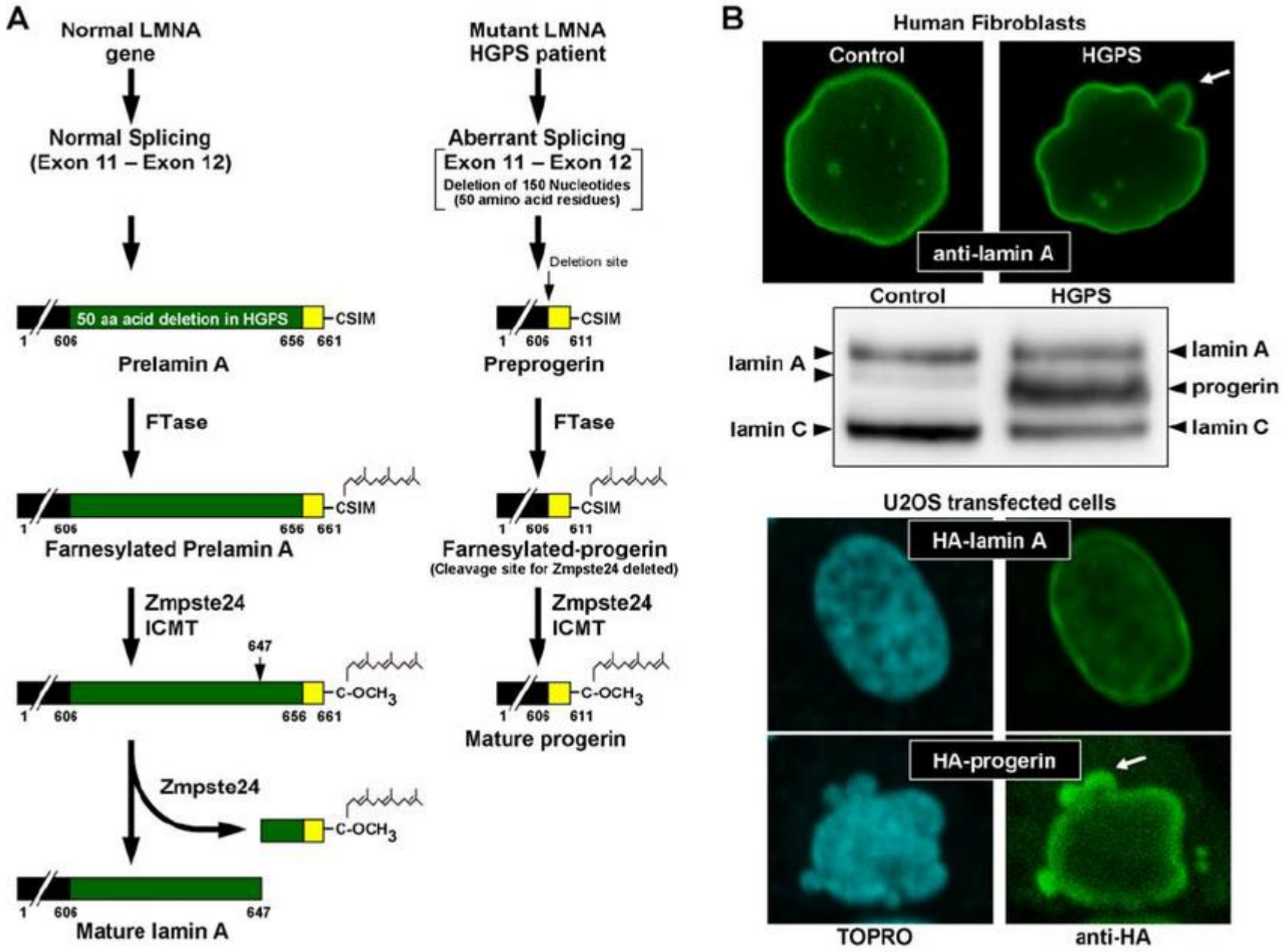
Hutchinson-Gilford progeria syndrome (HGPS)
PROGERIA

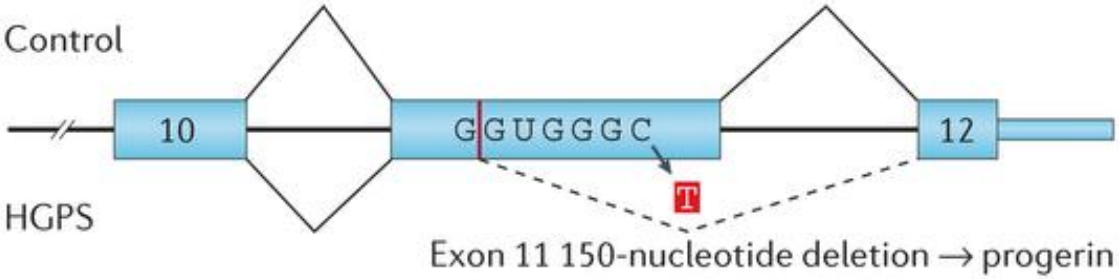
is caused by a **point mutation in the LMNA gene** that activates a cryptic donor splice site and yields a truncated form of prelamin A called progerin

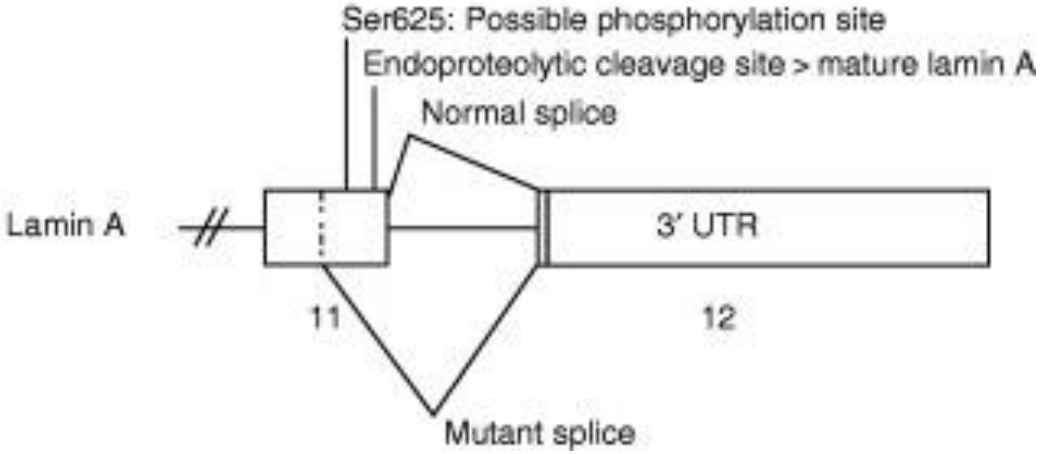

A
Normal LMNA gene
Normal Splicing (Exon 11 – Exon 12)
50 aa acid deletion in HGPS
CSIM
1 606 656 661
Prelamin A
FTase
CSIM
1 606 656 661
Farnesylated Prelamin A
Zmpste24 ICMT
647
C-OCH3
1 606 656 661
Zmpste24
C-OCH3
1 606 647
Mature lamin A
Mutant LMNA HGPS patient
Aberrant Splicing
Exon 11 – Exon 12
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)





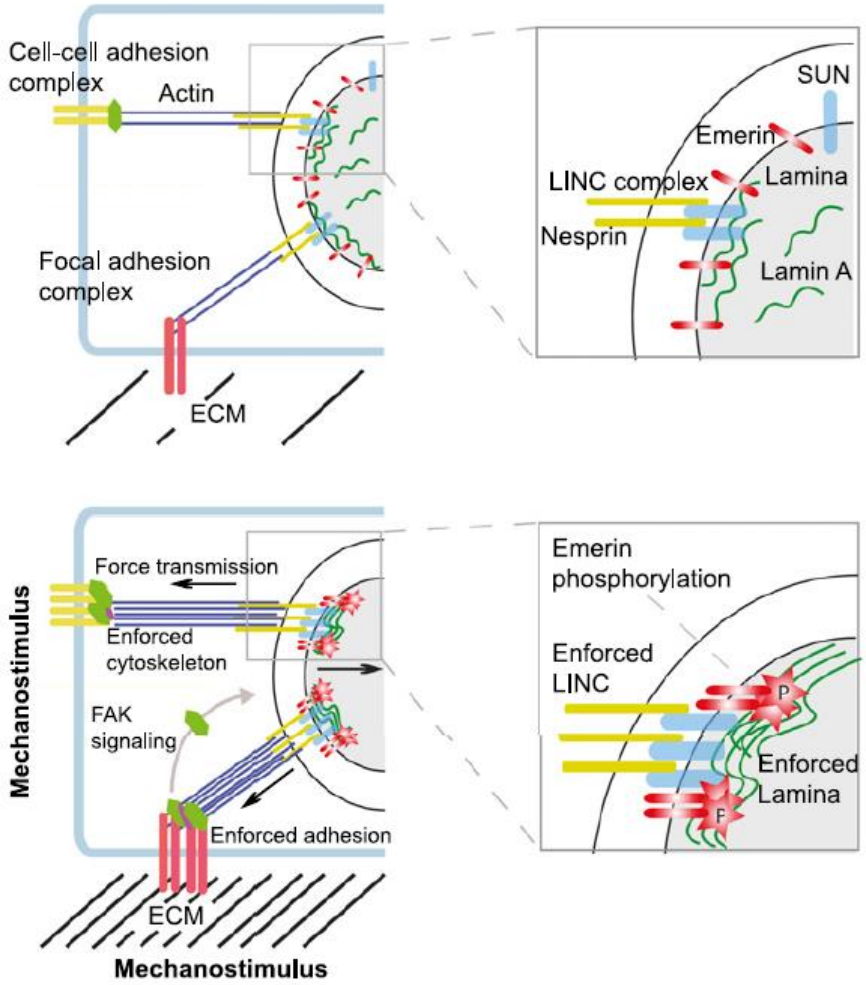
Loss of cleavage site for protease


Q
U
i
Z

Lamins at the crossroads of mechanosignaling

Selma Osmanagic-Myers, Thomas Dechat, and Roland Foisner

Max F. Perutz Laboratories, Department of Medical Biochemistry, Medical University Vienna, A-1030 Vienna, Austria



Lamins: Nuclear Intermediate Filament Proteins with Fundamental Functions in Nuclear Mechanics and Genome Regulation

Yosef Gruenbaum[1] and Roland Foisner[2]

Annu. Rev. Biochem. 2015. 84:131–64

- **Lamins** are intermediate filament proteins that **form a scaffold, termed nuclear lamina, at the nuclear periphery**. A small fraction of lamins also localize throughout the nucleoplasm.

- **Lamins bind to a growing number of nuclear protein complexes**

- **Lamins** are implicated in both **nuclear and cytoskeletal organization, mechanical stability, chromatin organization, gene regulation, genome stability, differentiation, and tissue-specific functions**.

- Mutation in lamins are involved in human **laminopathies**, ranging from muscular dystrophy to accelerated aging, as observed in Hutchinson–Gilford progeria and atypical Werner syndromes.

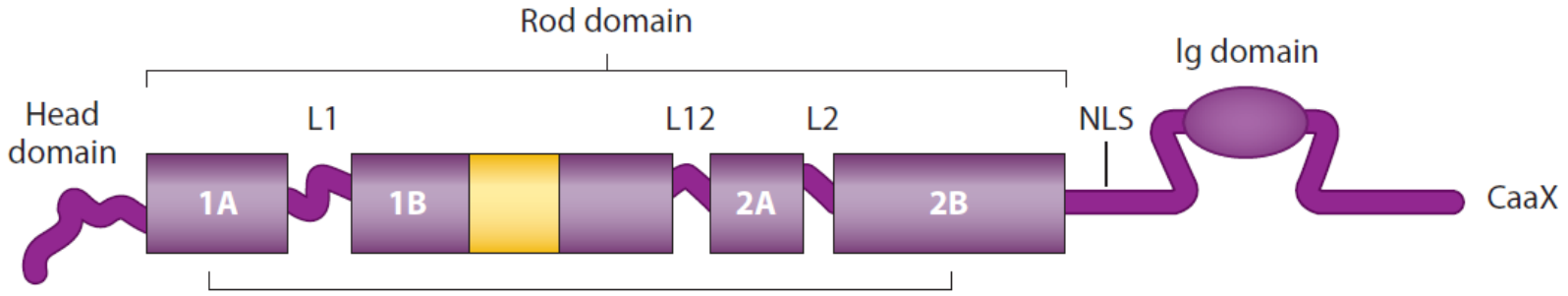
The domain organization of a lamin monomer

the **N-terminal (head)** domain;

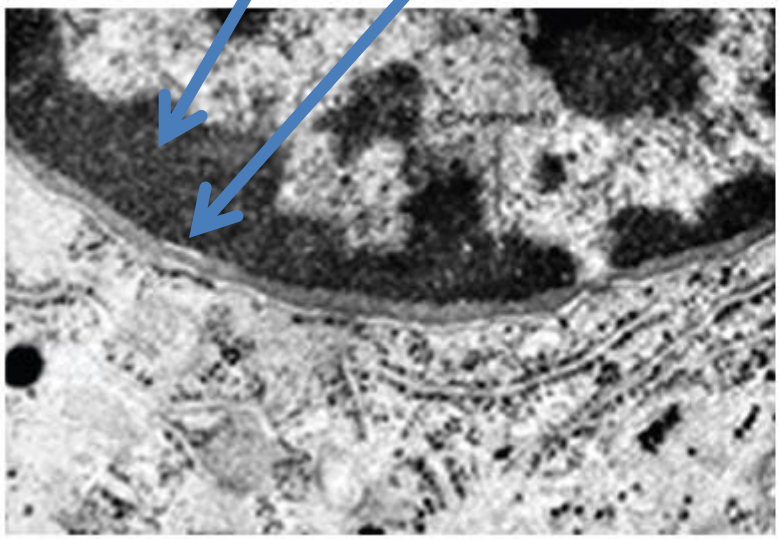
the **central rod domain**, which is composed of four α-helices (1A, 1B, 2A, 2B); three linker regions (L1, L12, L2);

C-terminal (tail) domain, which includes the nuclear localization signal (NLS), immunoglobulin (Ig) domain, and a CaaX motif (C, cysteine; a, aliphatic amino acid; X, any amino acid).

