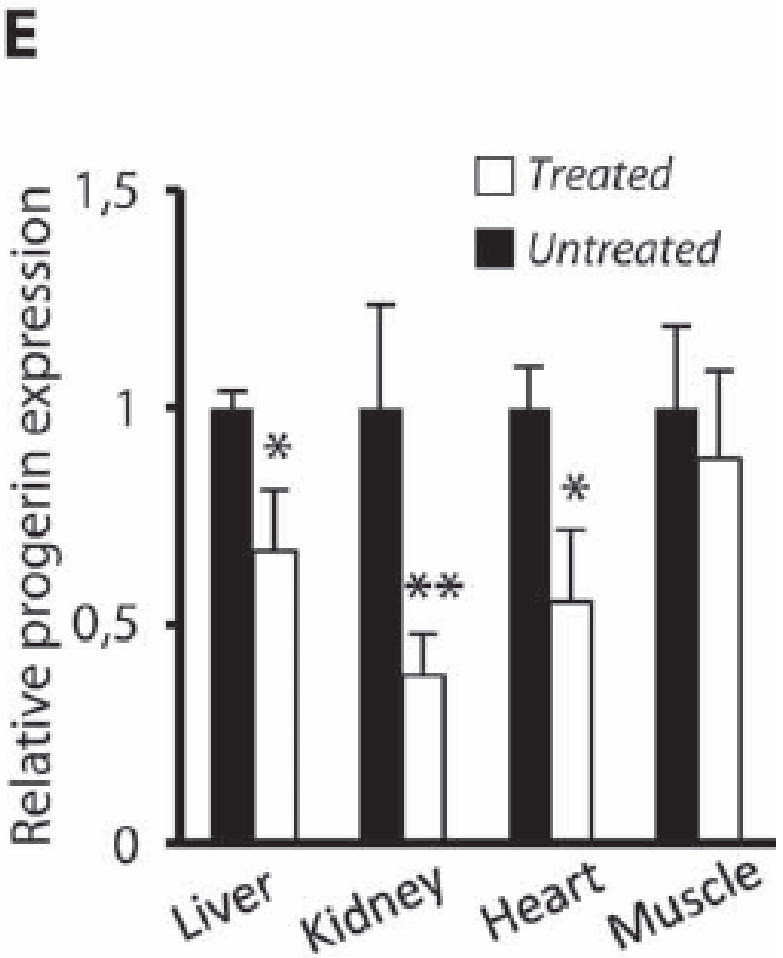


MmEx10 and MmEx11 induced reduction of nucleus abnormalities

MmEx10 and MmEx11 increased survival and restored normal body weight



MmEx10 and MmEx11 inhibited progerin production in several tissues



nature medicine

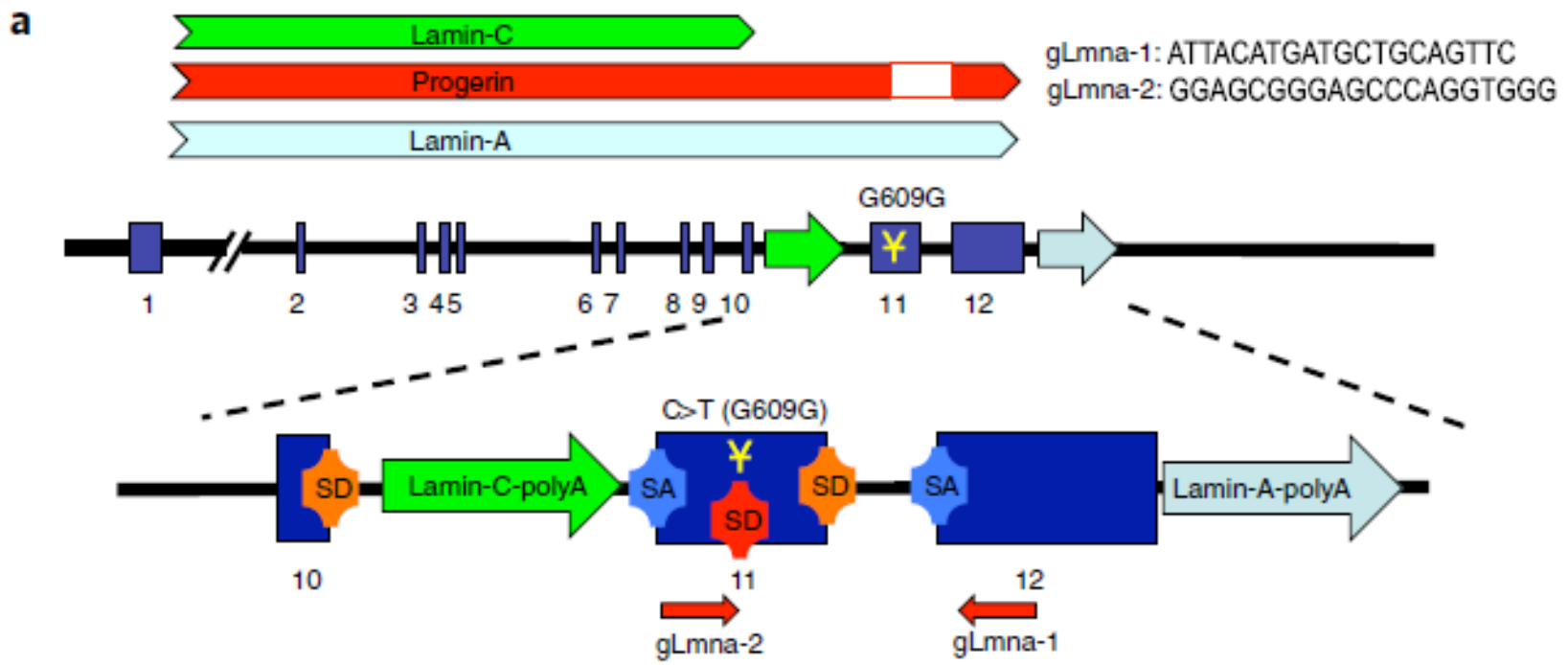
BRIEF COMMUNICATION

