

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

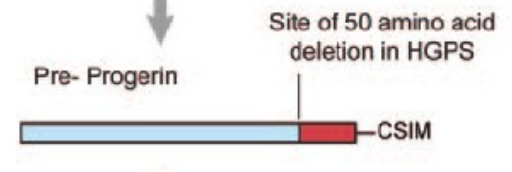
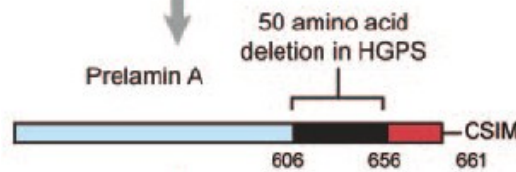
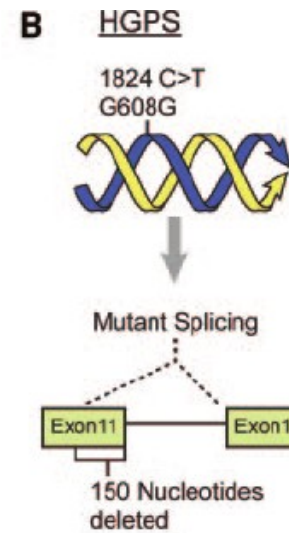
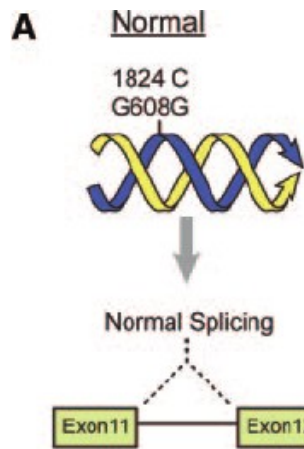
is caused by a **point mutation in the LMNA gene** that activates a cryptic donor splice site and yields a truncated form of prelamin A called progerin

LAMINA ALTERATIONS INDUCE DISEASE

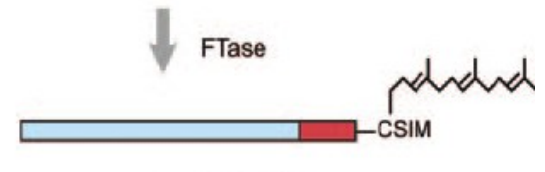
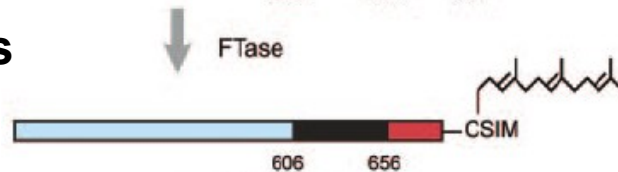
Progeria, or Hutchinson–Gilford progeria syndrome (HGPS), is a rare, fatal genetic disease characterized by an **appearance of accelerated aging in children**.

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

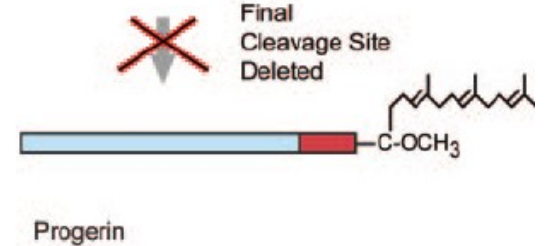
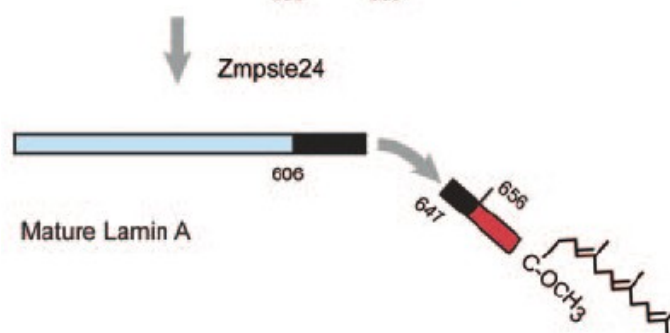
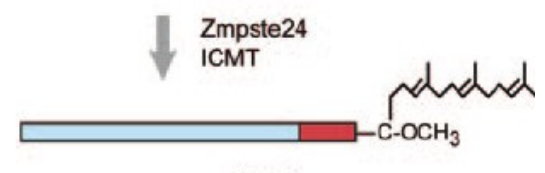
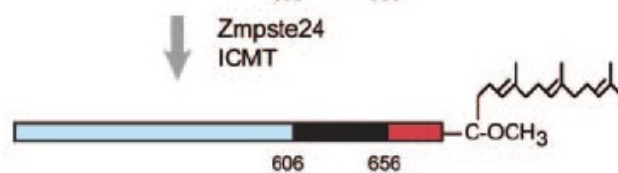
Progerin accumulates in cells causing progressive molecular defects, including nuclear shape abnormalities, chromatin disorganization, damage to DNA and delays in cell proliferation.



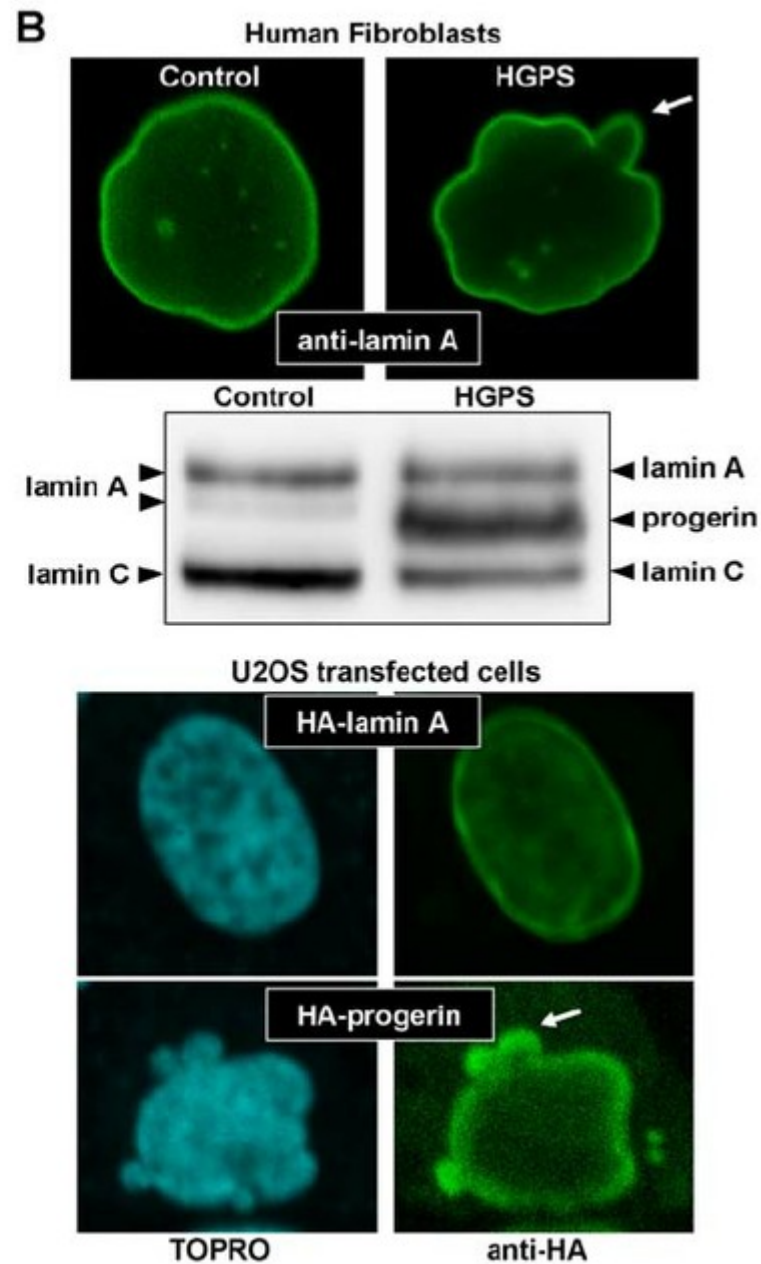
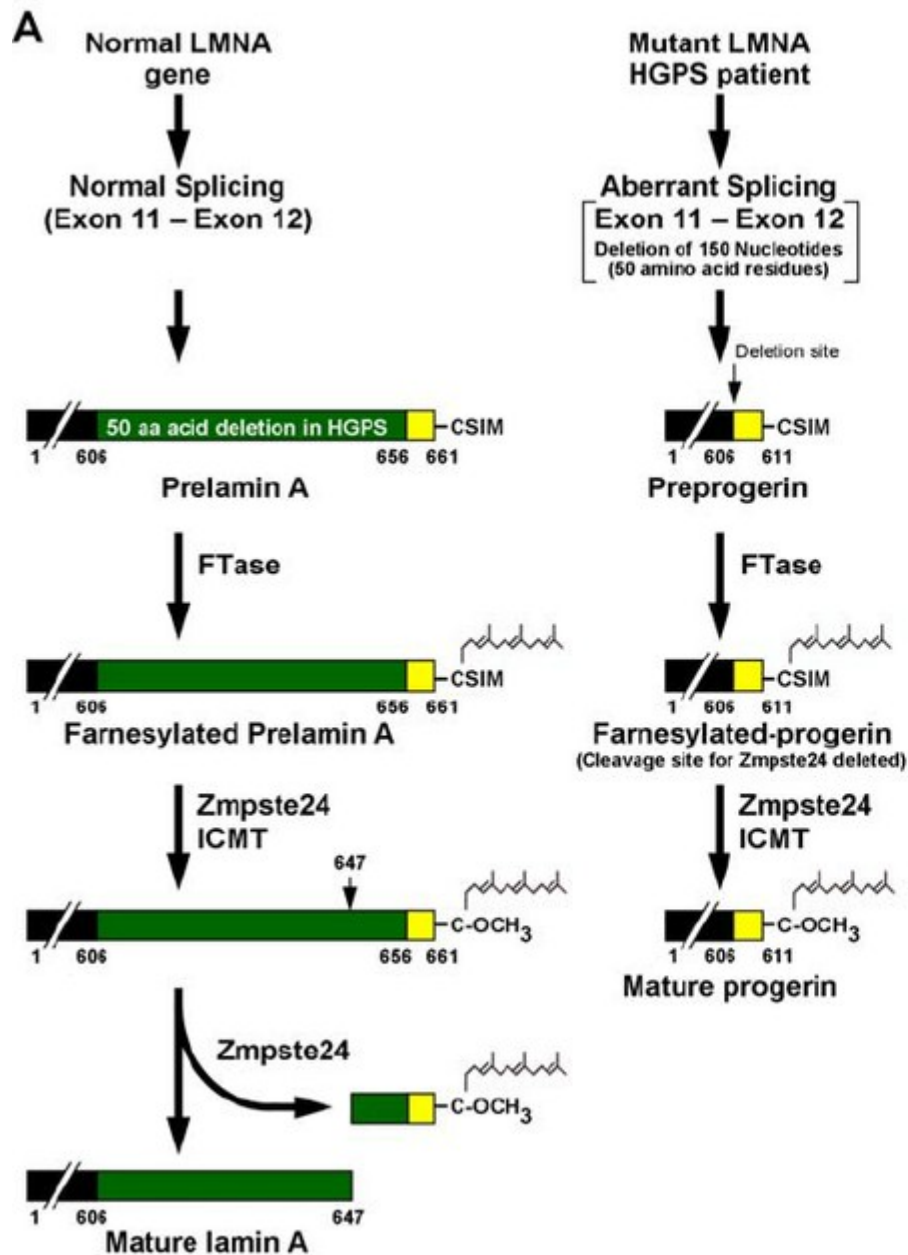
farnesylated C-terminus