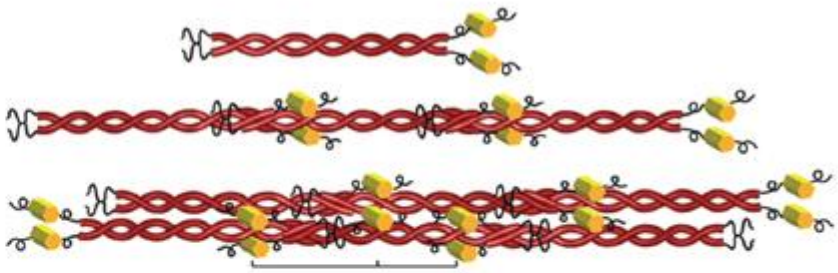
Six heptads present in lamins and absent in mammalian cytoplasmic intermediate filaments.



Peripheral heterochromatin (*black layer) that is underneath a thick nuclear lamina (gray layer).*

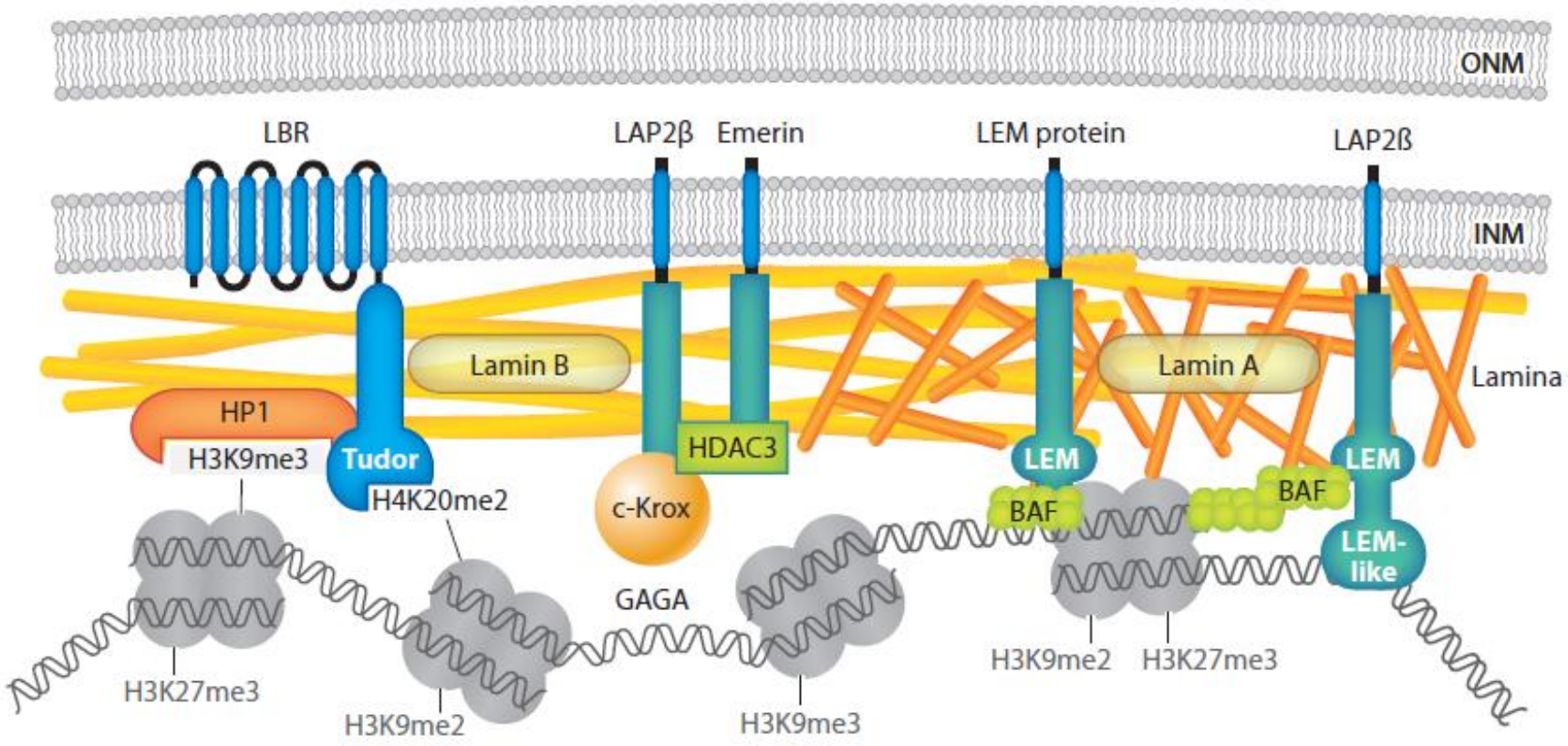


A transmission electron micrograph of nuclei from old rat liver



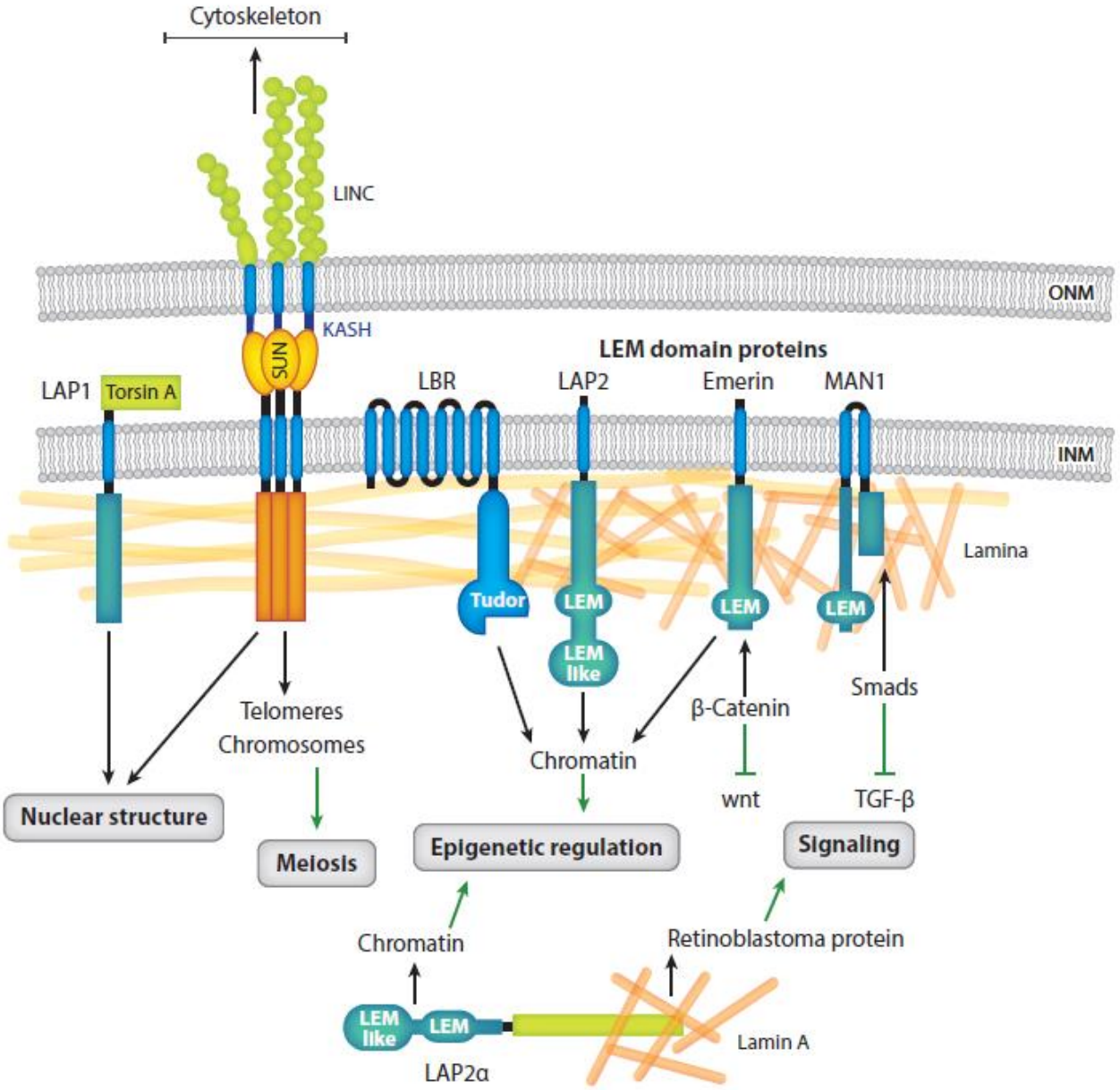
Lamin polypeptides assemble first into parallel dimers, and the dimers associate longitudinally to form polar head-to-tail polymer structures. Two head-to-tail polymers interact laterally in an antiparallel fashion to form protofilaments.

Lamina-associated domains (LADs) in the genome **are enriched in transcriptionally inactive genes and heterochromatic histone marks**