https://doi.org/10.1038/s41591-019-0343-4

Single-dose CRISPR–Cas9 therapy extends lifespan of mice with Hutchinson–Gilford progeria syndrome

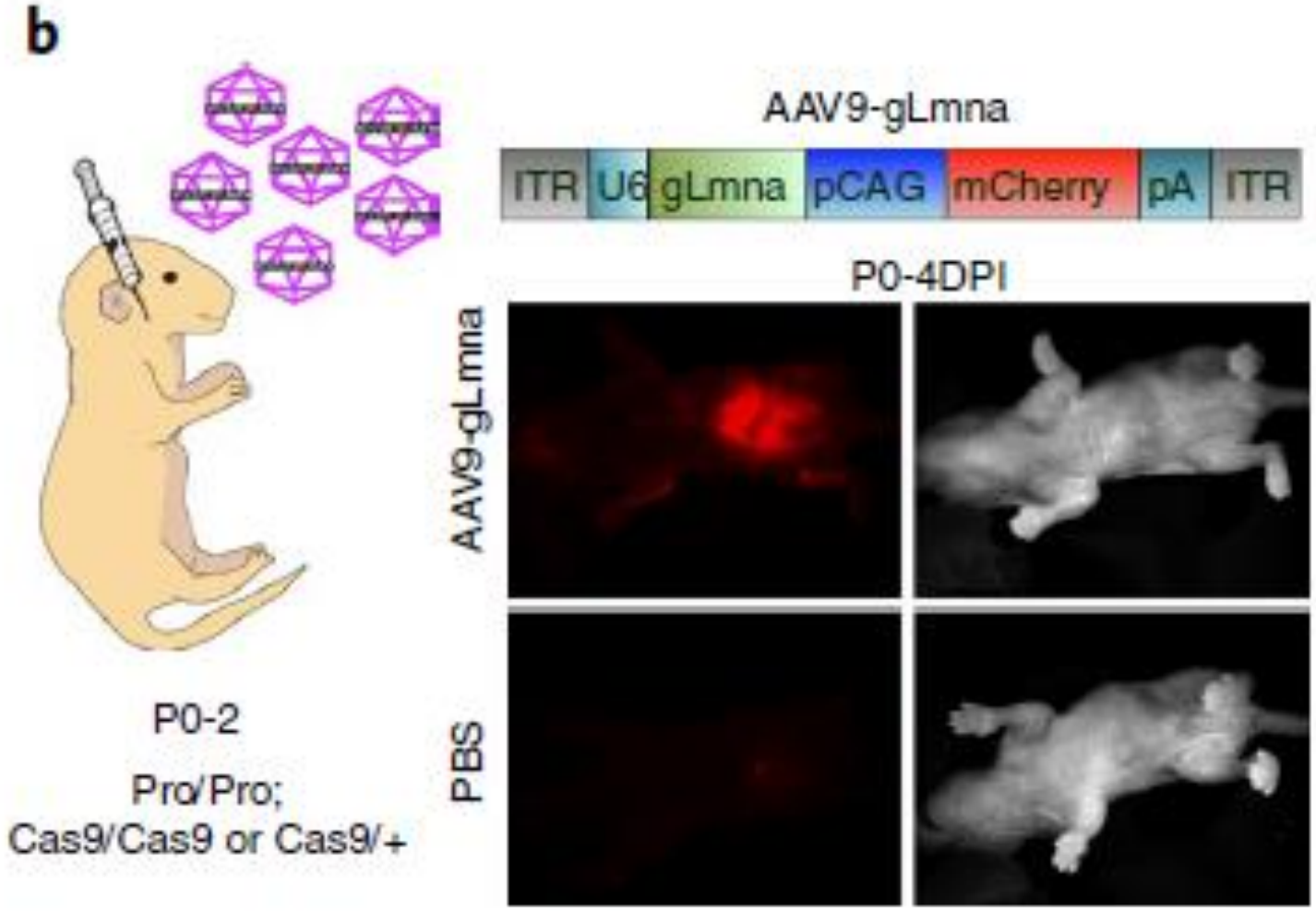
Ergin Beyret[1,3], Hsin-Kai Liao[1,3], Mako Yamamoto[1,2], Reyna Hernandez-Benitez[1], Yunpeng Fu[1], Galina Erikson[1], Pradeep Reddy[1] and Juan Carlos Izpisua Belmonte[1*]