ZMPSTE24 enzyme cleave the farnesylated C-terminus



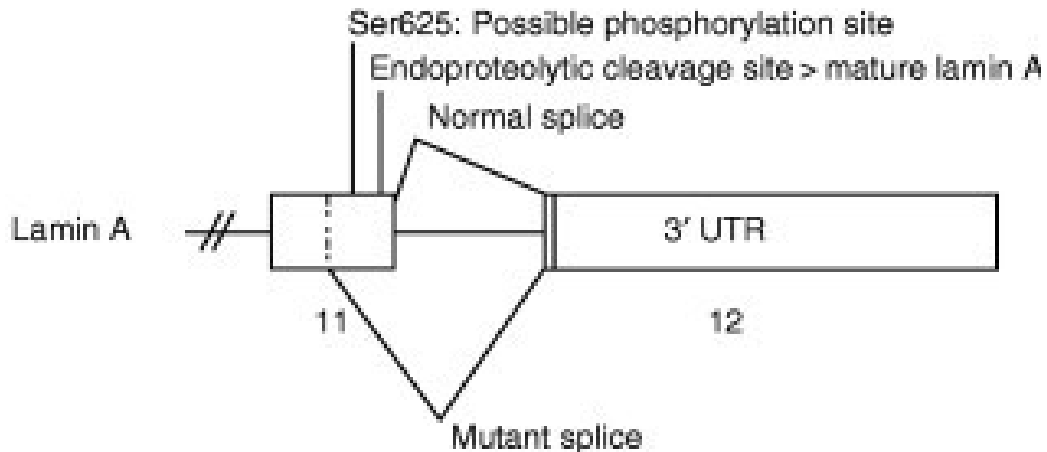
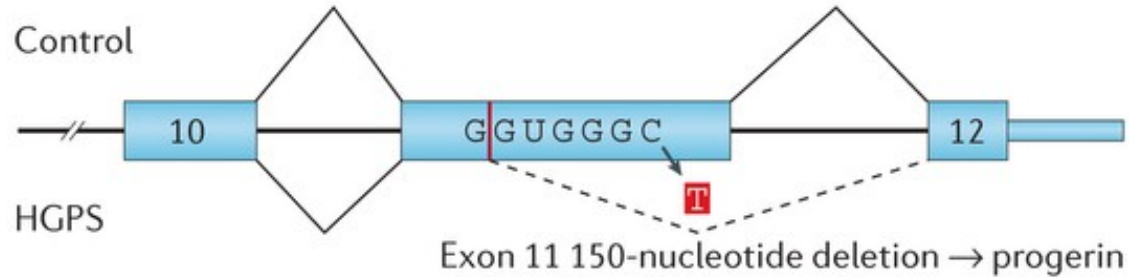
LAMININ A



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)

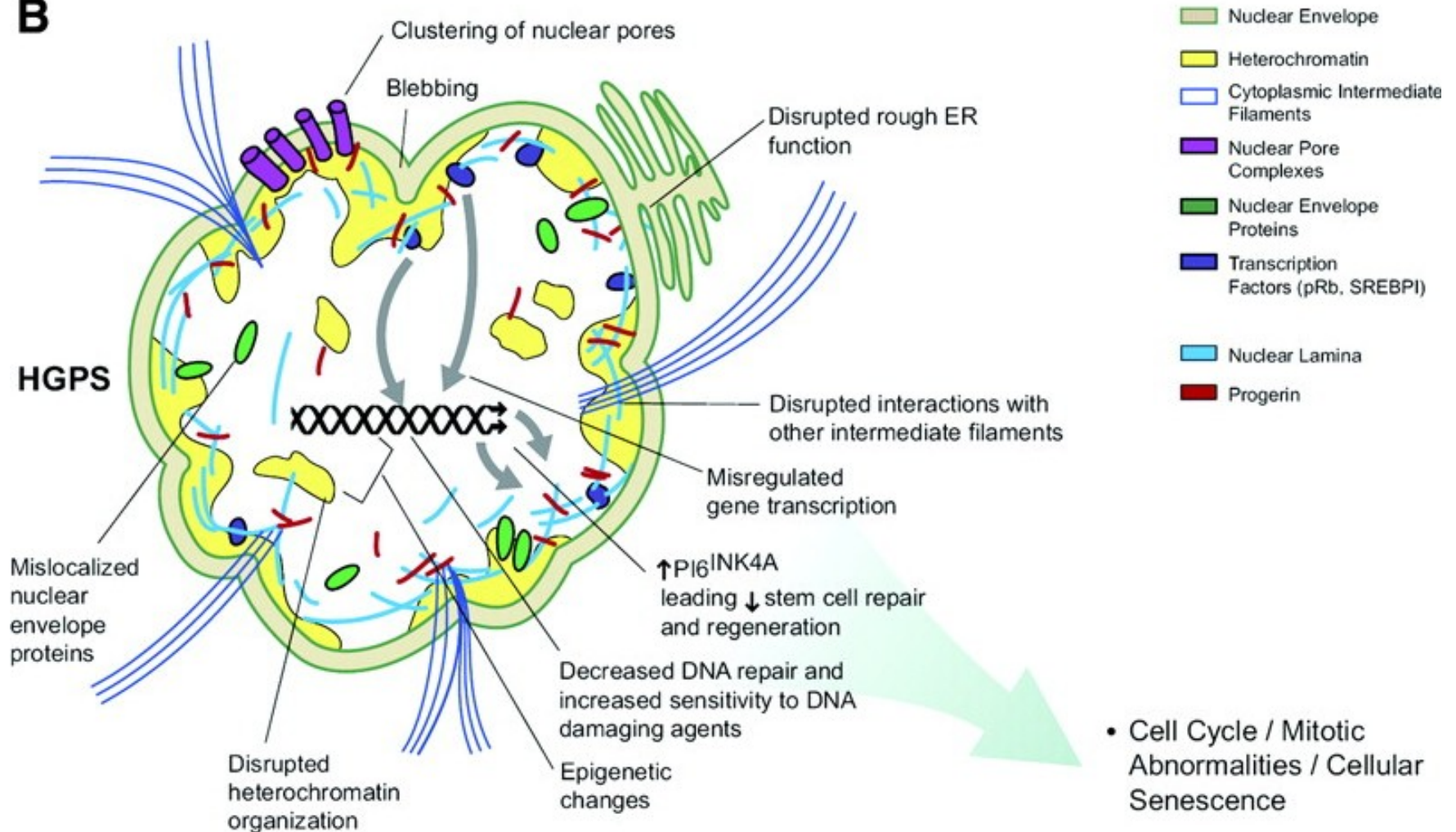
Alternative 5'ss (c. 1824C>T)



Loss of cleavage site for protease

PROGERIA EFFECTS ON THE BIOLOGICAL FUNCTIONS

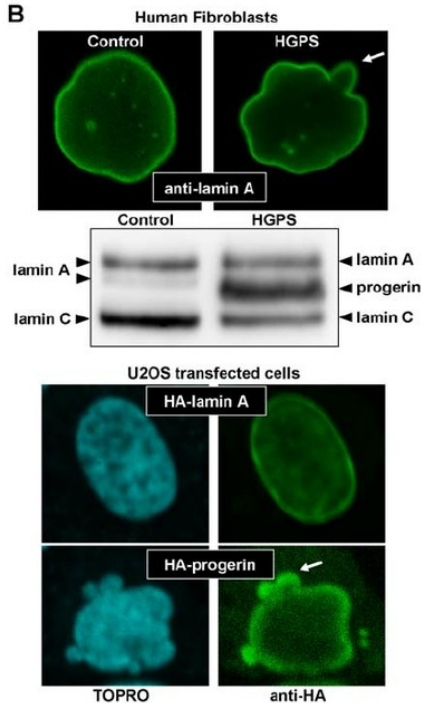
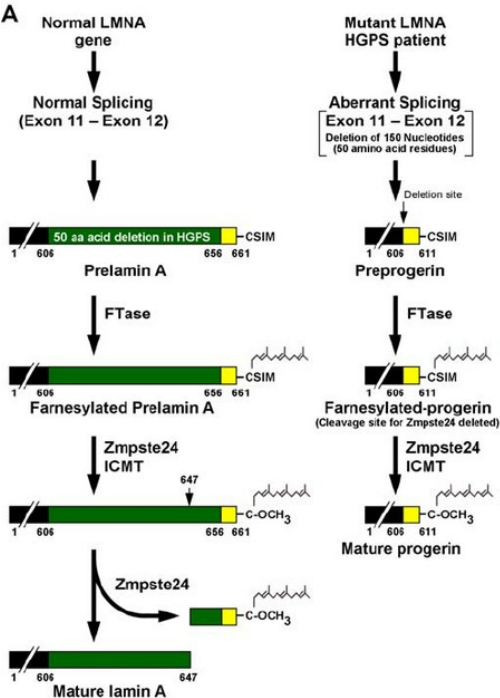
B



- Nuclear Envelope
- Heterochromatin
- Cytoplasmic Intermediate Filaments
- Nuclear Pore Complexes
- Nuclear Envelope Proteins
- Transcription Factors (pRb, SREBP1)
- Nuclear Lamina
- Progerin

- Cell Cycle / Mitotic Abnormalities / Cellular Senescence
- Genome Instability
- Accelerated Aging Cardiovascular Disease

THERAPY



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†}

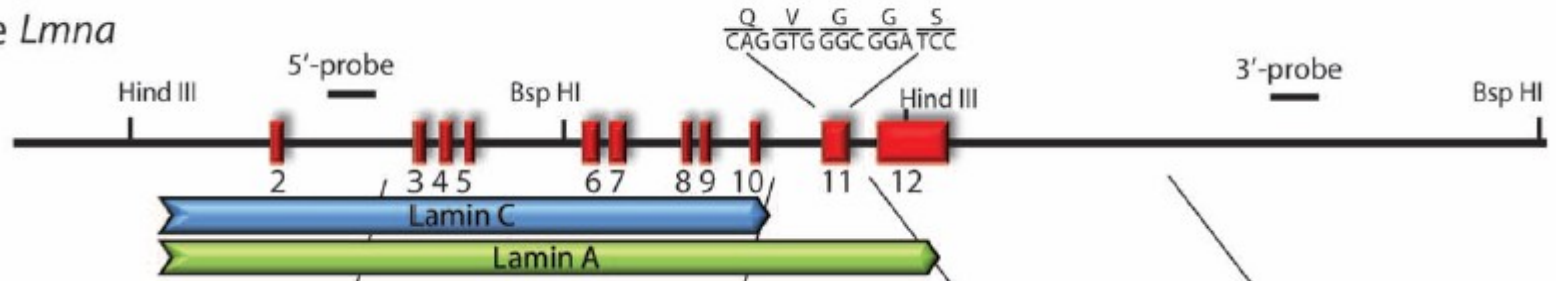
Hutchinson-Gilford progeria syndrome (HGPS) is caused by a point mutation in the *LMNA* gene that activates a cryptic donor splice site and yields a truncated form of prelamin A called progerin. Small amounts of progerin are also produced during normal aging. Studies with mouse models of HGPS have allowed 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

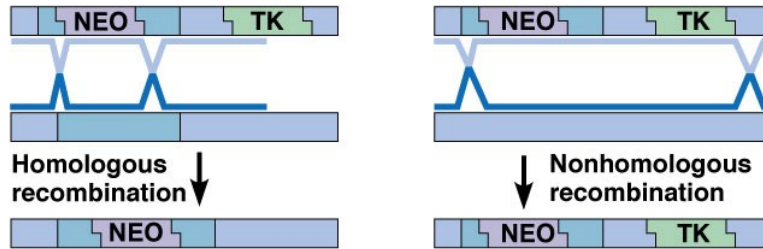
Wild type locus *Lmna* that encodes for lamin C and lamin A

Wild-type *Lmna*



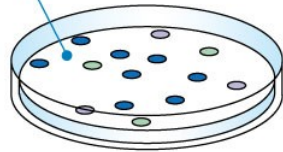
Enrichment for homologous recombinants

1 DNA is introduced into embryonic stem (ES) cells. The DNA contains a non-functional copy of the gene of interest, an antibiotic resistance gene (Neo) and a gene encoding a viral enzyme (TK).

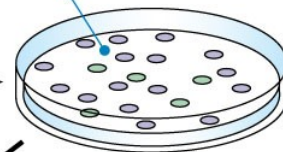


TK: thymidine kinase
Converts ganciclovir in a toxic product

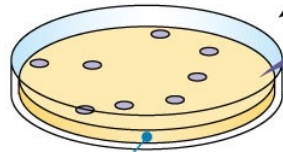
2 ES cells are grown in culture.



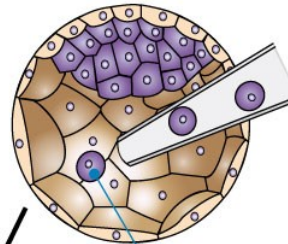
3 Cells containing DNA are selected using an antibiotic (neomycin).