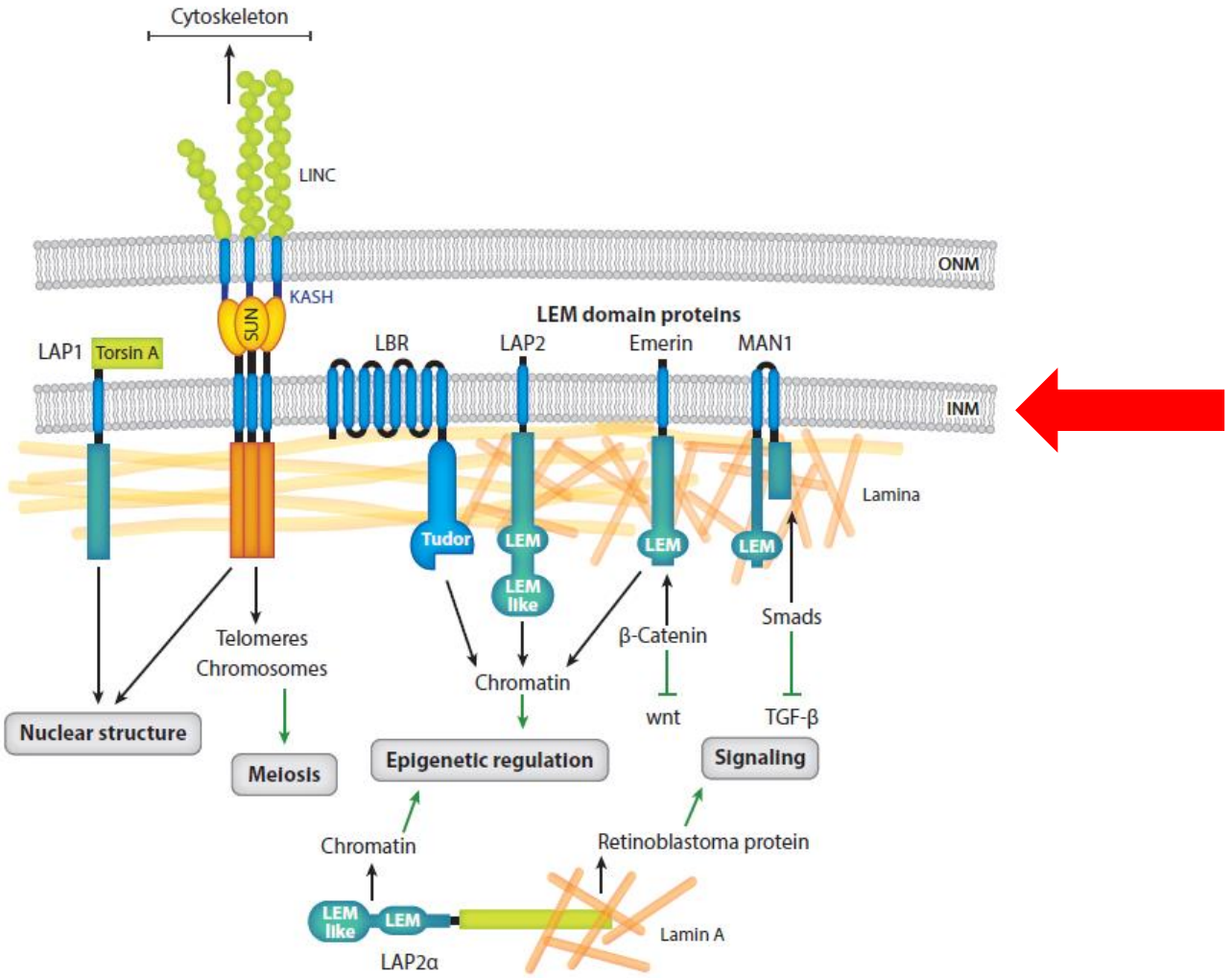


Silence epigenetic marks **Lamina-associated domain**

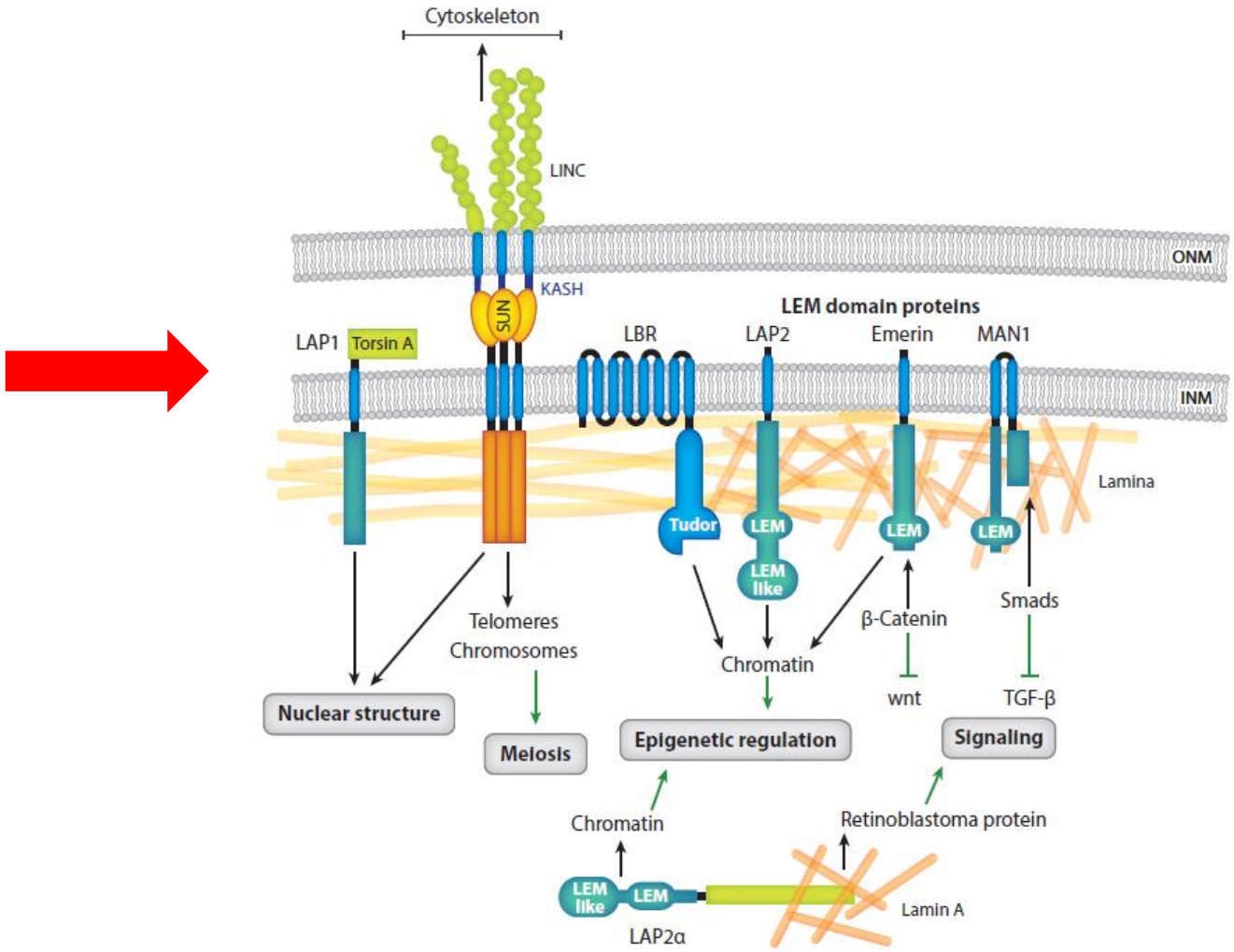
Characterized lamin-binding proteins and their functions.



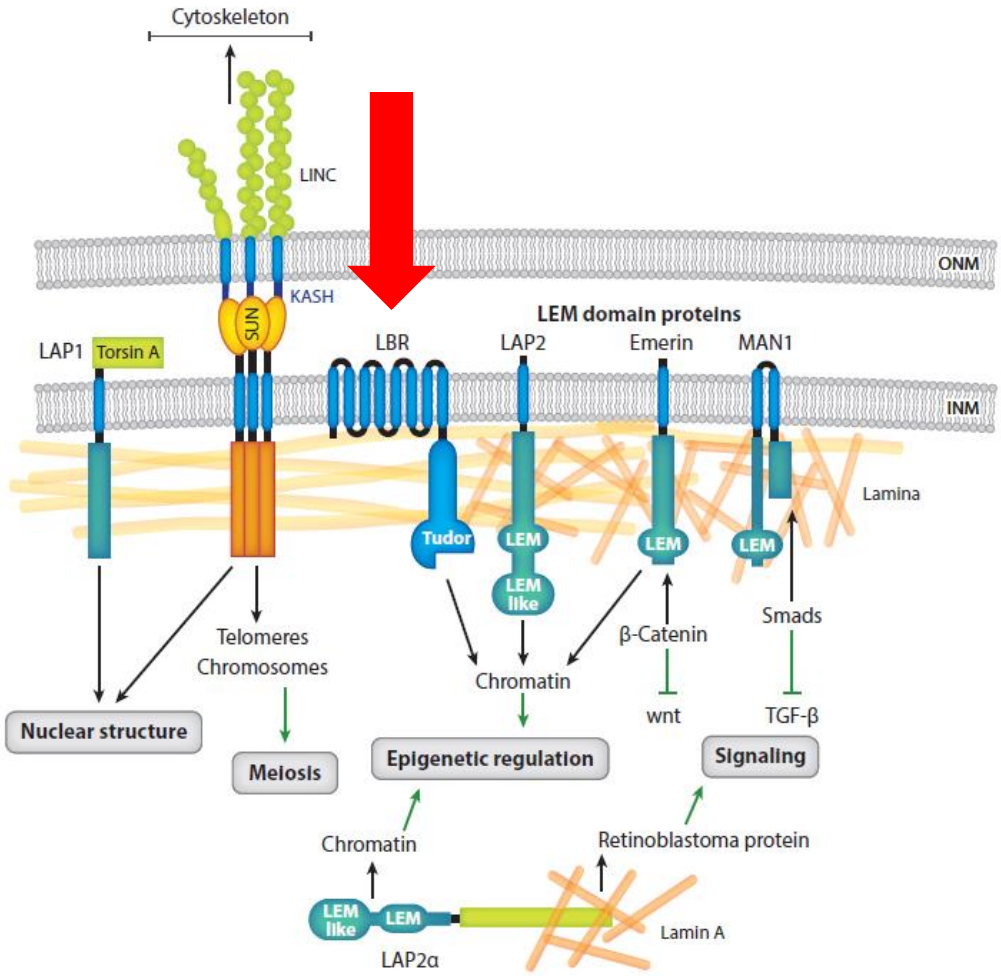
Complexes of tissue-specific nuclear envelope transmembrane proteins (NETs) with abundantly expressed lamins **may define the tissue-specific functions of lamin,** giving rise to tissue-specific phenotypes seen in lamin-linked diseases



Lamina-associated polypeptide 1 (LAP1), which also binds AAA+ ATPase torsin A in the perinuclear lumen and the LEM protein emerin in the INM. **LAP1 regulates torsin A ATPase activity** , and this interaction seems to be particularly important in neuronal cells because a torsin A mutant that exhibits stronger binding to LAP1 causes DYT1 dystonia, a disease of the central nervous system.



LBR is an INM protein with eight transmembrane domains and has sterol reductase activity. Mutations in this gene cause a Pelger-Huet anomaly, most likely linked to nuclear defects, and Greenberg skeletal dysplasia, linked to a deficiency in sterol reductase activity. Recent studies have revealed an essential **role of LBR in tethering chromatin to the lamina and in epigenetic gene silencing.**

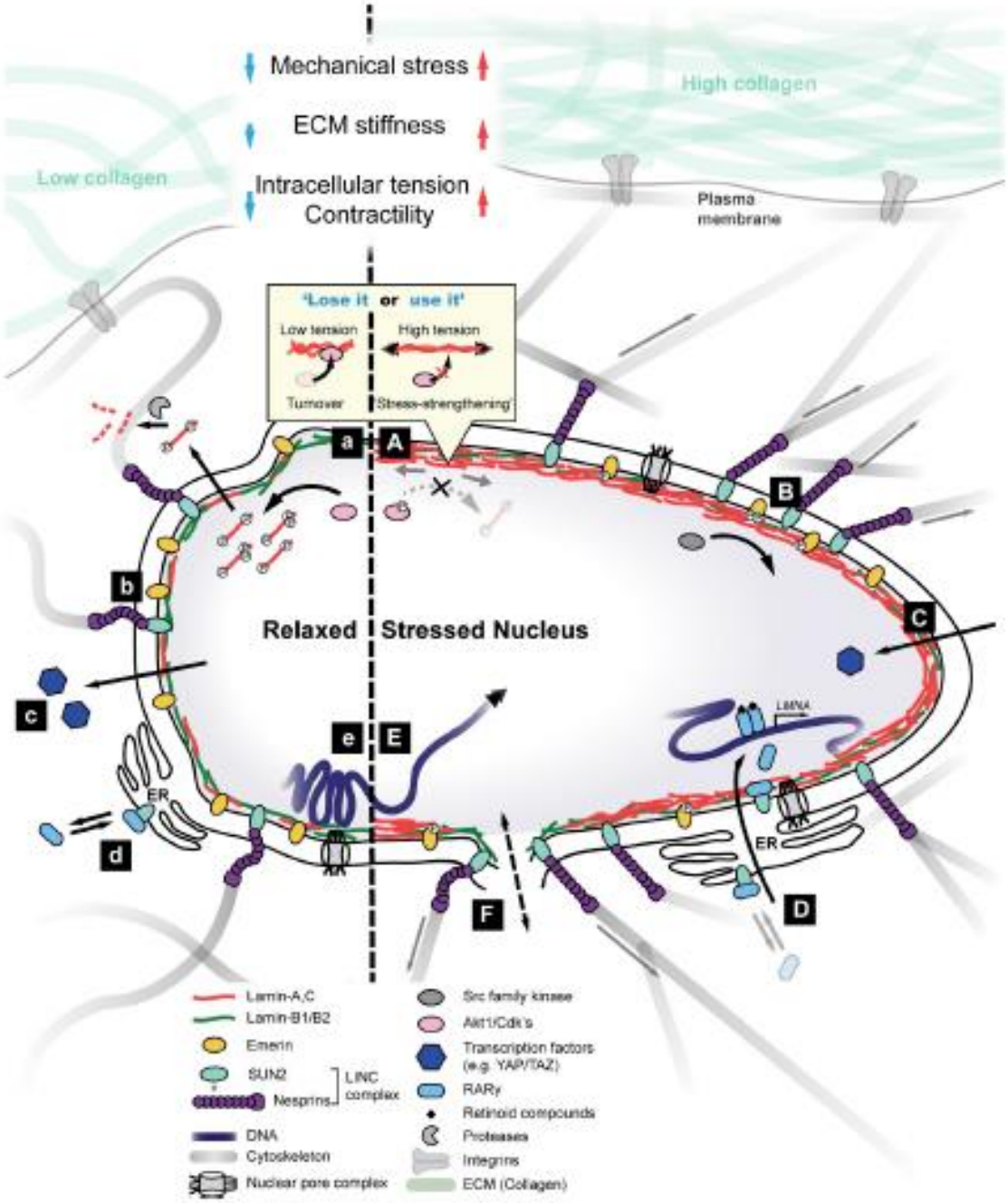


Mechanosensing by the nucleus: From pathways to scaling relationships

Sangkyun Cho, Jerome Irianto, and Dennis E. Discher

Nucleocytoskeletal coupling of the lamina with the cytoskeleton via proteins **allows force transmission** from the extracellular matrix (ECM) through cell adhesion complexes and the cytoskeleton into the nucleus and contributes to **mechanosignal transduction**