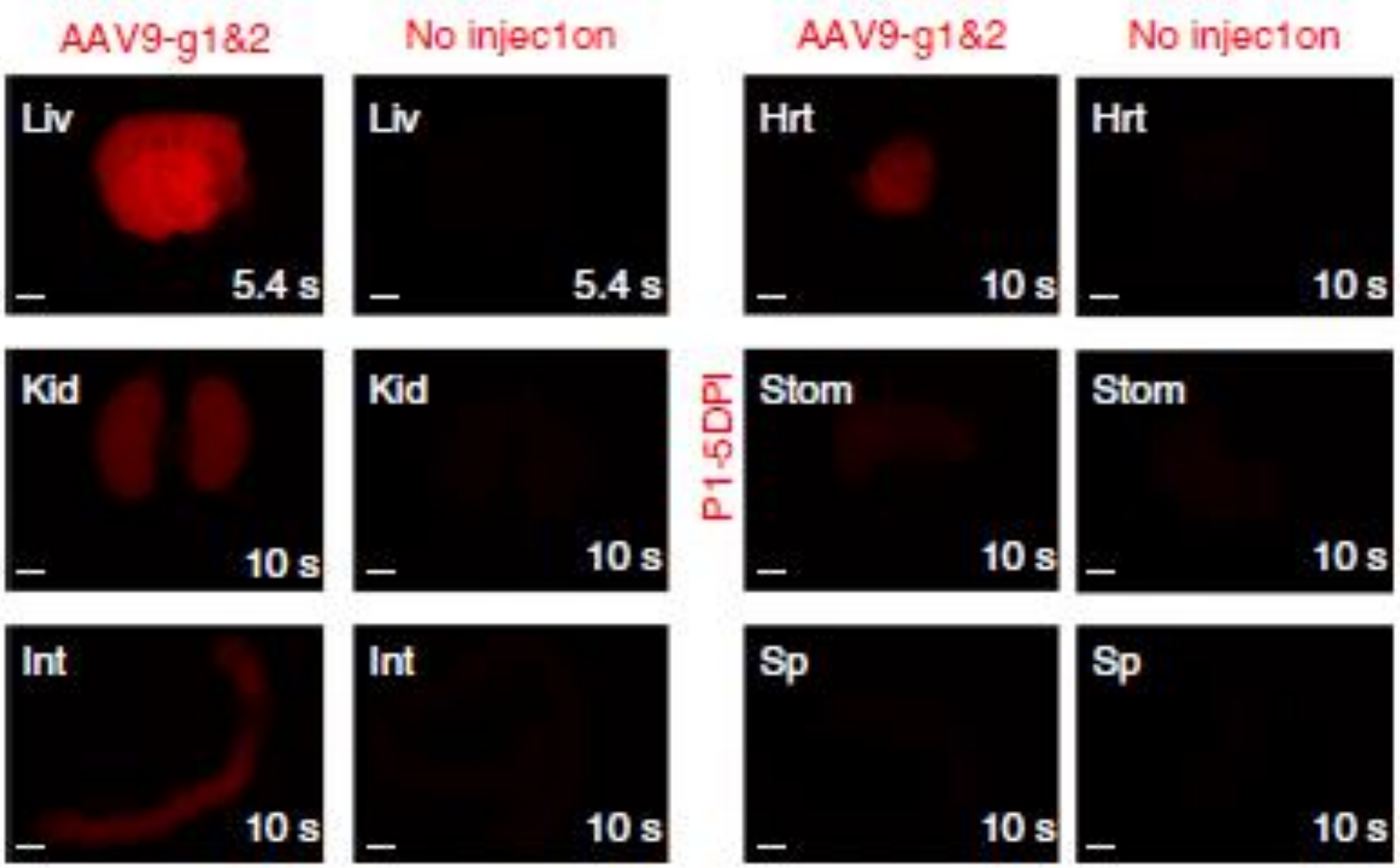
Induction of the corresponding **mutation in the mouse (Gly609Gly)** induces phenotypes similar to those in human patients. On the other hand, lamin A appears to be dispensable, possibly due to compensation from its shorter isoform, lamin C14,15, and mice without lamin A live longer than wild-type (WT) mice, indicating that HGPS results not from lack of lamin A but from the accumulation of progerin. Therefore, **HGPS can be treated by CRISPR–Cas9-targeted disruption of lamin A/progerin.**