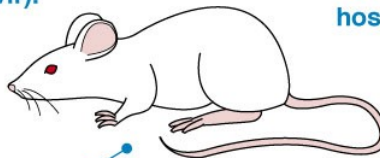
4 Cells in which the DNA has inserted by recombination are selected using an antiviral drug (ganciclovir).



5 "Knockout" cells are inserted into a host embryo.

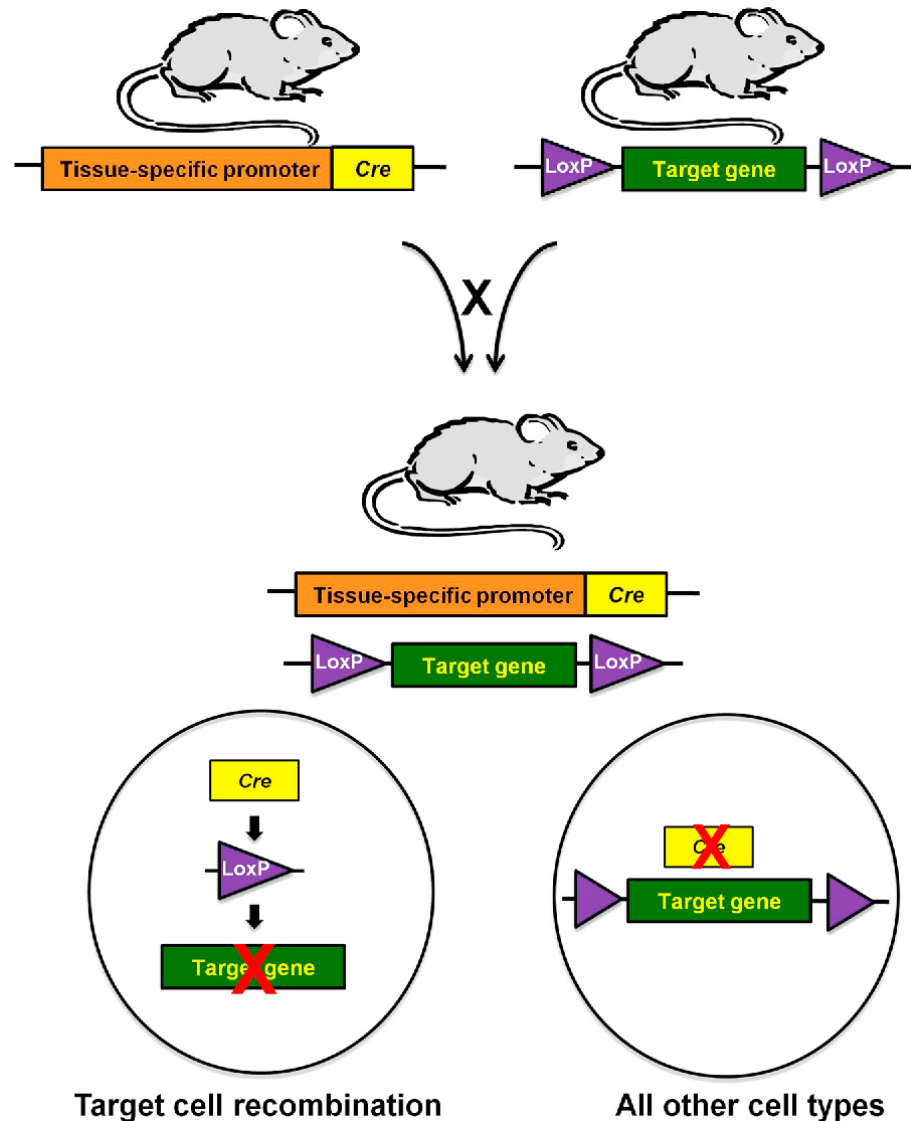


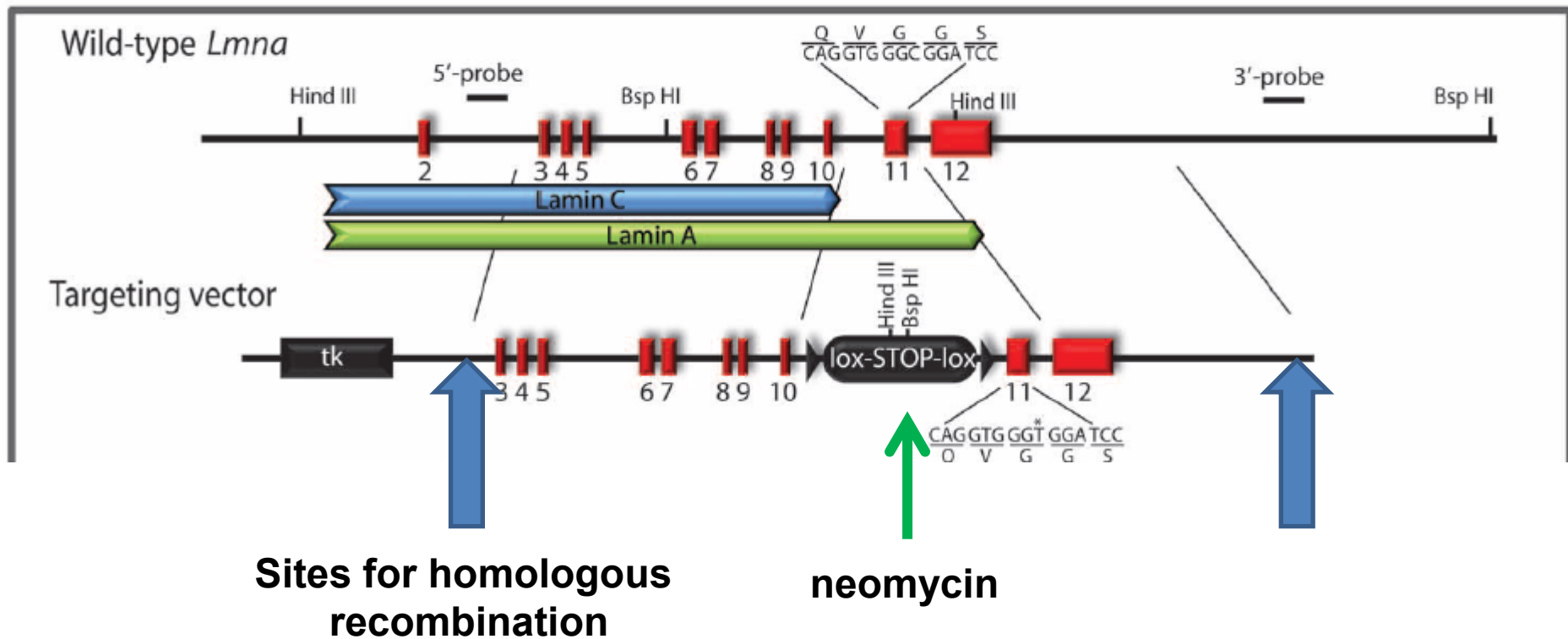
6 Resulting mice are bred to produce "knockout" mice.



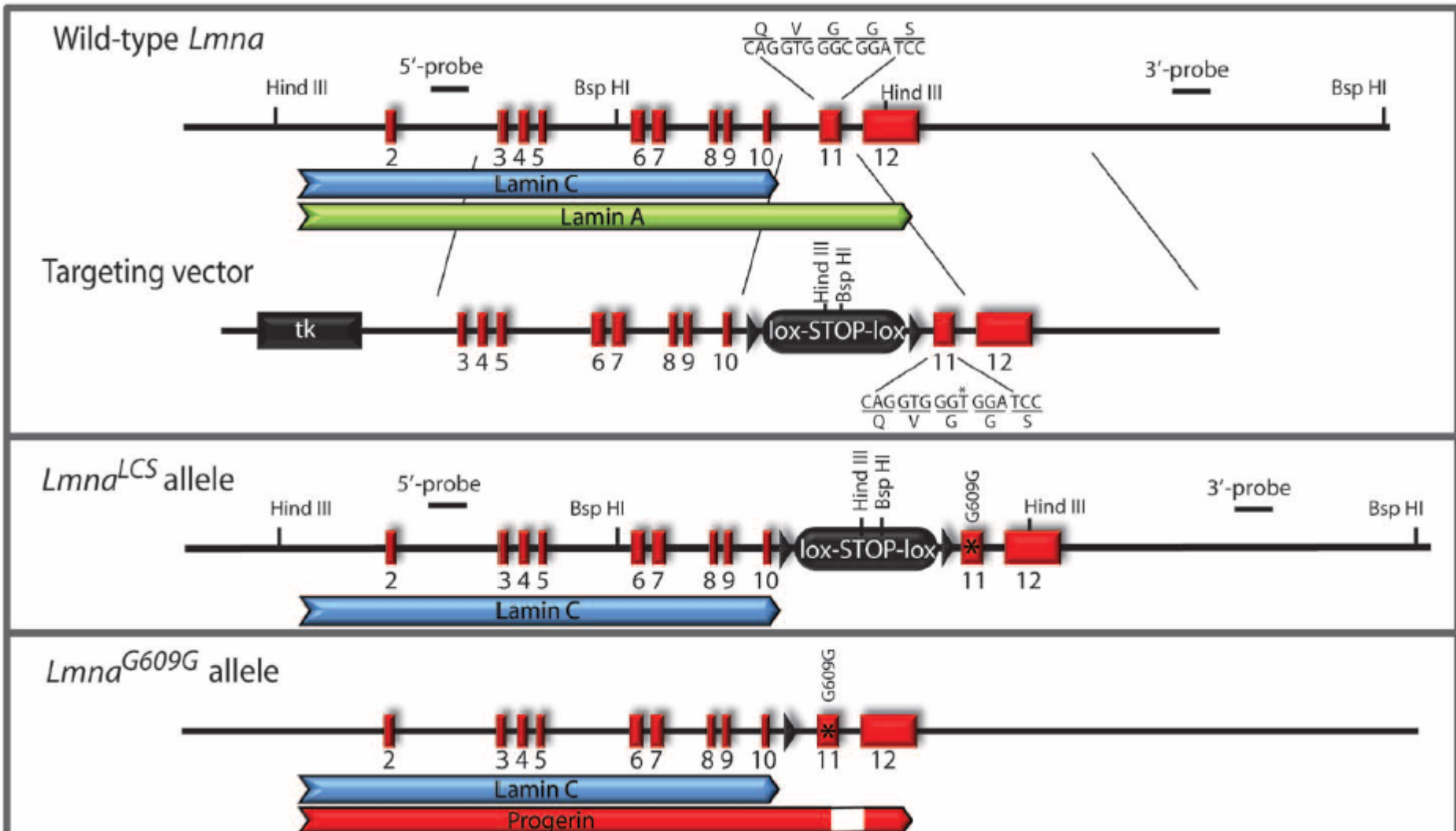
Cre/LoxP system

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





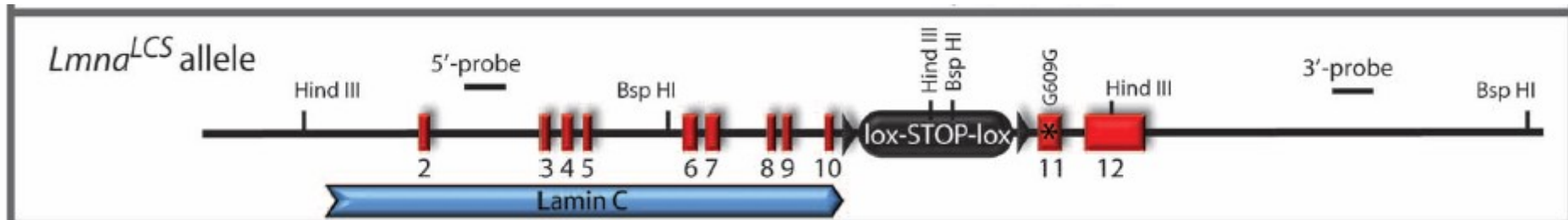
A conditional mutant allele with a **neomycin resistance gene** flanked by **two loxP sites** close to *Lmna* intron 10. This cassette was able to prevent the formation of prelamina A transcripts by blocking lamin A-specific splicing and is obtained the transcript for lamin C.