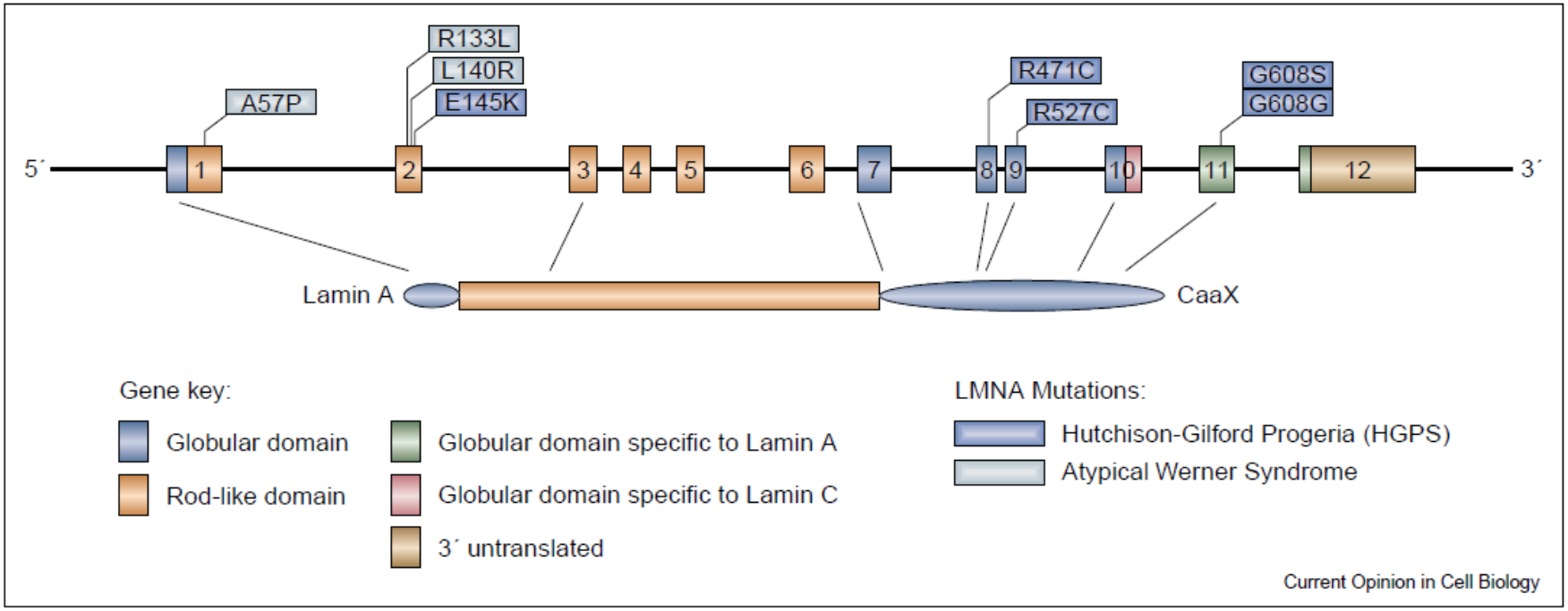
J. Cell Biol. Vol. 216 No. 2 305–315
https://doi.org/10.1083/jcb.201610042


Mechanical stress
ECM stiffness
Intracellular tension
Contractility
Low collagen
High collagen
Plasma membrane
'Lose it or use it'
Low tension
High tension
Turnover
Stress-strengthening
Relaxed
Stressed Nucleus
a
A
B
b
C
c
e
E
d
F
D
ER
LMNA
Lamin-A,C
Lamin-B1/B2
Emerin
SUN2
Nesprins
LINC complex
DNA
Cytoskeleton
Nuclear pore complex
Src family kinase
Akt1/Cdk's
Transcription factors (e.g. YAP/TAZ)
RARγ
Retinoid compounds
Proteases
Integrins
ECM (Collagen)

Aging and nuclear organization: lamins and progeria

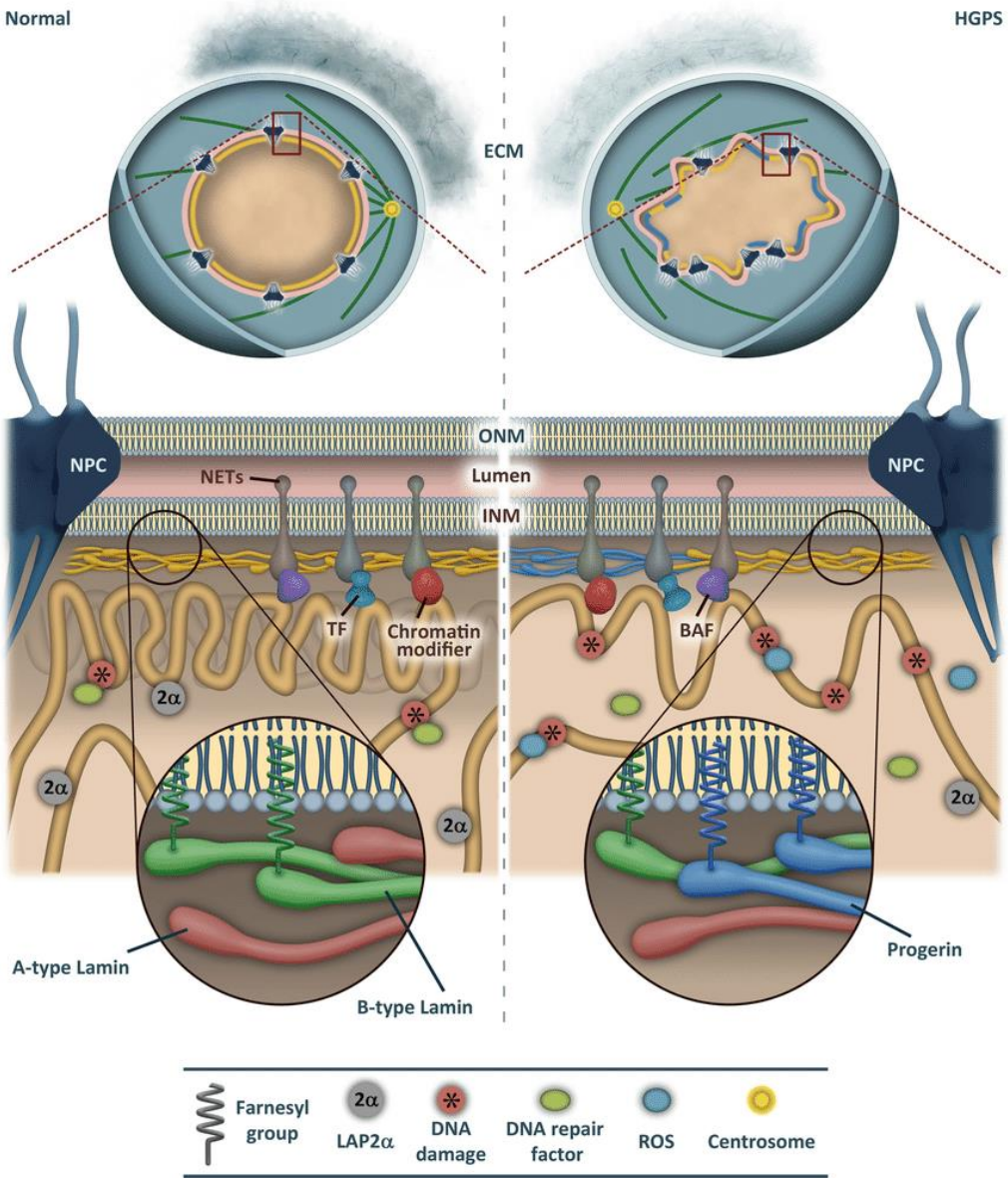
Leslie C Mounkes and Colin L Stewart[1]

Figure 1

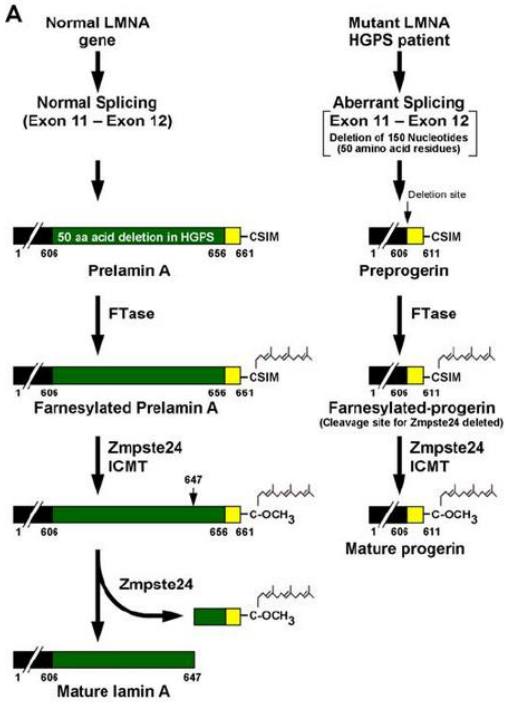


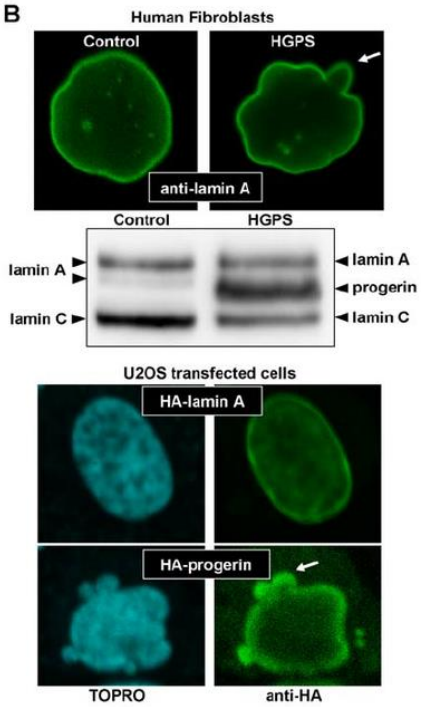
The distribution of mutations in the *LMNA* gene that result in HGPS and atypical Werner's syndrome are shown in relation to the gene and protein structure. The most common HGPS-causing mutation is the splicing mutation at G608 in exon 11.