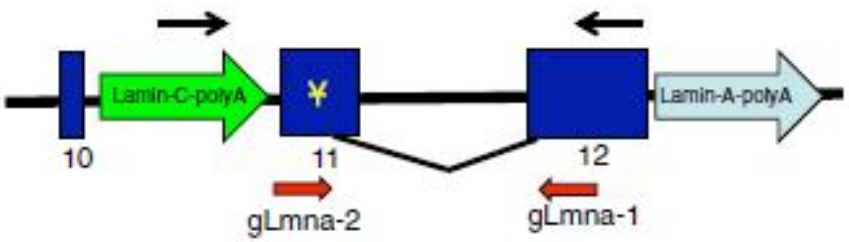


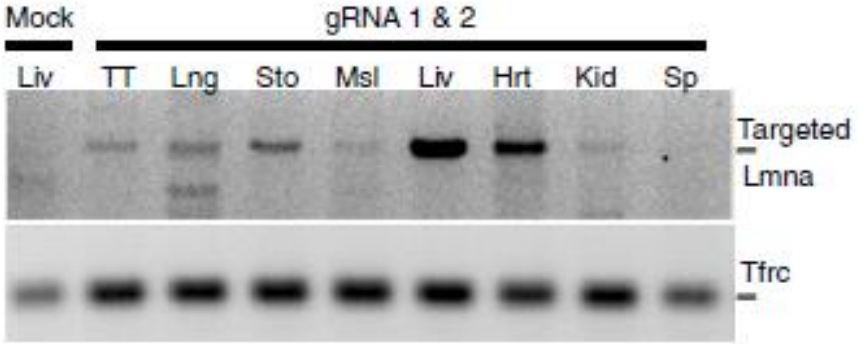
The in vivo gene therapy scheme.