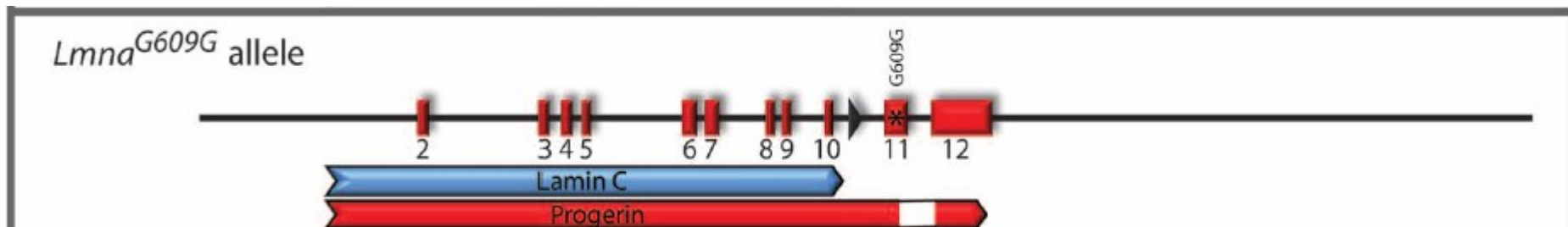
Lmna^{LCS}/+mice and crossed them with a **Cre-deleter mouse strain** to obtain germline removal of the neomycin resistance cassette. *Lmna*^{G609G} knock-in allele, which expressed lamin C, lamin A, and progerin.

Plan to create Progerin model:

- *Lmna*^{LCS} (Lamin C-Stop), directs only the expression of lamin C and allows study of the potential effects of lamin A deficiency.

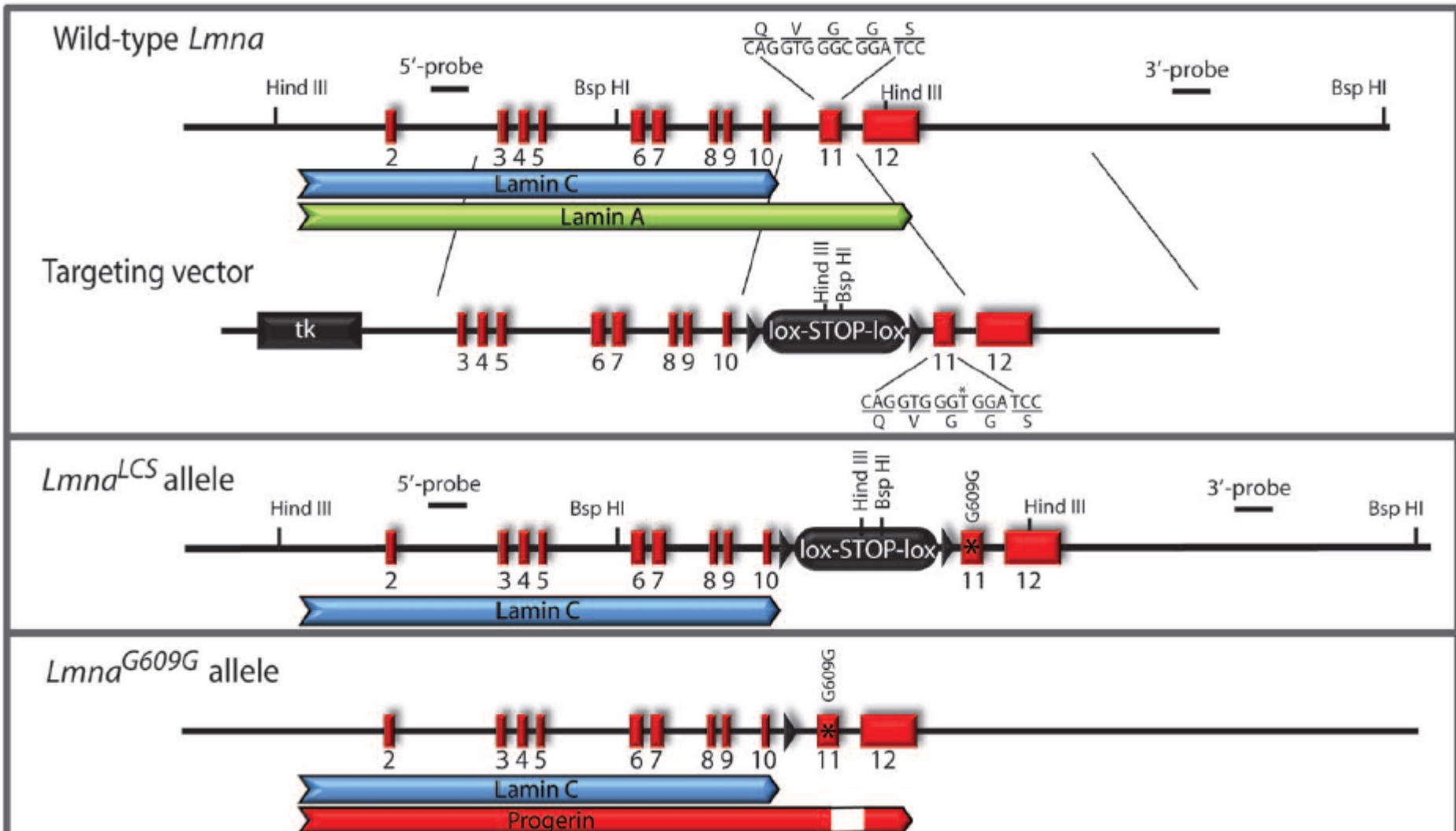


- *Lmna*^{G609G} knock-in allele, which expressed lamin C, lamin A, and progerin

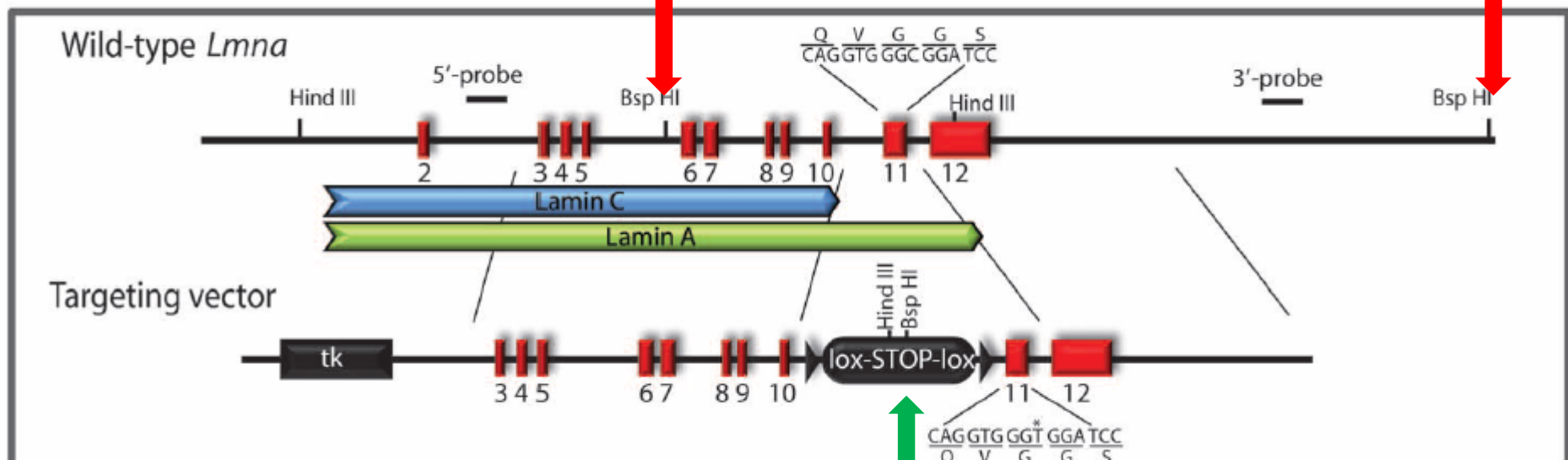


CONDITIONAL TRANSGENIC MICE

**GENOTYPING
AND
CHARACTERIZATION**

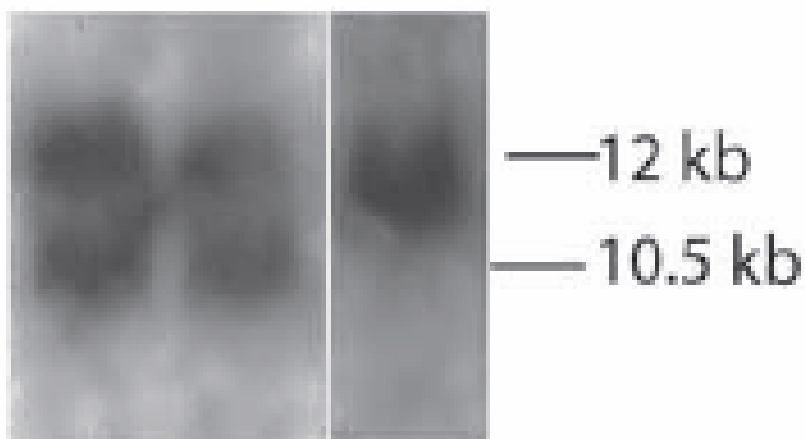


Lmna^{LCS}/+mice and crossed them with a **Cre-deleter mouse strain** to obtain germline removal of the neomycin resistance cassette. *Lmna*^{G609G} knock-in allele, which expressed lamin C, lamin A, and progerin.

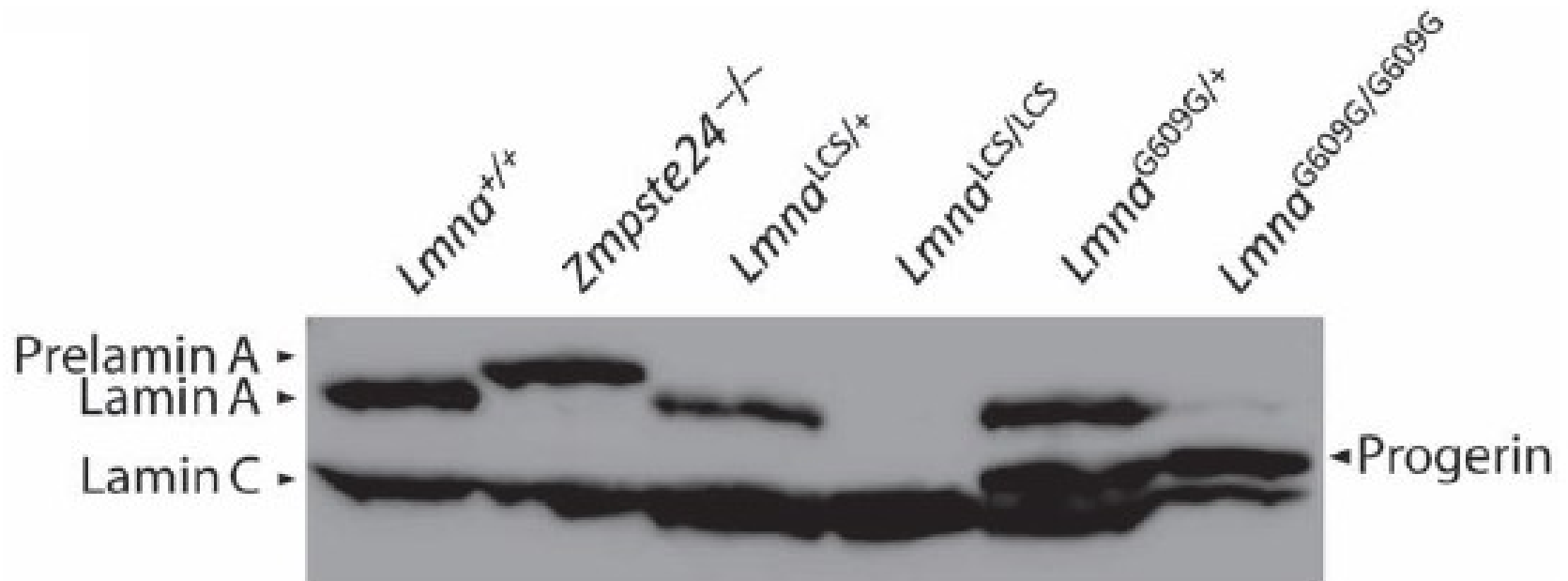


3'-probe (Bsp HI)