Progerin creates a disorganization in the nucleus structure and changes in the protein association that control biological function



THERAPHY


A
Normal LMNA gene
Normal Splicing (Exon 11 – Exon 12)
50 aa acid deletion in HGPS
CSIM
Prelamin A
FTase
Farnesylated Prelamin A
Zmpste24
ICMT
Mature lamin A
Mutant LMNA HGPS patient
Aberrant Splicing Exon 11 – Exon 12
Deletion of 150 Nucleotides (50 amino acid residues)
Deletion site
Preprogerin
Farnesylated-progerin
(Cleavage site for Zmpste24 deleted)
Mature progerin


B
Human Fibroblasts
Control
HGPS
anti-lamin A
lamin A
lamin C
progerin
U2OS transfected cells
HA-lamin A
HA-progerin
TOPRO
anti-HA


Q
U
i
Z

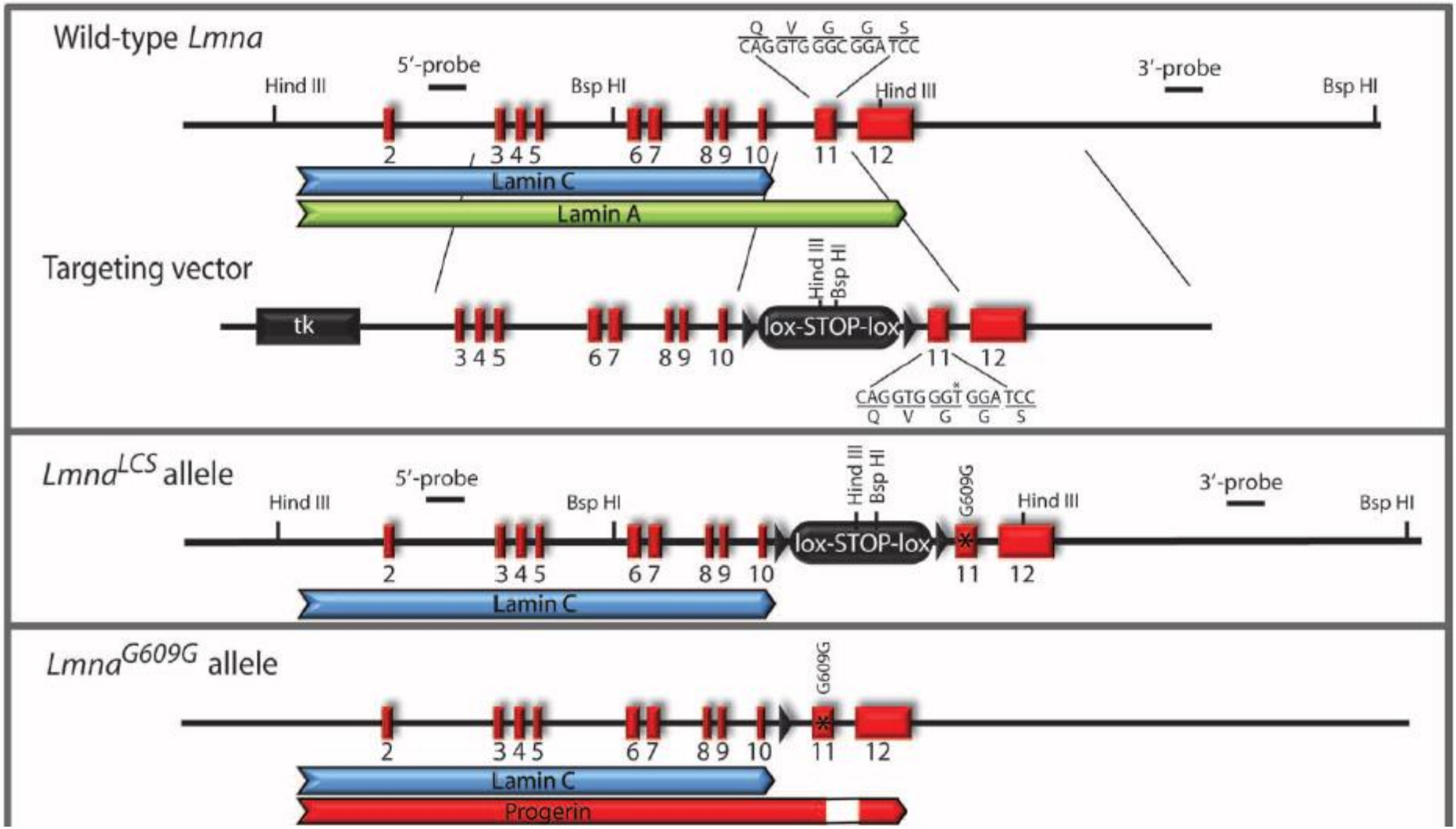
Splicing-Directed Therapy in a New Mouse Model of Human Accelerated Aging

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.

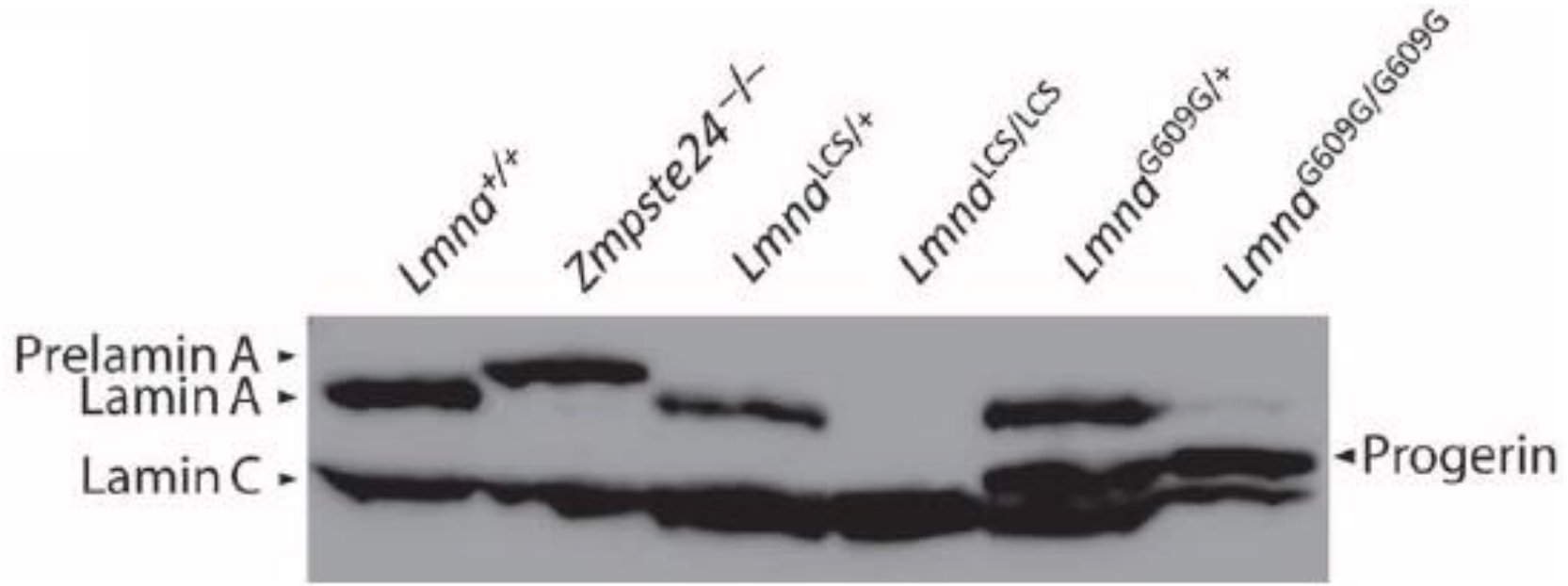
CONDITIONAL TRANSGENIC MICE

PROGERIN MODEL



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.

PROGERIN AND LAMININ TYPES PROTEIN EXPRESSION

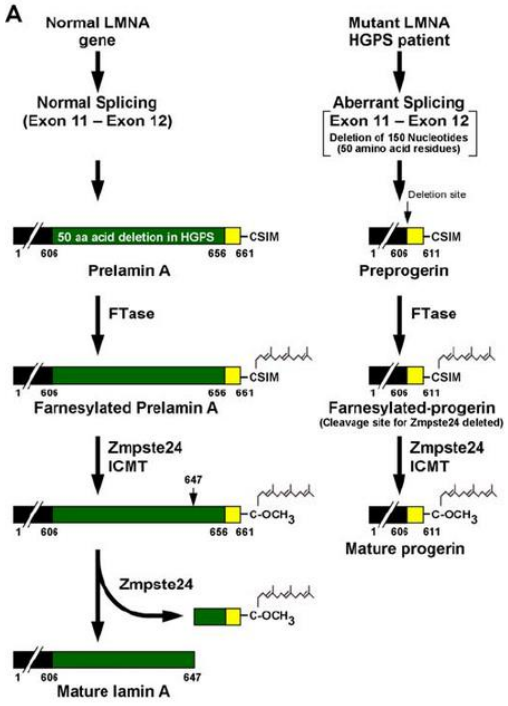


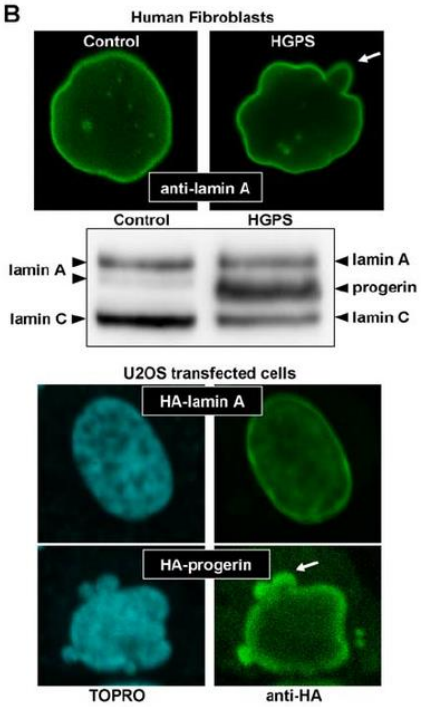
Western (immuno) blot analysis of mouse adult fibroblasts obtained from the mice with the various genotypes used in the study. Lamin A, lamin C, prelamin A, and progerin were detected with a monoclonal antibody against lamin A/C (Manlac-1).

PROGERIA MODEL
CONDITIONAL TRANSGENIC MICE

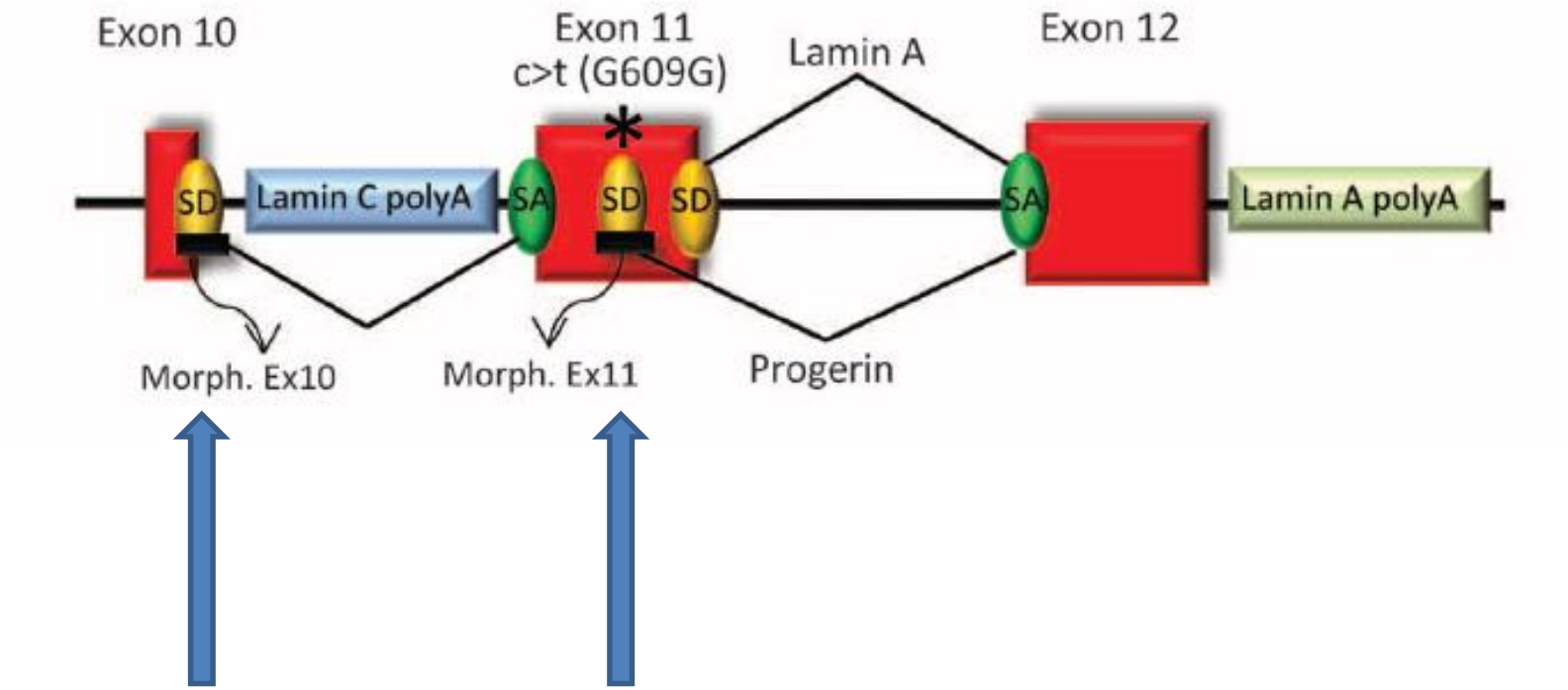
- Lmna G609G has reduction in growth rates and body weight
- Lmna G609G changes in cytoskeleton structure
- Lmna G609G is associated with blebbing membrane (bleb is a protrusion of cell membrane) and DNA damage
- Lmna G609G has tissues senescence and changes in organs size