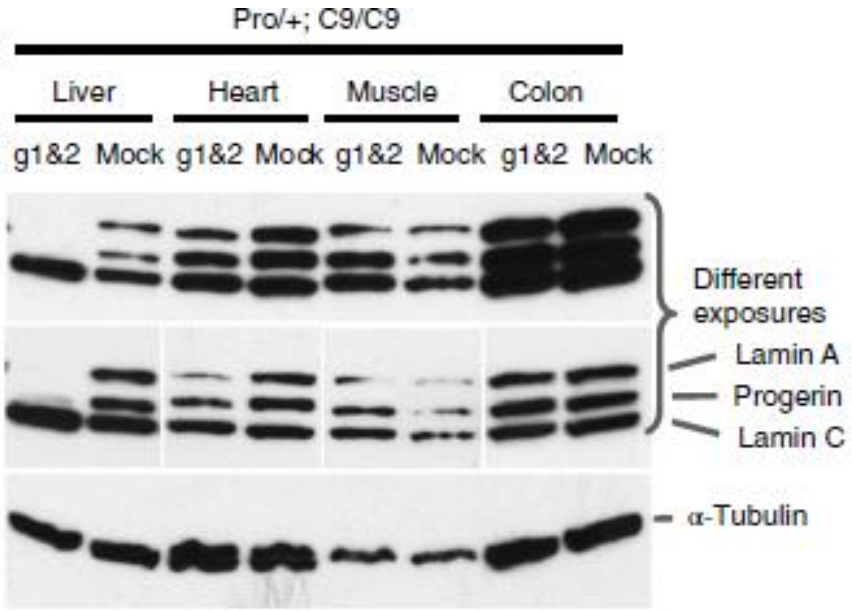
AAV9-mCherry-gLmna was injected into 0- to 2-day-old mice (P0-2). Upper panels show the mCherry signal 4 days post-injection (DPI) of a P0 mouse (P0-4DPI) versus the PBS-injected control (lower panels).



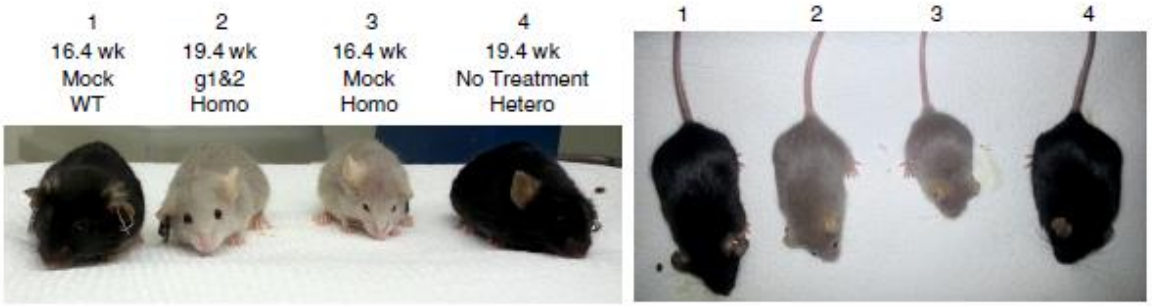

AAV9-g1&2
No injec1on
AAV9-g1&2
No injec1on
Liv
5.4 s
Liv
5.4 s
Hrt
10 s
Hrt
10 s
Kid
10 s
Kid
10 s
P1-5DPI
Stom
10 s
Stom
10 s
Int
10 s
Int
10 s
Sp
10 s
Sp
10 s