1 2 wt

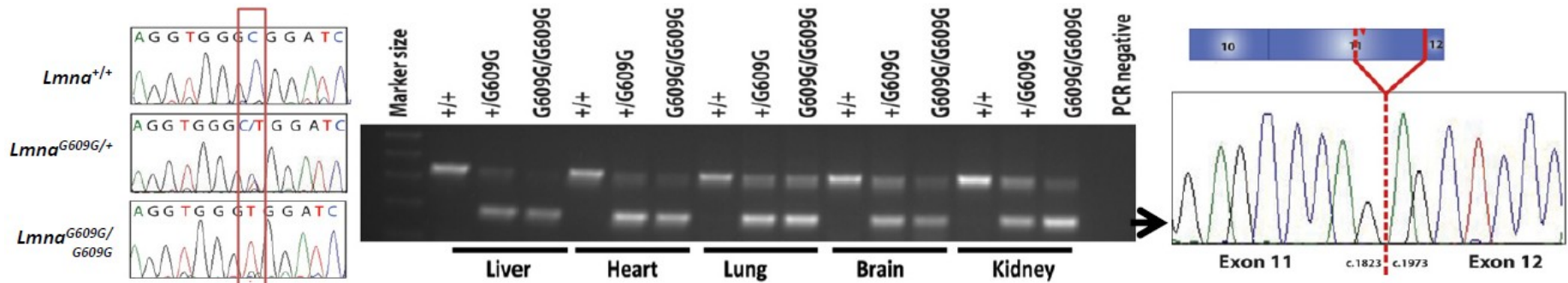


PROGERIN AND LAMININ TYPES PROTEIN EXPRESSION

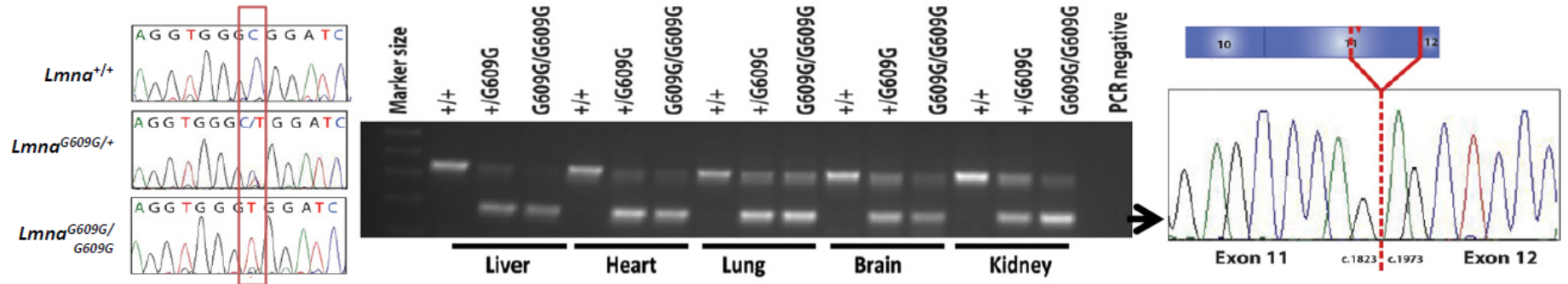


Western (immuno) blot analysis of mouse adult fibroblasts obtained from the mice with the various genotypes used in the study. Lamin A, lamin C, prelamin A, and progerin were detected with a monoclonal antibody against lamin A/C (Manlac-1).

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**

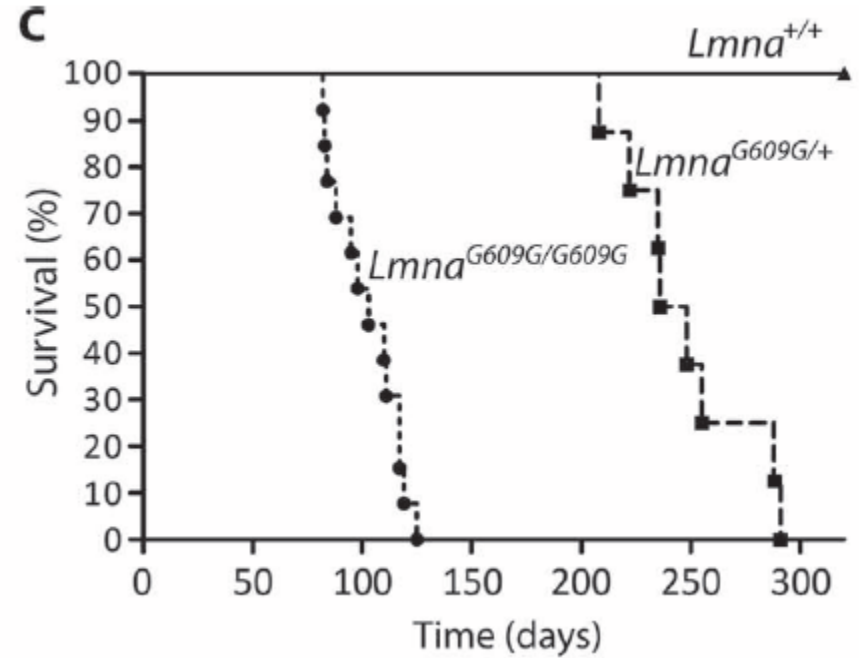
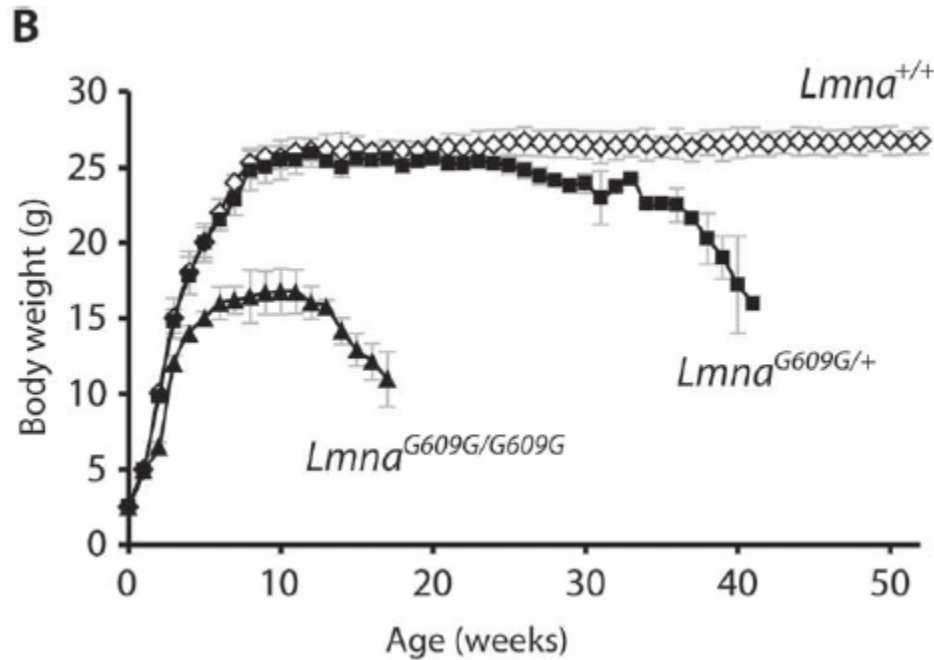
PHENOTYPE

Lmna G609G has reduction in growth rates

Lmna^{+/+} *Lmna*^{G609G/+} *Lmna*^{G609G/G609G}

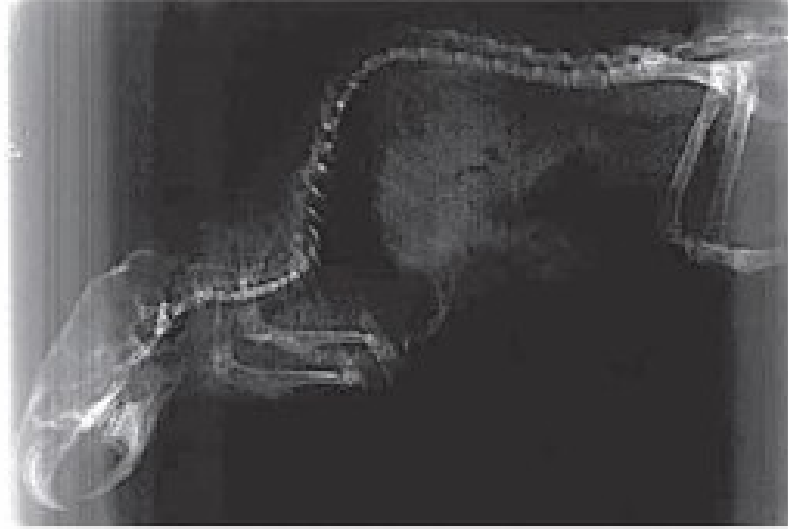


Lmna G609G has loss of weight and short life

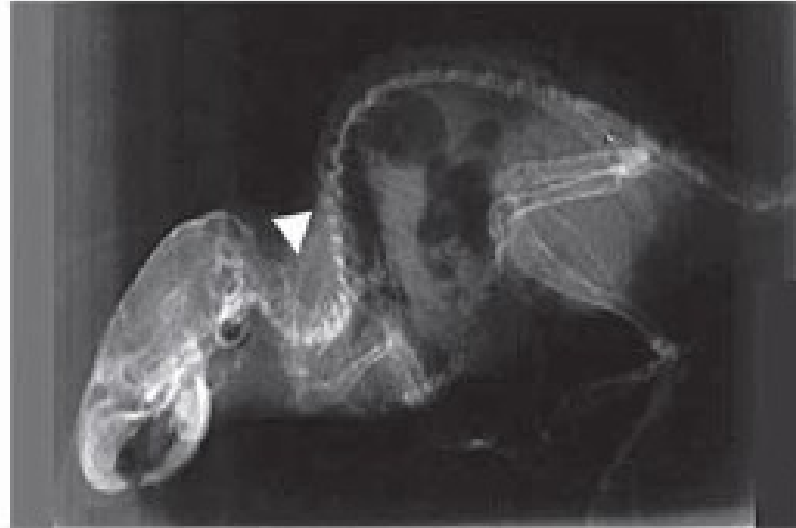


**Lmna G609G has a marked curvature of the spine
(cervicothoracic lordokyphosis)**

+/+

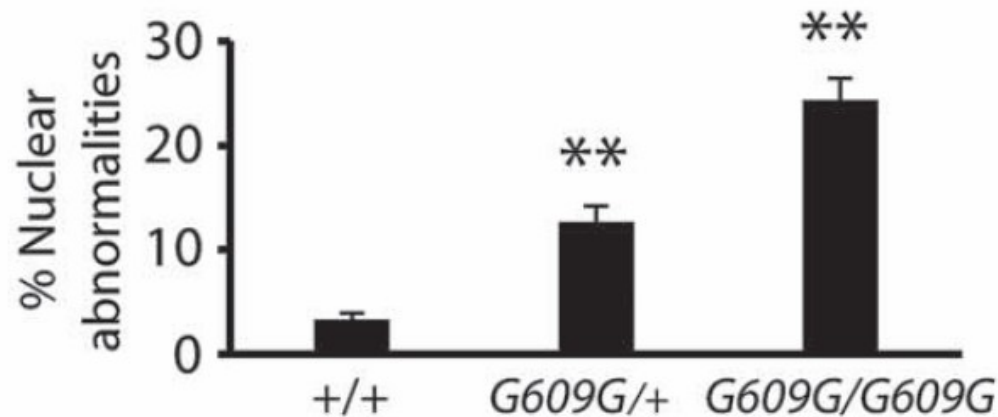
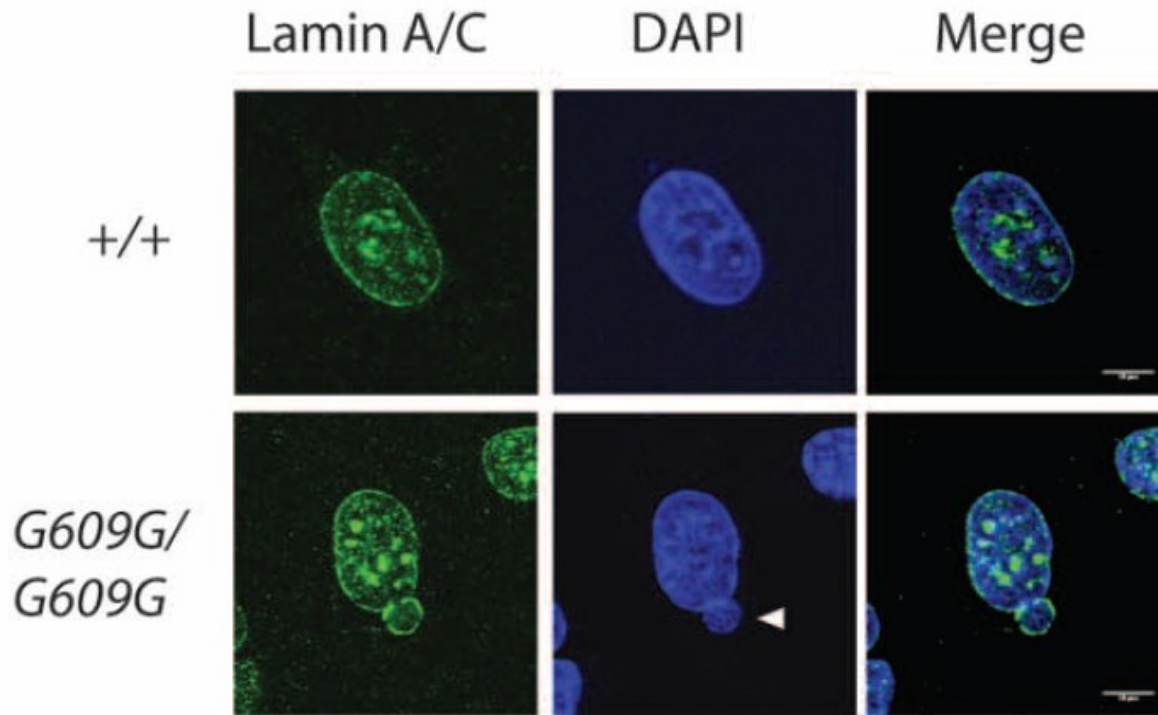