THERAPHY


A
Normal LMNA gene
Normal Splicing (Exon 11 – Exon 12)
50 aa acid deletion in HGPS
CSIM
Prelamin A
FTase
Farnesylated Prelamin A
Zmpste24
ICMT
Mature lamin A
Mutant LMNA HGPS patient
Aberrant Splicing Exon 11 – Exon 12
Deletion of 150 Nucleotides (50 amino acid residues)
Deletion site
Preprogerin
Farnesylated-progerin
(Cleavage site for Zmpste24 deleted)
Mature progerin


B
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

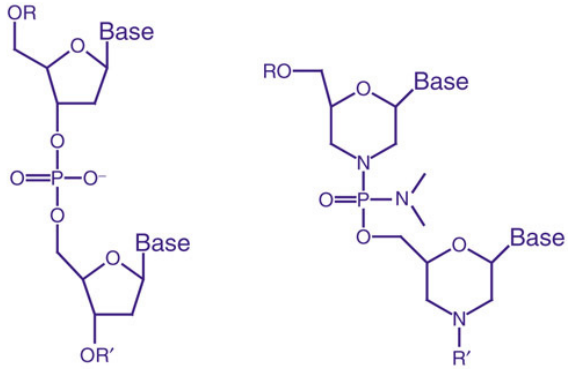
MORFOLINO

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**,

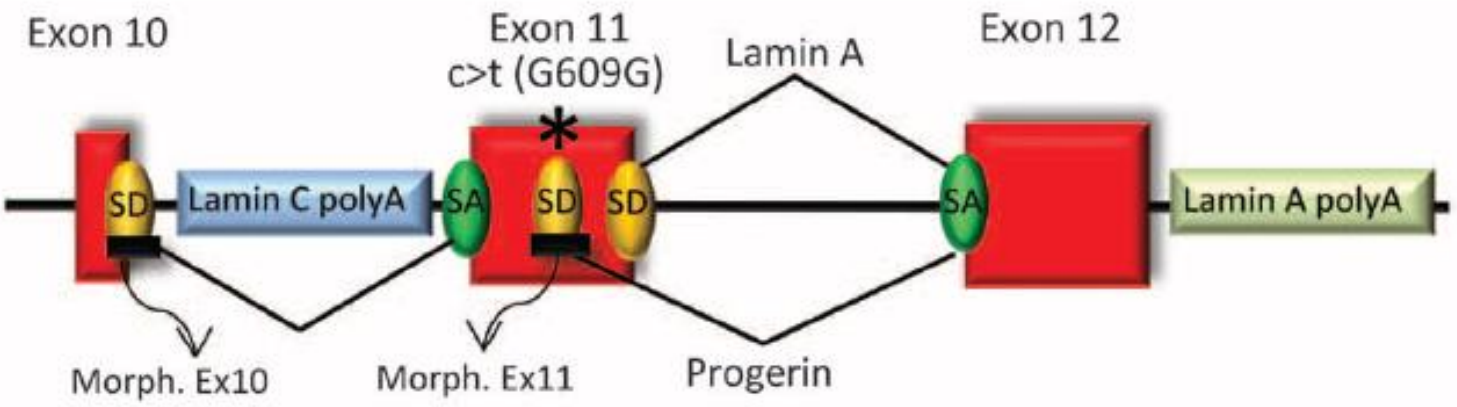
phosphorodiamidate morpholino oligomers (PMOs or Morpholinos) do not result in degradation of their target RNA: not recognized by cellular nucleases.



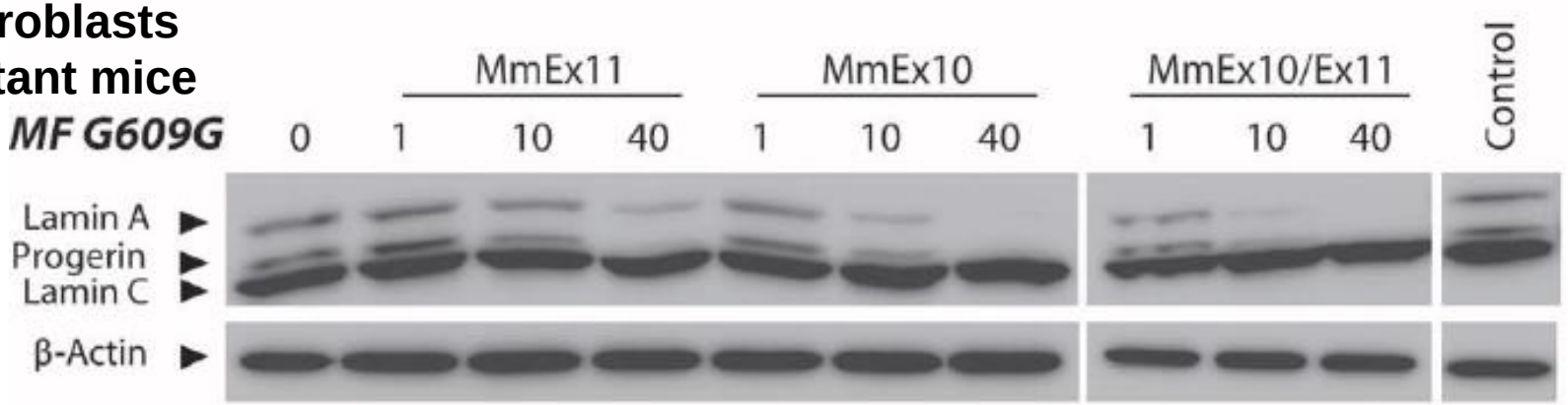
Phosphodiester DNA

Morpholino

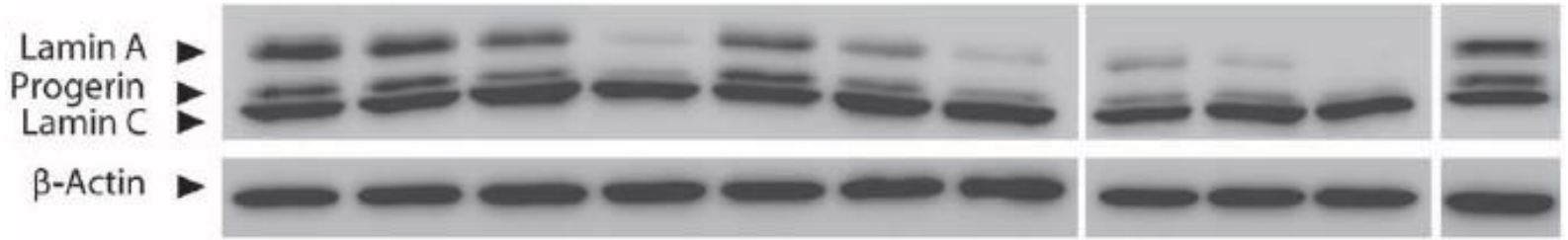
Both MmEx10 and MmEx11 morpholinos each reduced progerin amount in a dose-dependent manner