Lamin-C-polyA
Lamin-A-polyA
10
11
12
gLmna-2
gLmna-1

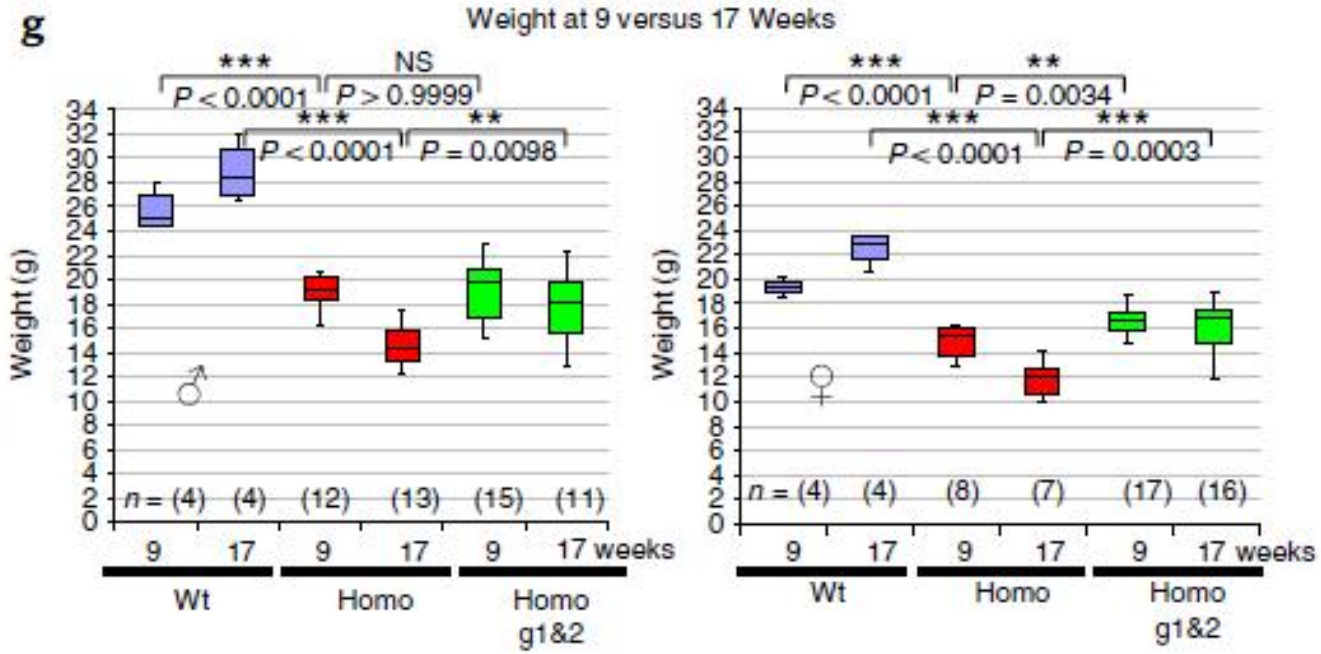

Mock
gRNA 1 & 2
Liv
TT
Lng
Sto
Msl
Liv
Hrt
Kid
Sp
Targeted
Lmna
Tfrc


Pro/+; C9/C9
Liver
Heart
Muscle
Colon
g1&2
Mock
g1&2
Mock
g1&2
Mock
g1&2
Mock
Different exposures
Lamin A
Progerin
Lamin C
α-Tubulin