G609G/G609G

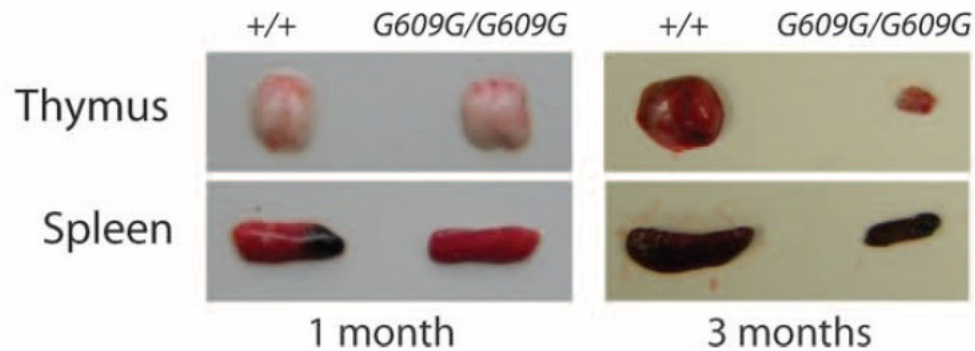
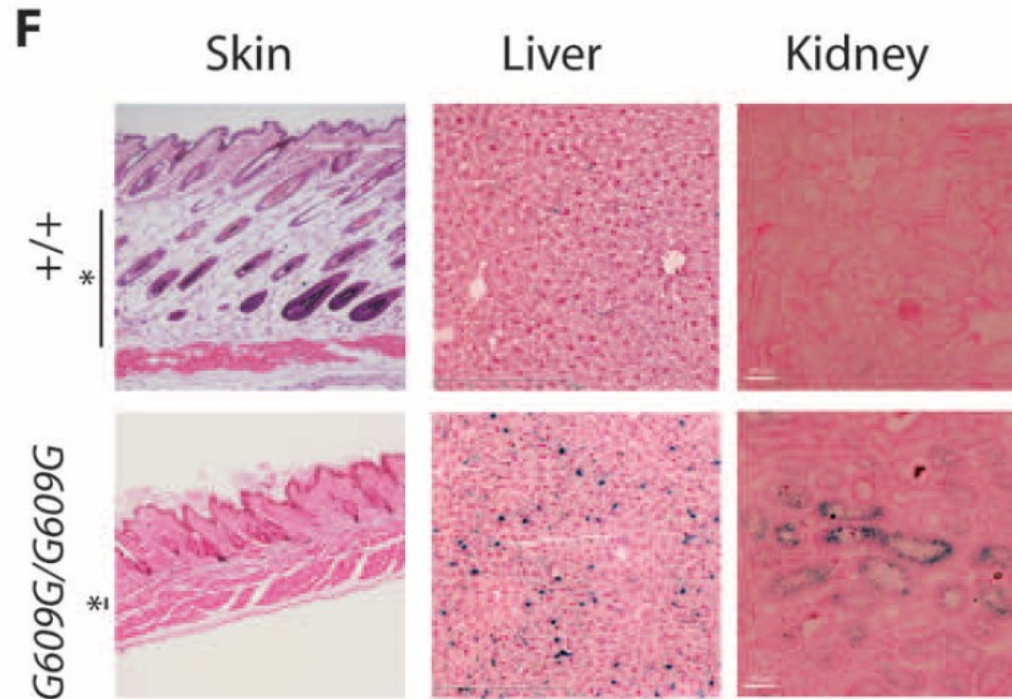


Progerin distribution is altered in mutant mice

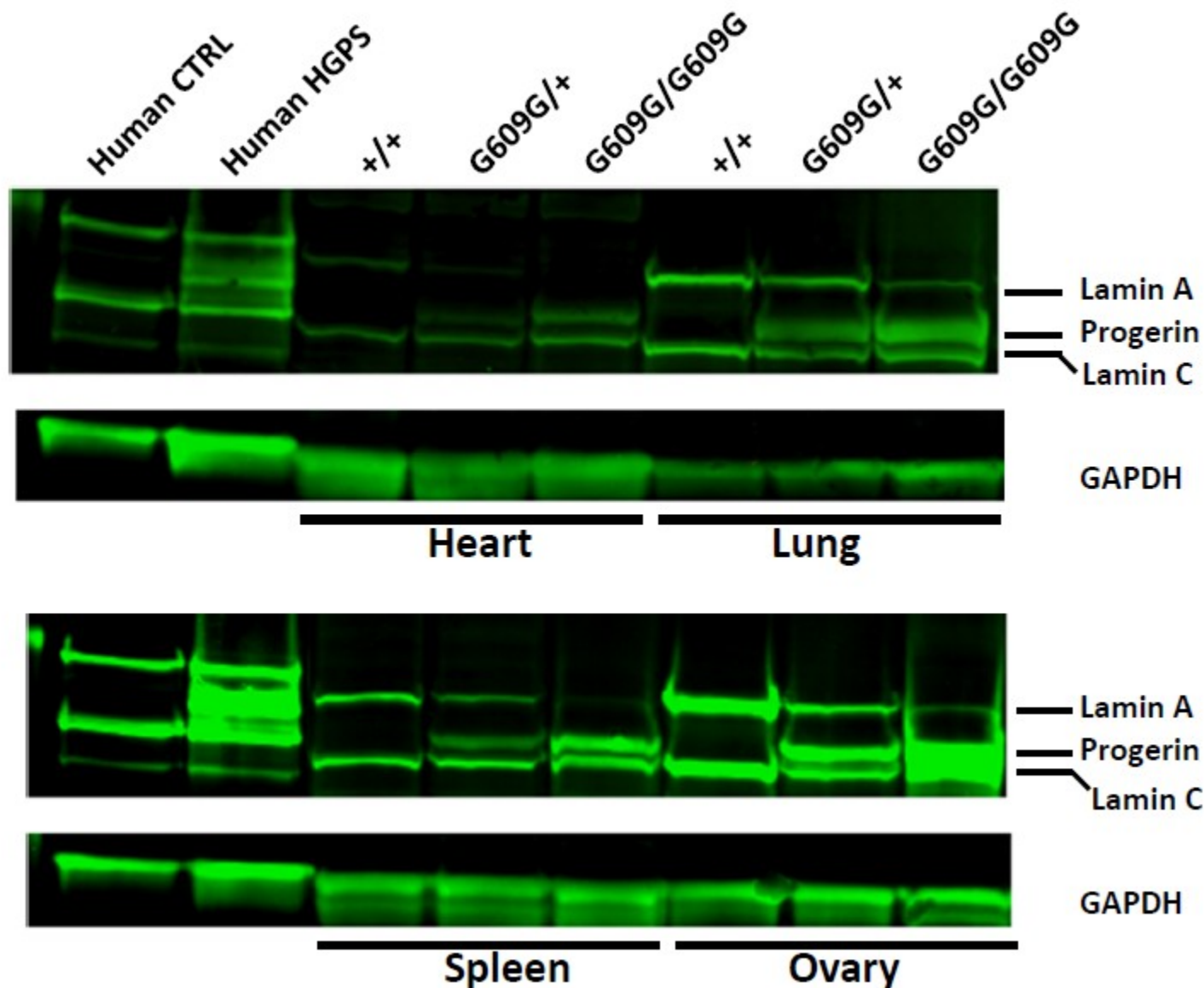
Using immunofluorescence assay by confocal microscopy



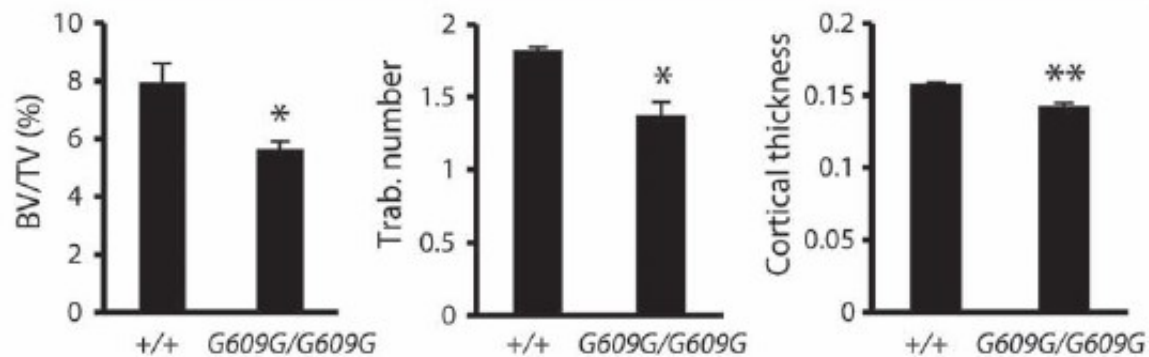
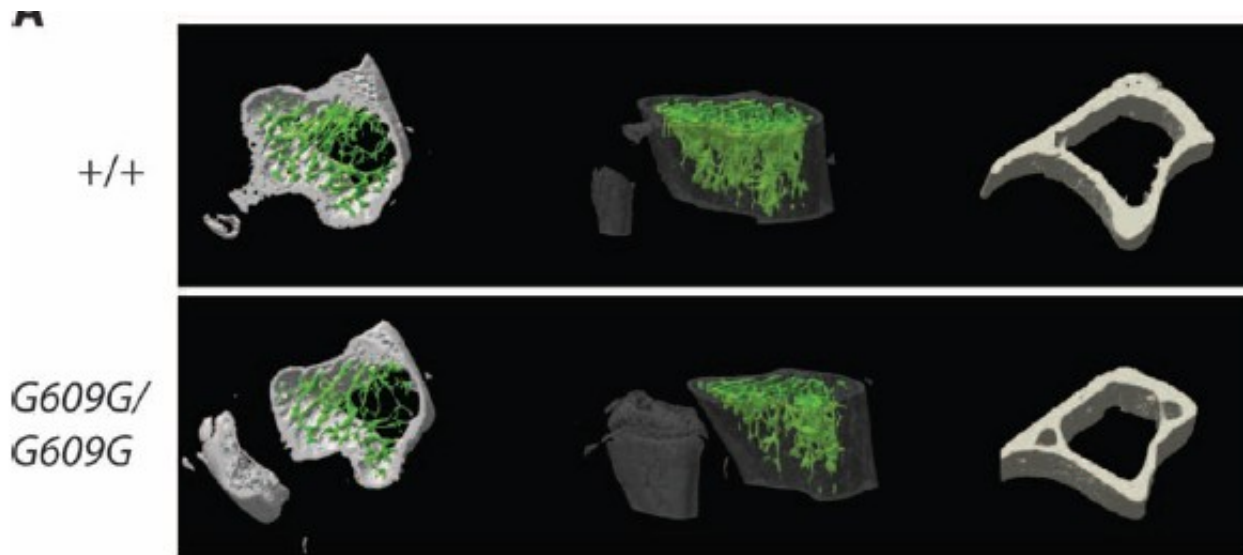
Tissue Senescence are tested using b-galattosidase