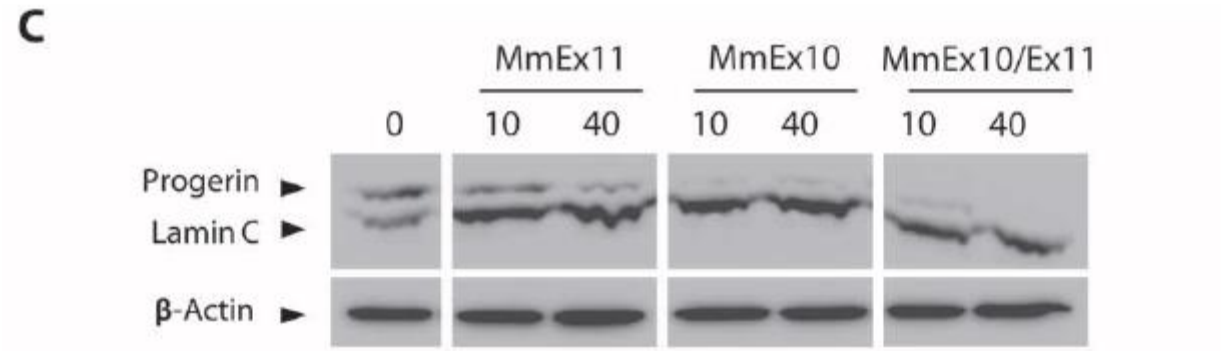
Fibroblasts mutant mice

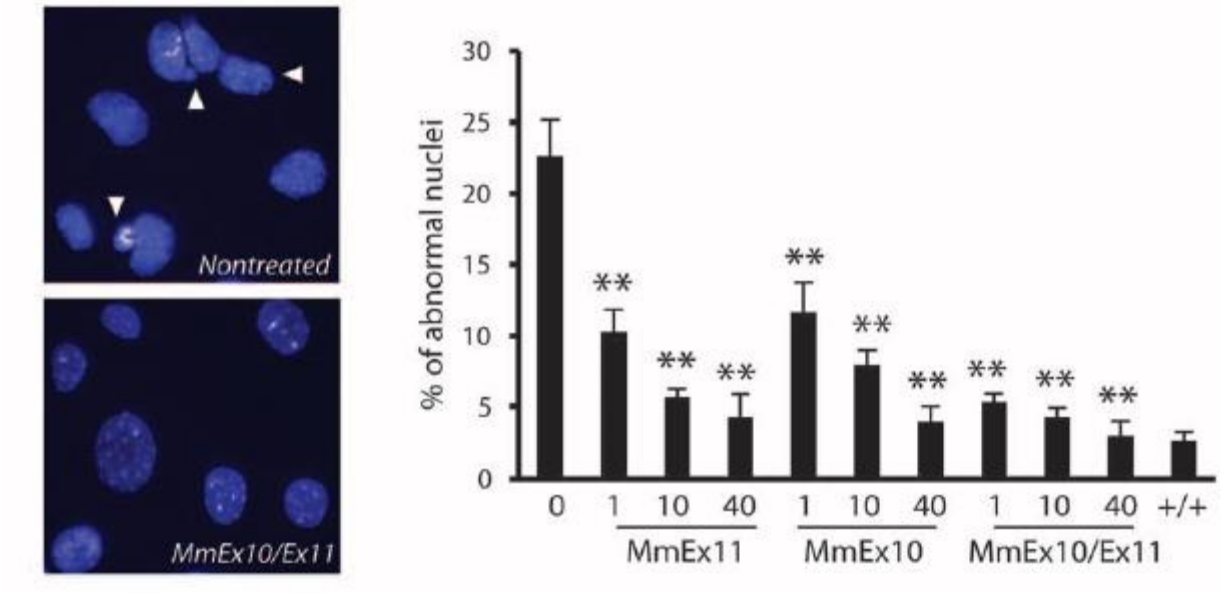


HF G608G **Fibroblasts from patients**



MmEx10 and MmEx11 inhibited progerin production





MmEx10 and MmEx11 induced reduction of nucleus abnormalities

nature medicine

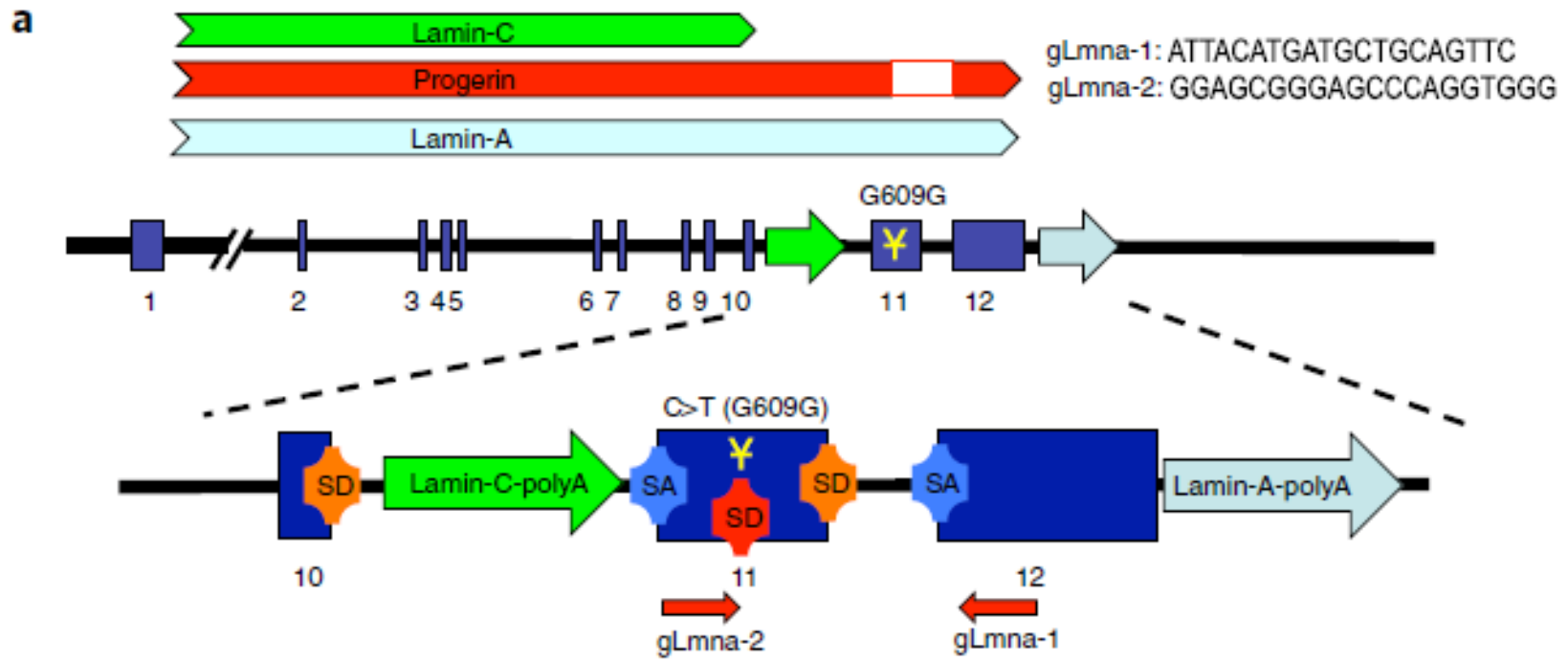
BRIEF COMMUNICATION

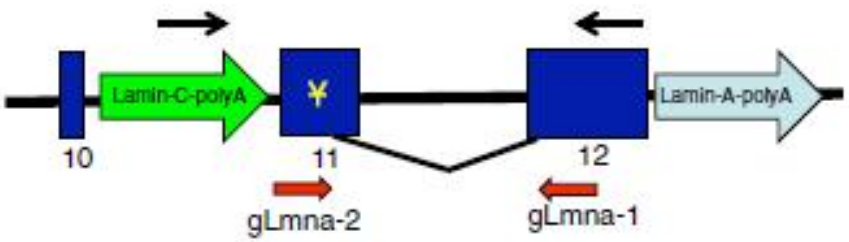
https://doi.org/10.1038/s41591-019-0343-4

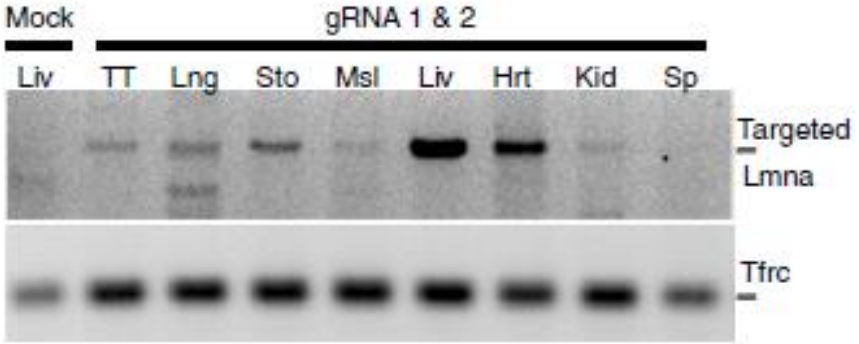
Single-dose CRISPR–Cas9 therapy extends lifespan of mice with Hutchinson–Gilford progeria syndrome

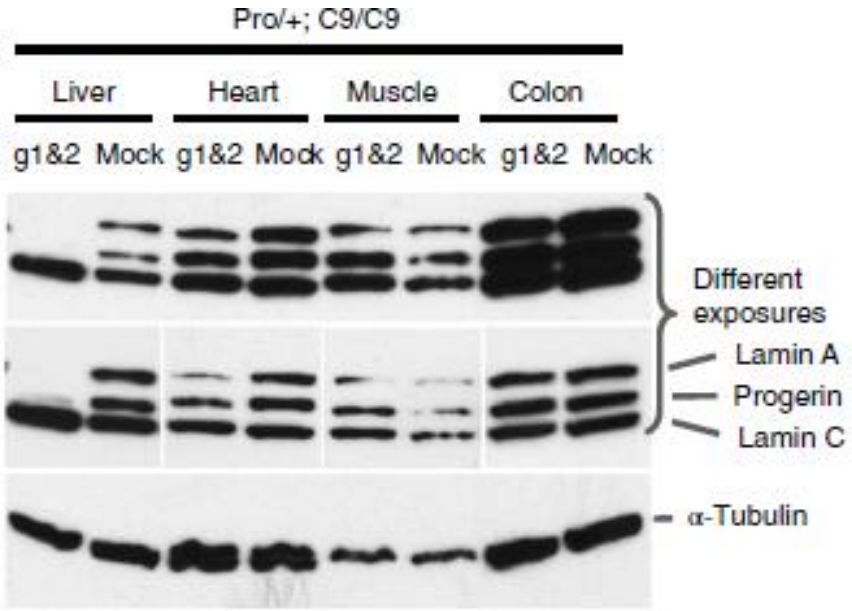
Ergin Beyret[1,3], Hsin-Kai Liao[1,3], Mako Yamamoto[1,2], Reyna Hernandez-Benitez[1], Yunpeng Fu[1], Galina Erikson[1], Pradeep Reddy[1] and Juan Carlos Izpisua Belmonte[1*]