f

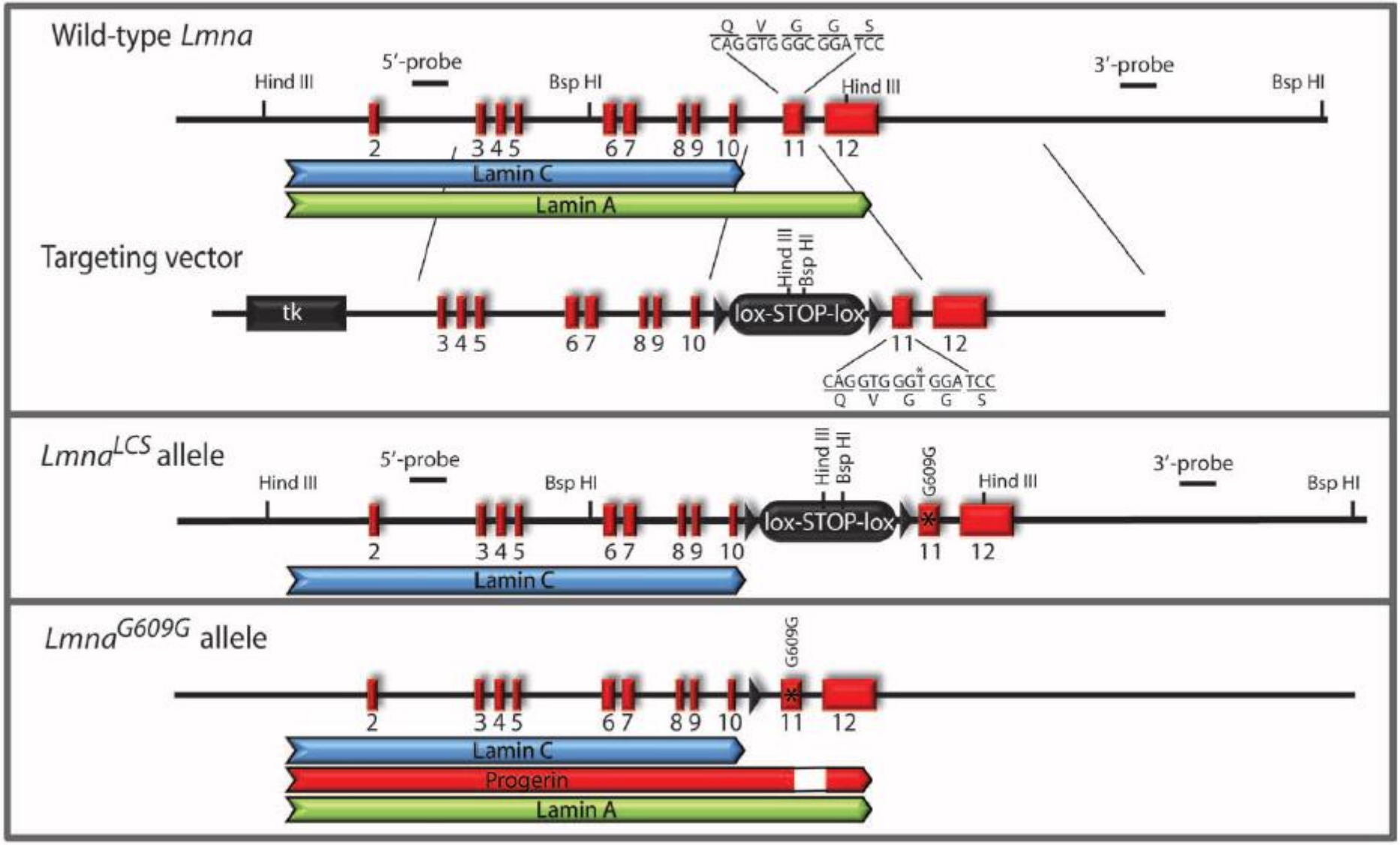

1
16.4 wk
Mock
WT
2
19.4 wk
g1&2
Homo
3
16.4 wk
Mock
Homo
4
19.4 wk
No Treatment
Hetero
1
2
3
4