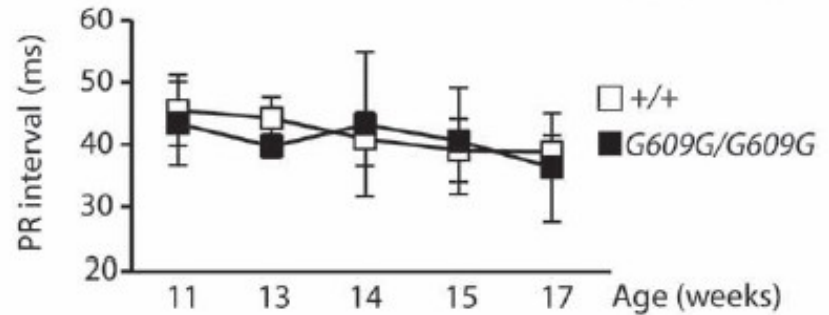
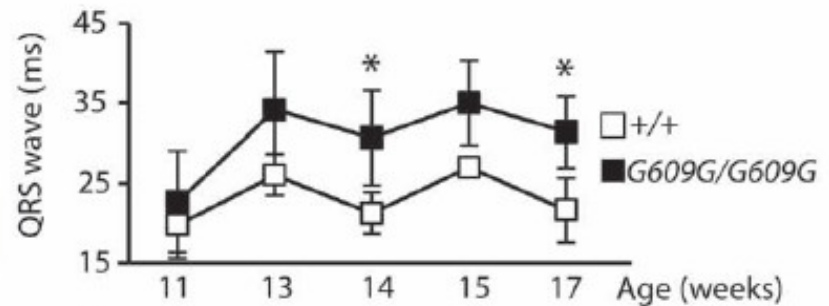
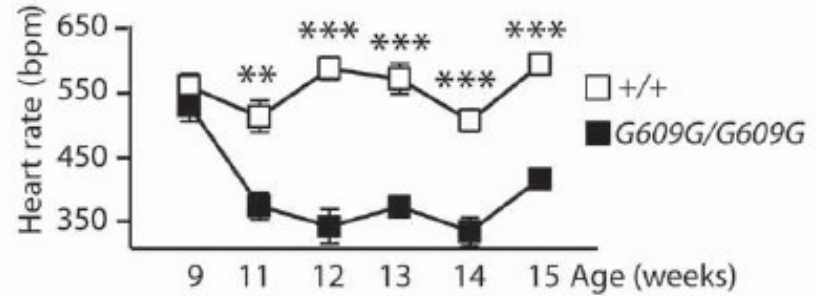
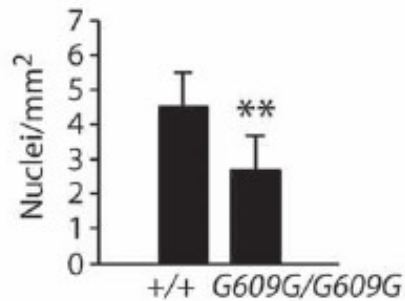
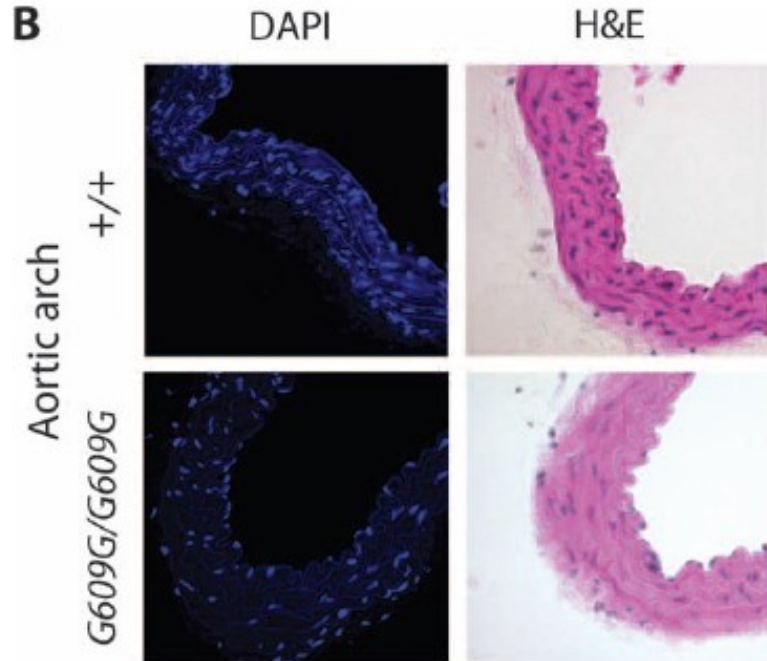
Progerin and laminin types are tested in several mice tissues



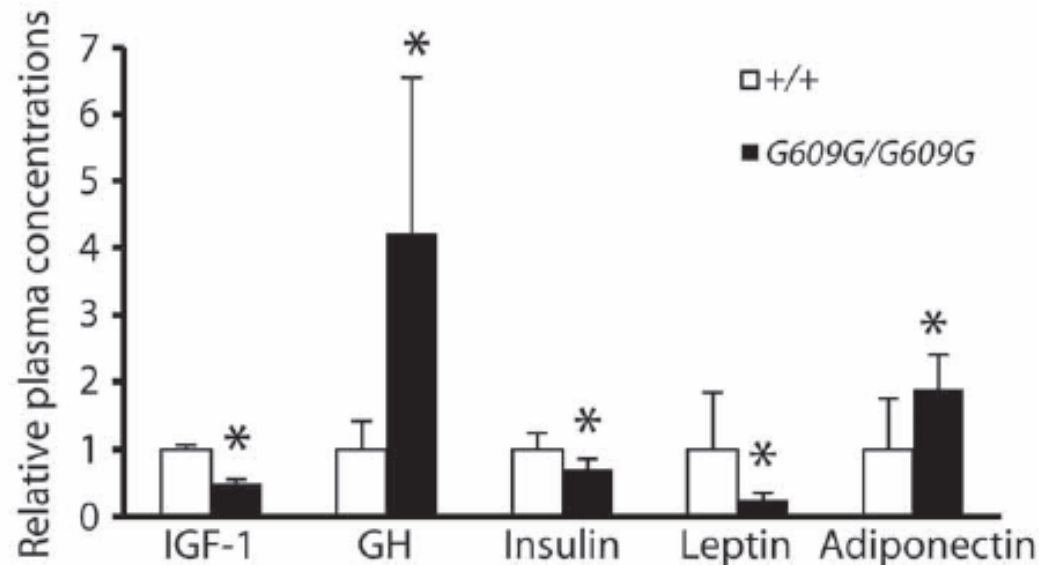
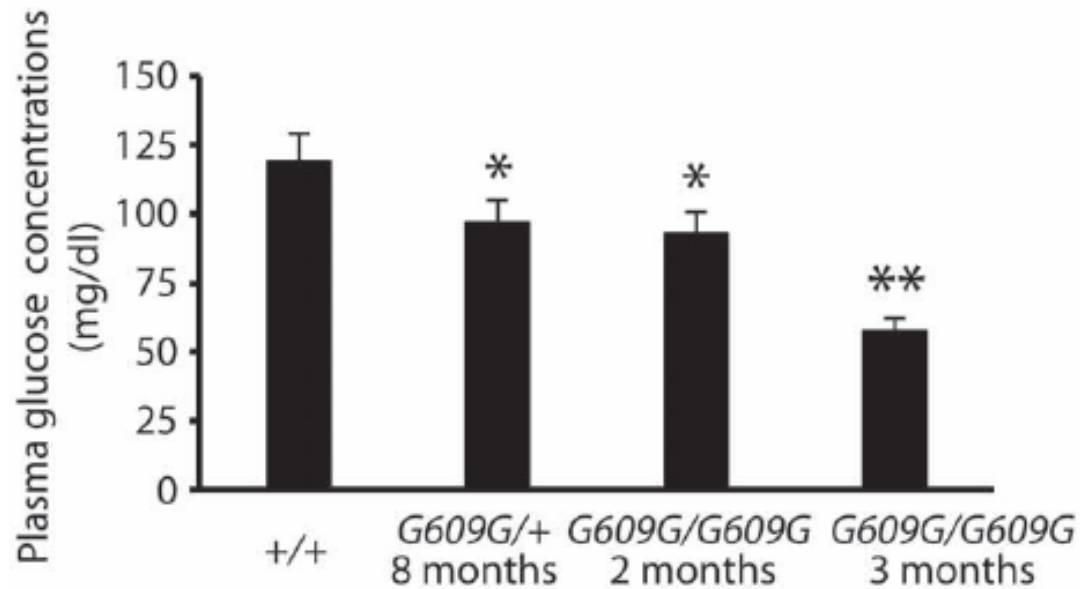
Tibias of mutant mice showed a reduction in bone density and cortical thickness as well as an increased porosity



Alteration of heart parameters functionality

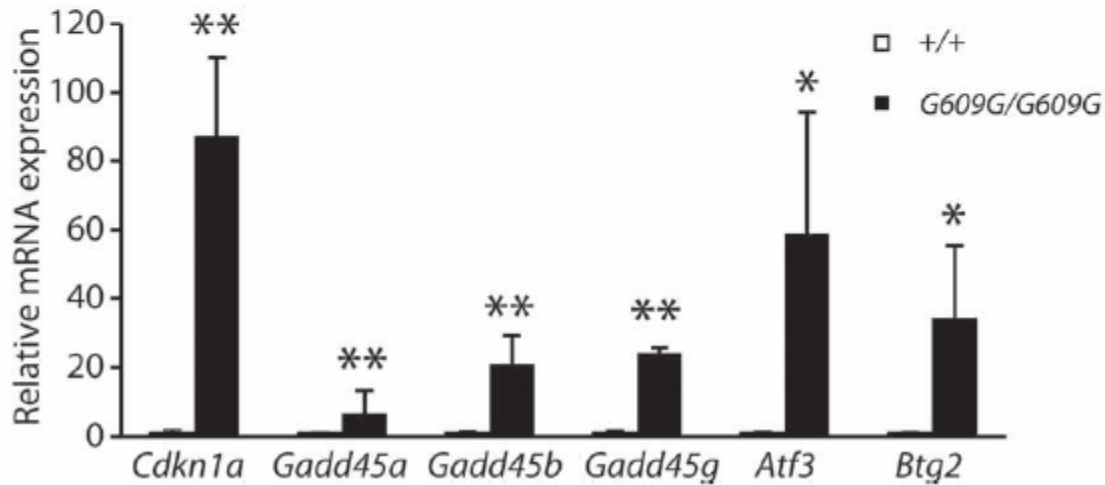
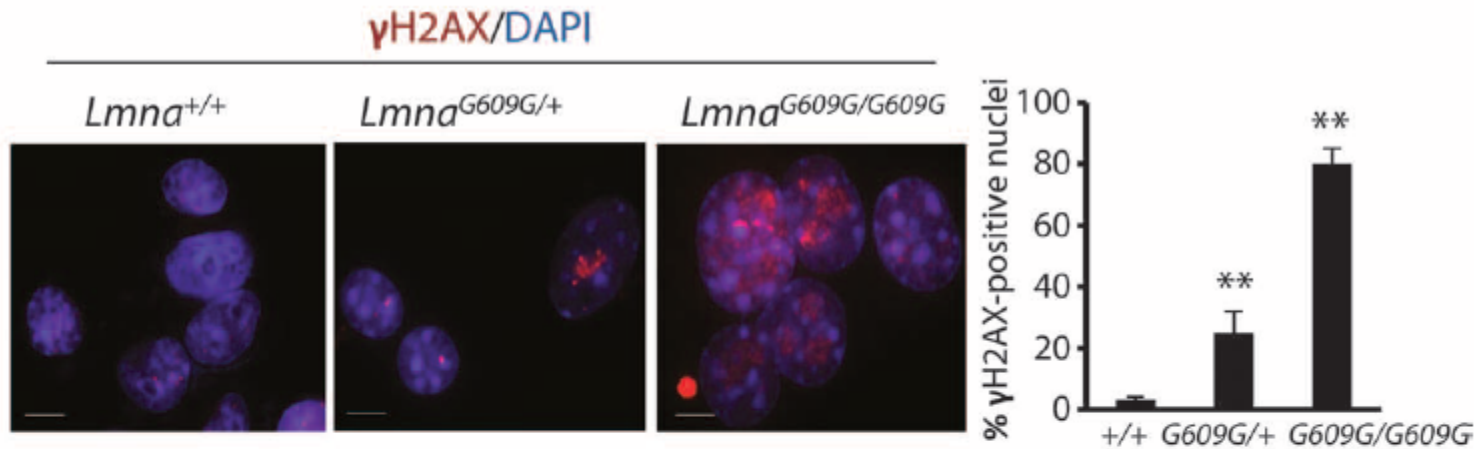


Alteration of glucose concentration and hormones associated with metabolism



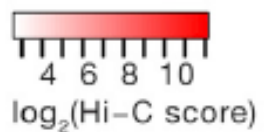
DNA damage and gene validation of microarray

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



IN SUMMARY:

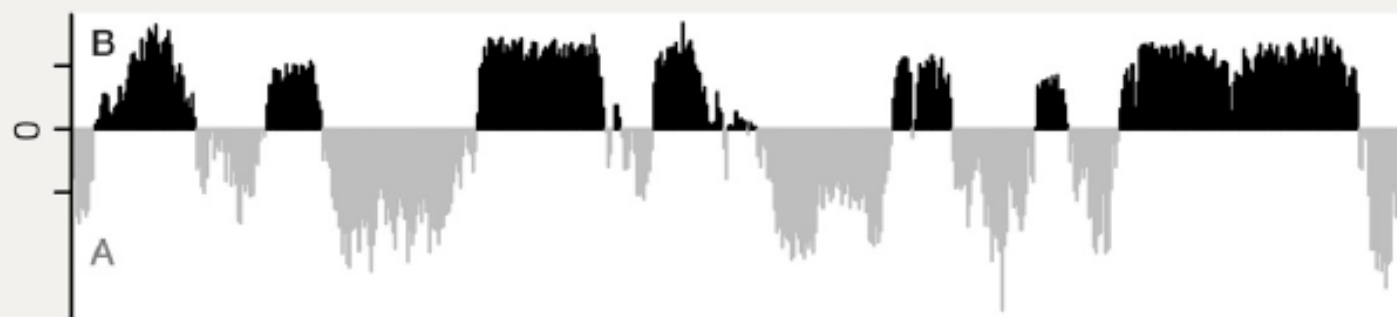
- **High order of chromatin structure is a component for TRANSCRIPTION REGULATION**
- **Alteration of LAMININ A, important in chromatin organization, induces PROGERIA**
- **MICE MODEL IS USED TO STUDY MOLECULAR MECHANISM IN DISEASE PROGRESSION**



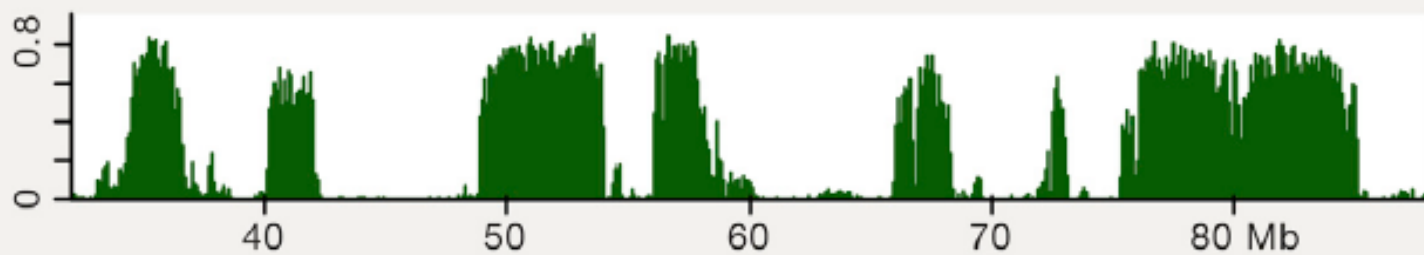
TADs



Compartment score

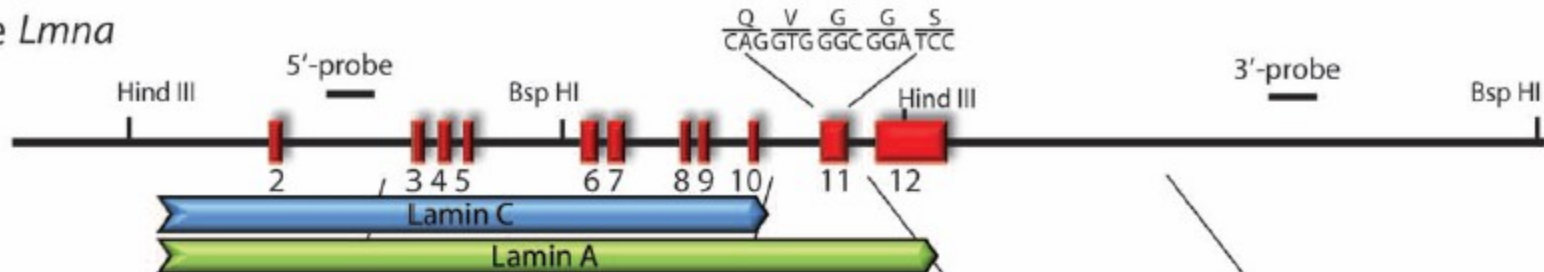


NL contact frequency

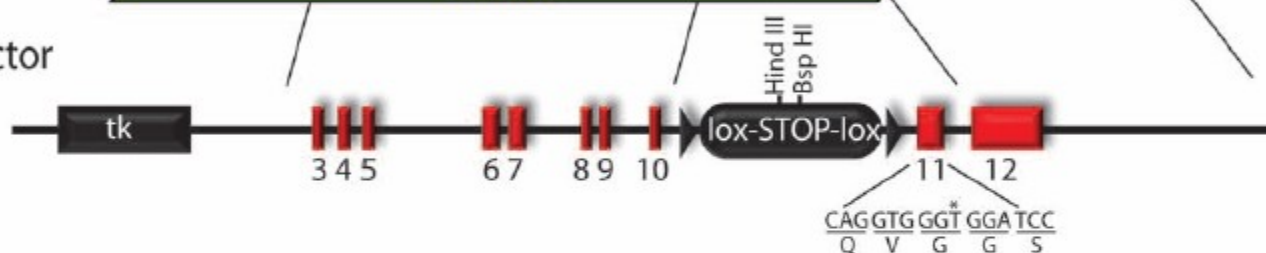


Position on chromosome 2

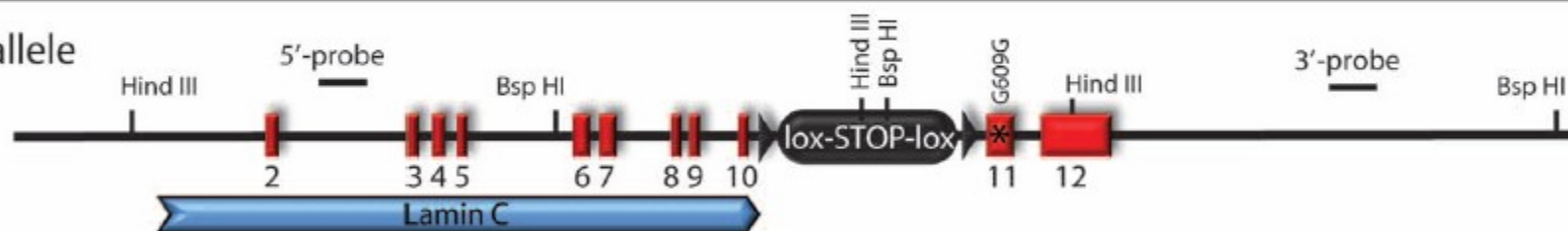
Wild-type *Lmna*



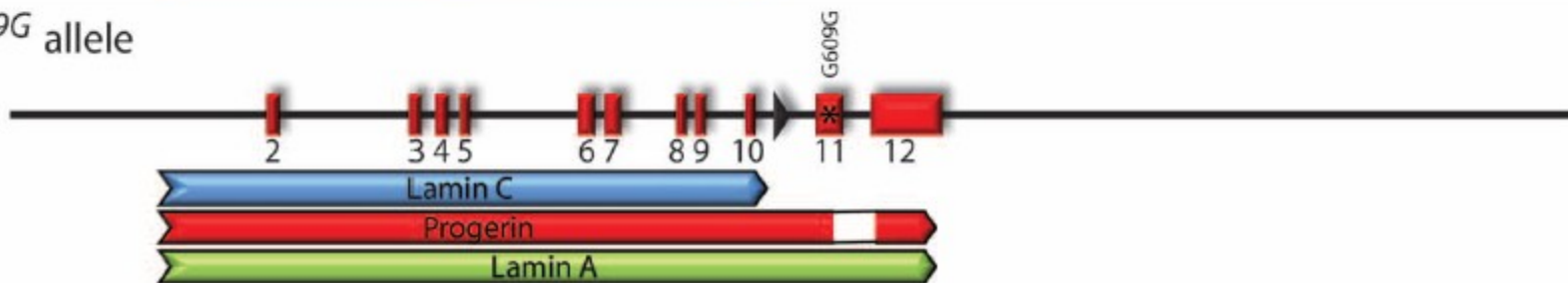
Targeting vector

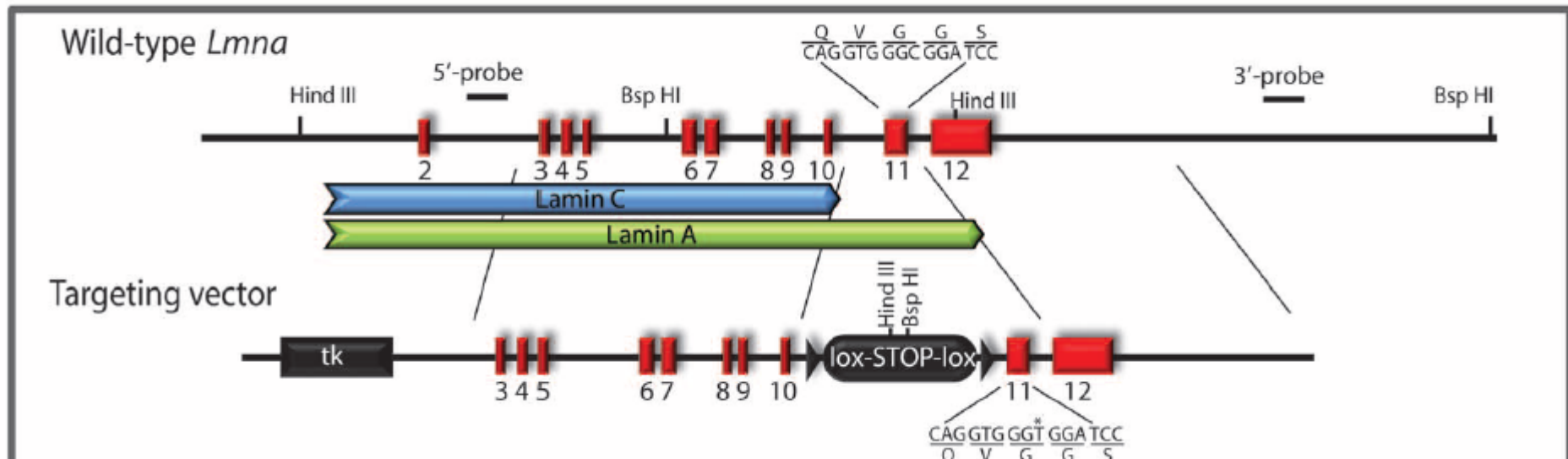


Lmna^{LCS} allele



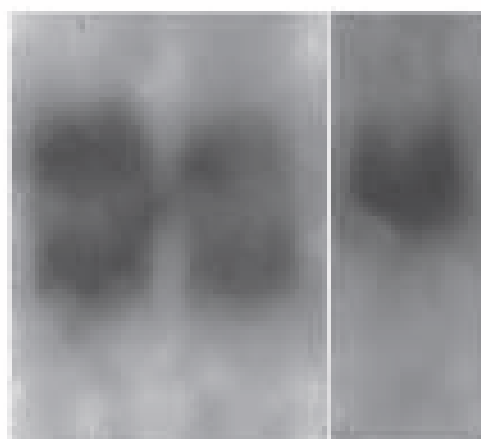
Lmna^{G609G} allele





3'-probe (Bsp HI)

1 2 wt



—12 kb

—10.5 kb