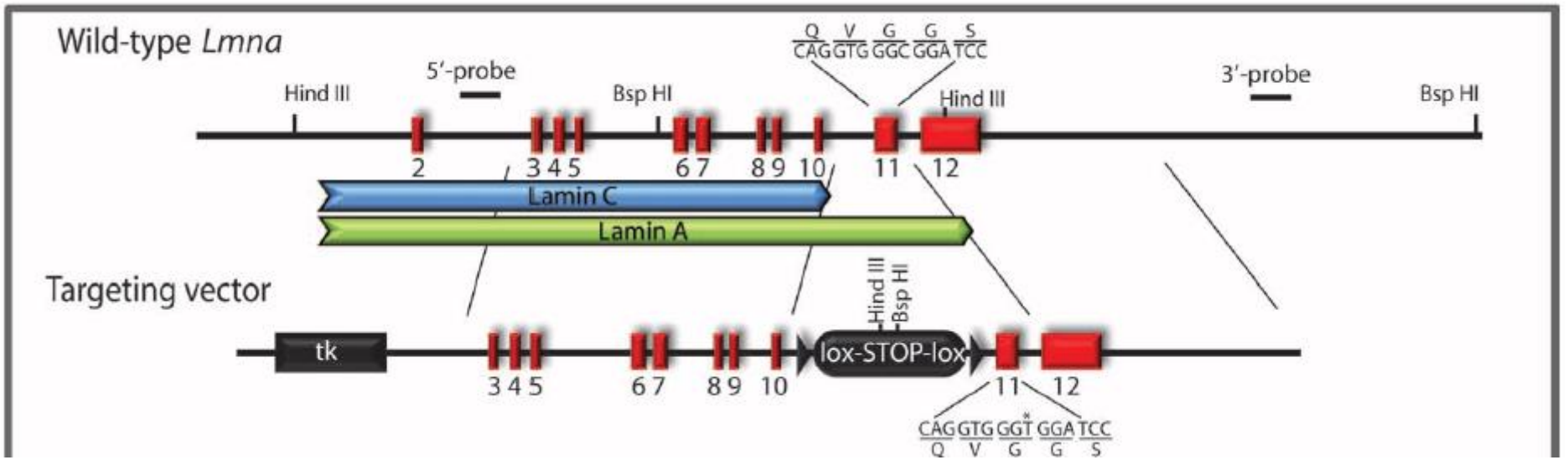
g

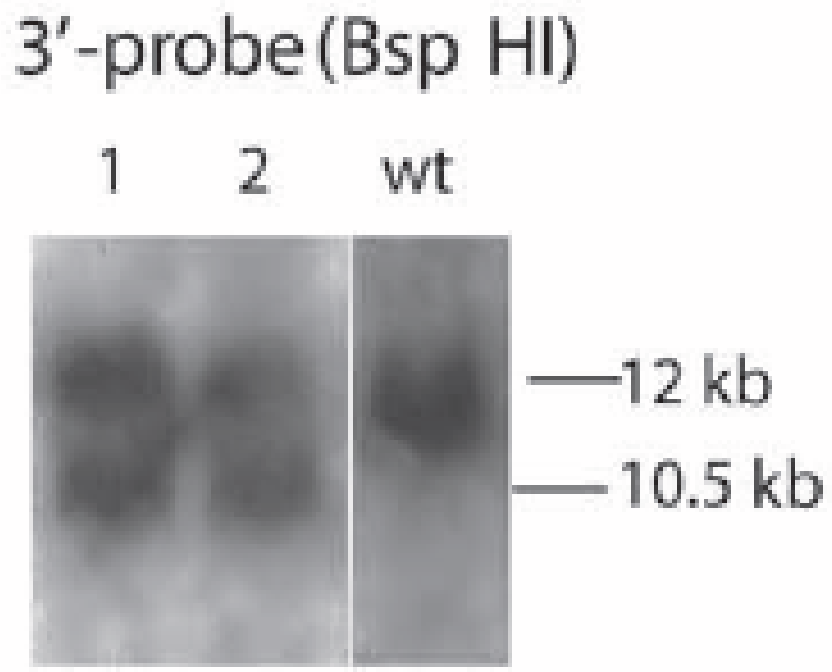

Weight at 9 versus 17 Weeks
***
P < 0.0001
NS
P > 0.9999
***
P < 0.0001
**
P = 0.0098
Weight (g)
n = (4) (4) (12) (13) (15) (11)
9
17
9
17
9
17 weeks
Wt
Homo
Homo g1&2
***
P < 0.0001
**
P = 0.0034
***
P < 0.0001
***
P = 0.0003
Weight (g)
n = (4) (4) (8) (7) (17) (16)
9
17
9
17
9
17 weeks
Wt
Homo
Homo g1&2

IN SUMMARY:

- **High order of chromatin structure is a component for TRANSCRIPTION REGULATION**
- **Alteration of LAMININ A, important in chromatin organization, induces PROGERIA**
- **MICE MODEL IS USED TO STUDY MOLECULAR MECHANISM IN DISEASE PROGRESSION**


Wild-type Lmna
Q V G G S
CAG GTG GGC GGA TCC
5′-probe
3′-probe
Hind III
Bsp HI
Hind III
Bsp HI
2 3 4 5 6 7 8 9 10 11 12
Lamin C
Lamin A
Targeting vector
Hind III
Bsp HI
tk
lox-STOP-lox
3 4 5 6 7 8 9 10 11 12
CAG GTG GGT GGA TCC
Q V G G S
Lmna^LCS allele
5′-probe
3′-probe
Hind III
Bsp HI
Hind III
Bsp HI
G609G
Hind III
Bsp HI
lox-STOP-lox
2 3 4 5 6 7 8 9 10 11 12
Lamin C
Lmna^G609G allele
G609G
2 3 4 5 6 7 8 9 10 11 12
Lamin C
Progerin
Lamin A


Wild-type Lmna
Q V G G S
CAG GTG GGC GGA TCC
Hind III
5′-probe
Bsp HI
Hind III
3′-probe
Bsp HI
2
3 4 5
6 7
8 9 10
11
12
Lamin C
Lamin A
Targeting vector
Hind III
Bsp HI
tk
lox-STOP-lox
3 4 5
6 7
8 9 10
11
12
CAG GTG GGT GGA TCC
Q V G G S


3′-probe (Bsp HI)
1
2
wt
12 kb
10.5 kb