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.**

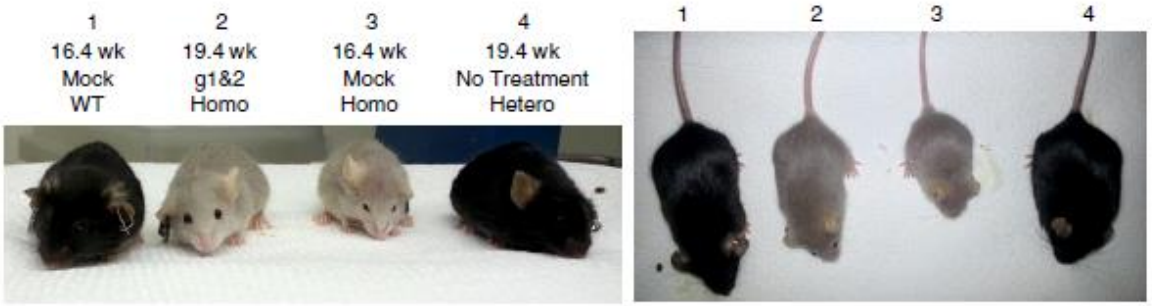



Lamin-C-polyA
Lamin-A-polyA
10
11
12
gLmna-2
gLmna-1

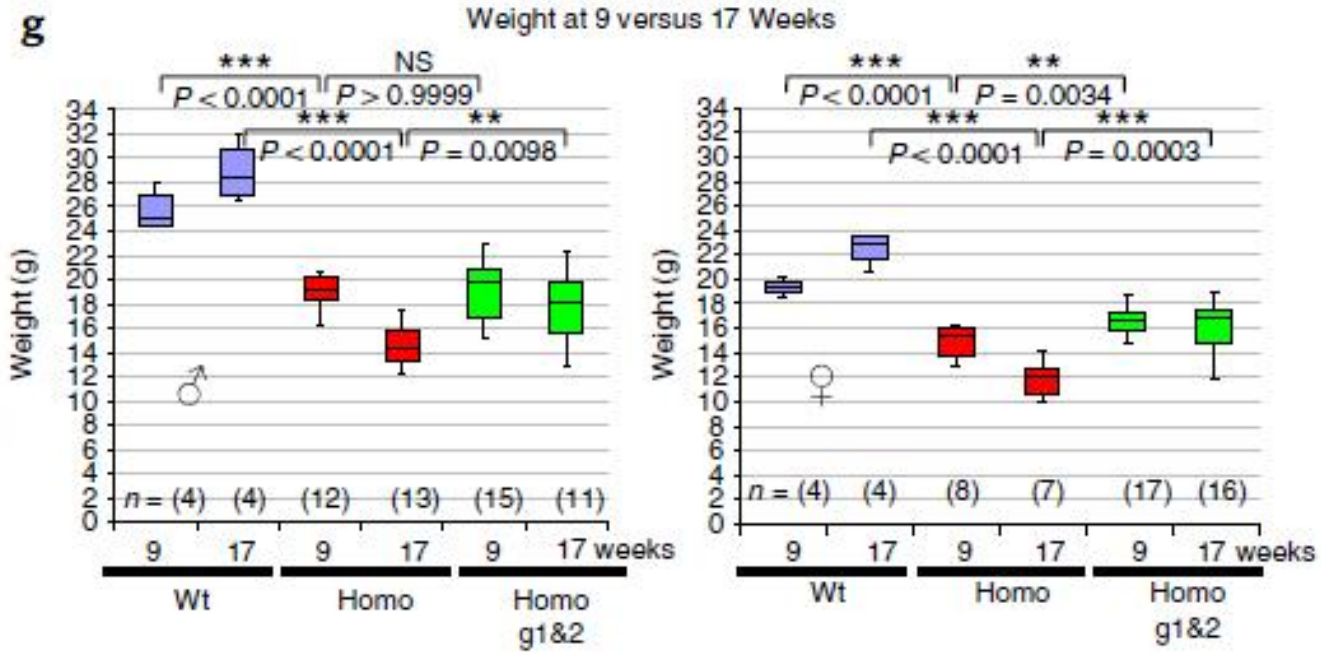

Mock
gRNA 1 & 2
Liv
TT
Lng
Sto
Msl
Liv
Hrt
Kid
Sp
Targeted
Lmna
Tfrc


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

nature medicine

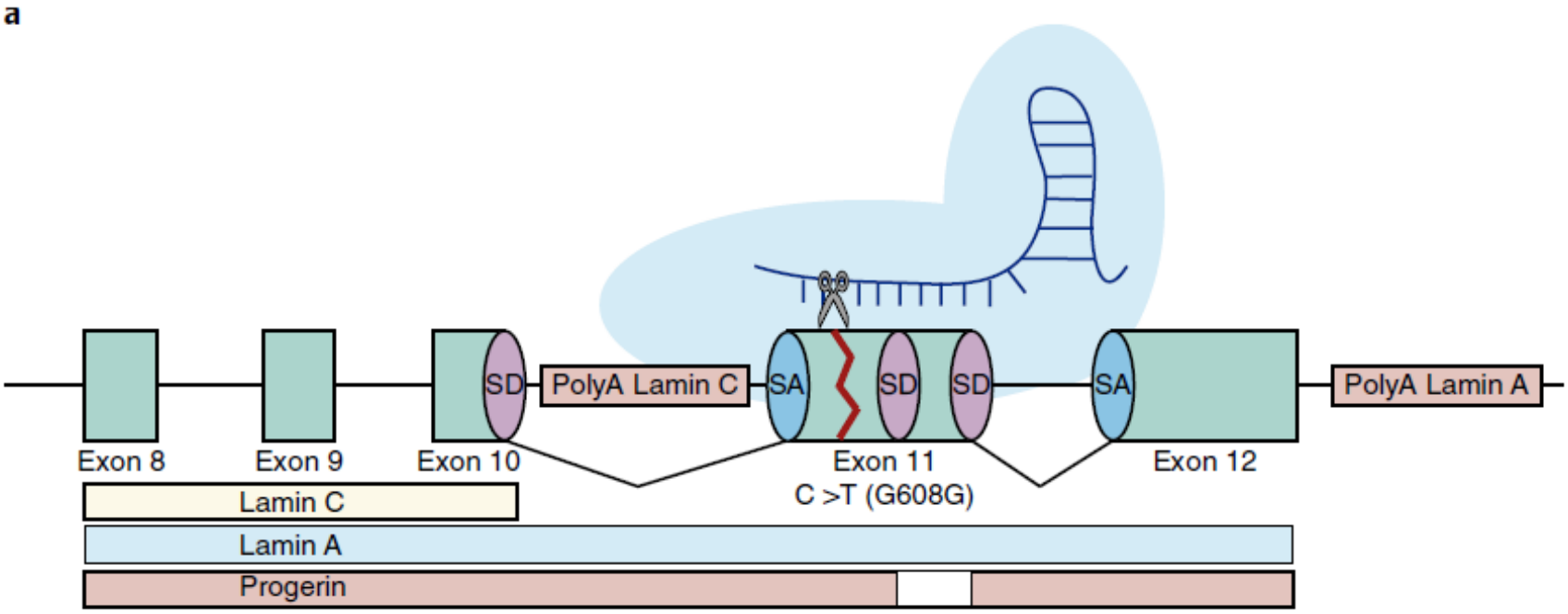
BRIEF COMMUNICATION

https://doi.org/10.1038/s41591-018-0338-6

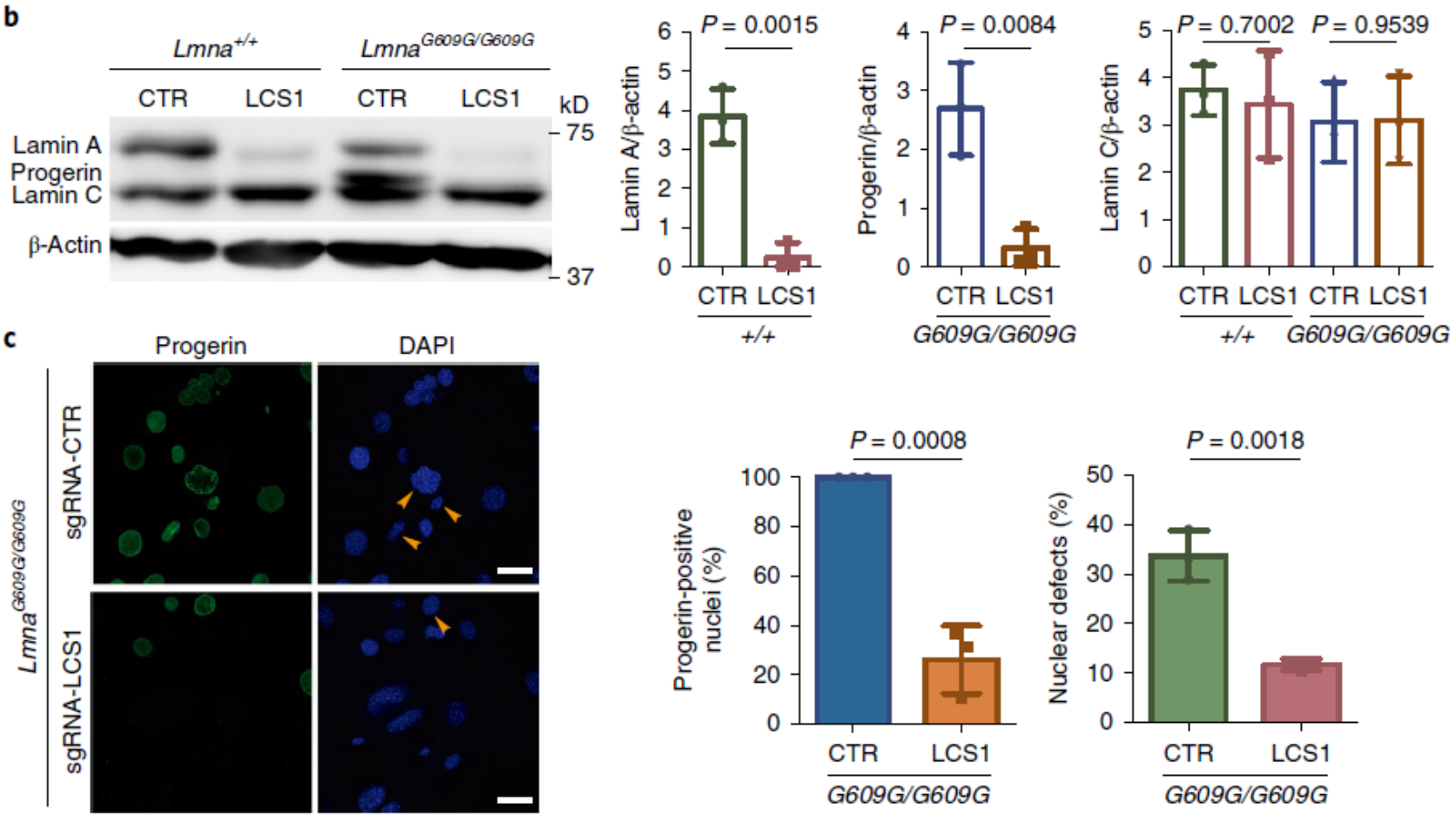
Development of a CRISPR/Cas9-based therapy for Hutchinson–Gilford progeria syndrome

Olaya Santiago-Fernández[1], Fernando G. Osorio[1], Víctor Quesada[1,2], Francisco Rodríguez[1], Sammy Basso[1], Daniel Maeso[1], Loïc Rolas[3], Anna Barkaway[3], Sussan Nourshargh[3], Alicia R. Folgueras[1], José M. P. Freije[1,2*] and Carlos López-Otín[1,2*]

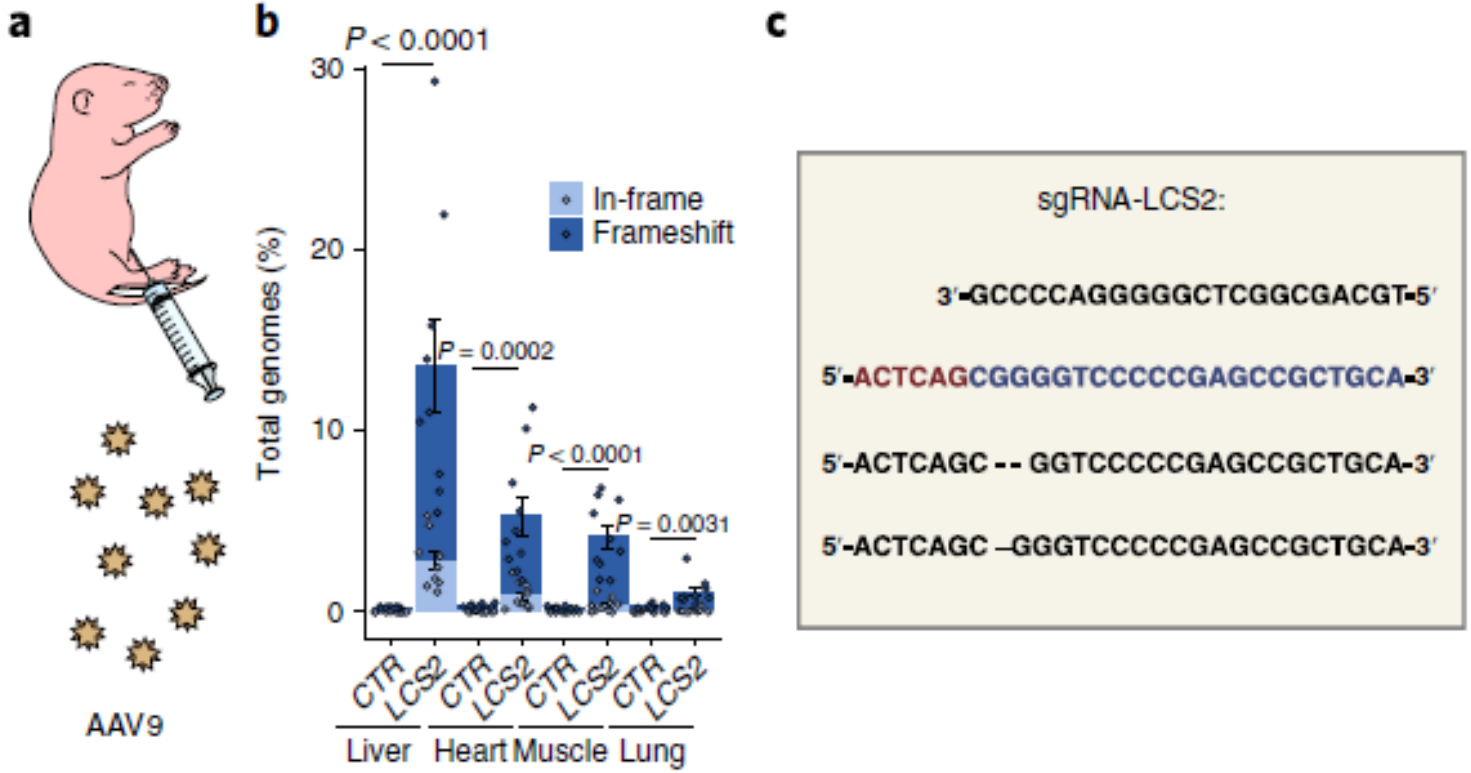
CRISPR/Cas9-based therapies hold considerable promise for the treatment of genetic diseases. Among these, Hutchinson-Gilford progeria syndrome, caused by a point mutation in the *LMNA* gene, stands out as a potential candidate. Here, we explore the efficacy of a CRISPR/Cas9-based approach that reverts several alterations in Hutchinson-Gilford progeria syndrome cells and mice by introducing frameshift mutations in the *LMNA* gene.


a
SD
PolyA Lamin C
SA
SD
SD
SA
PolyA Lamin A
Exon 8
Exon 9
Exon 10
Exon 11
C >T (G608G)
Exon 12
Lamin C
Lamin A
Progerin

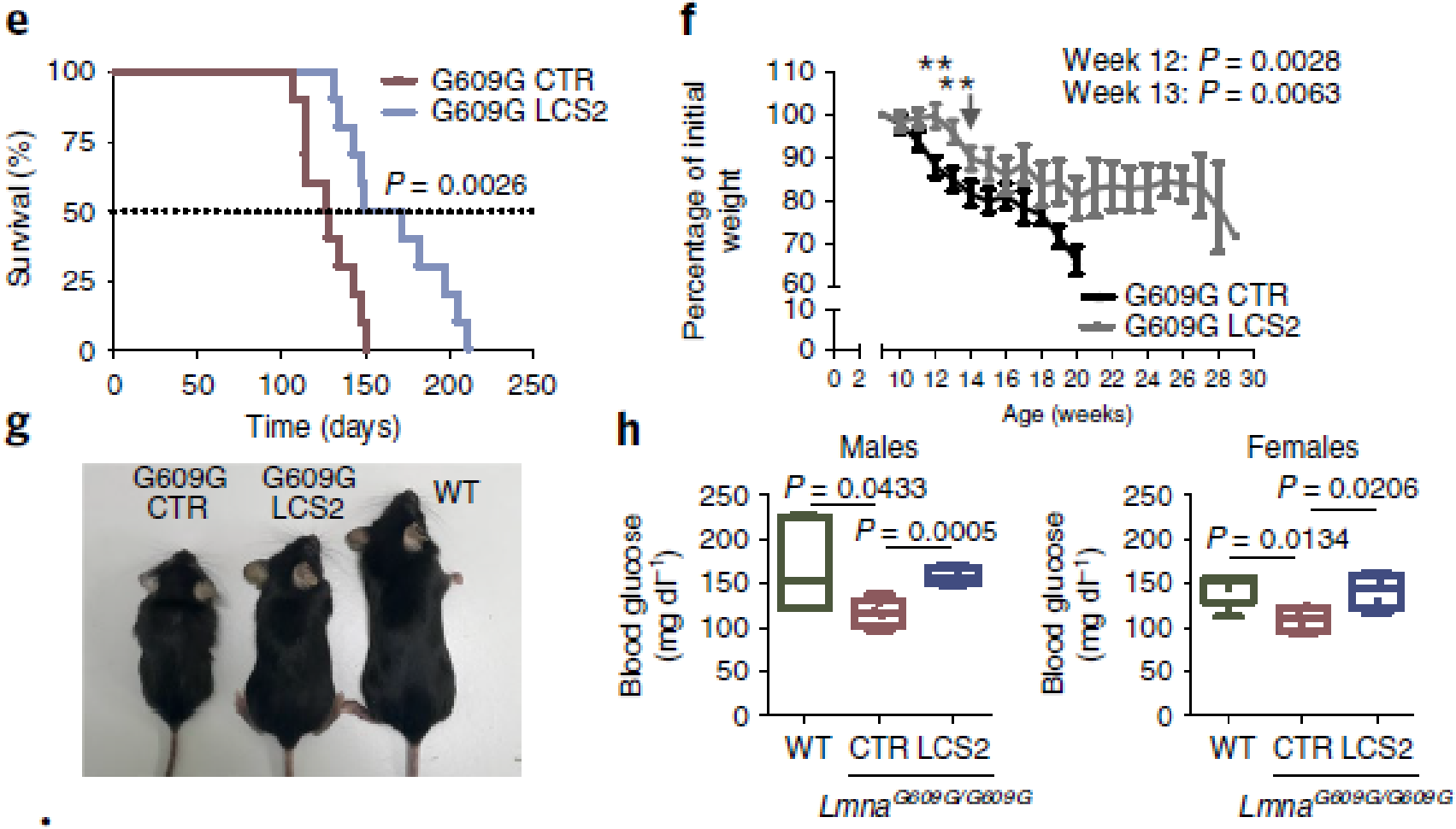
LCS1 reduced Lamin A and progerin, no changed LaminC, and nuclear blebbing

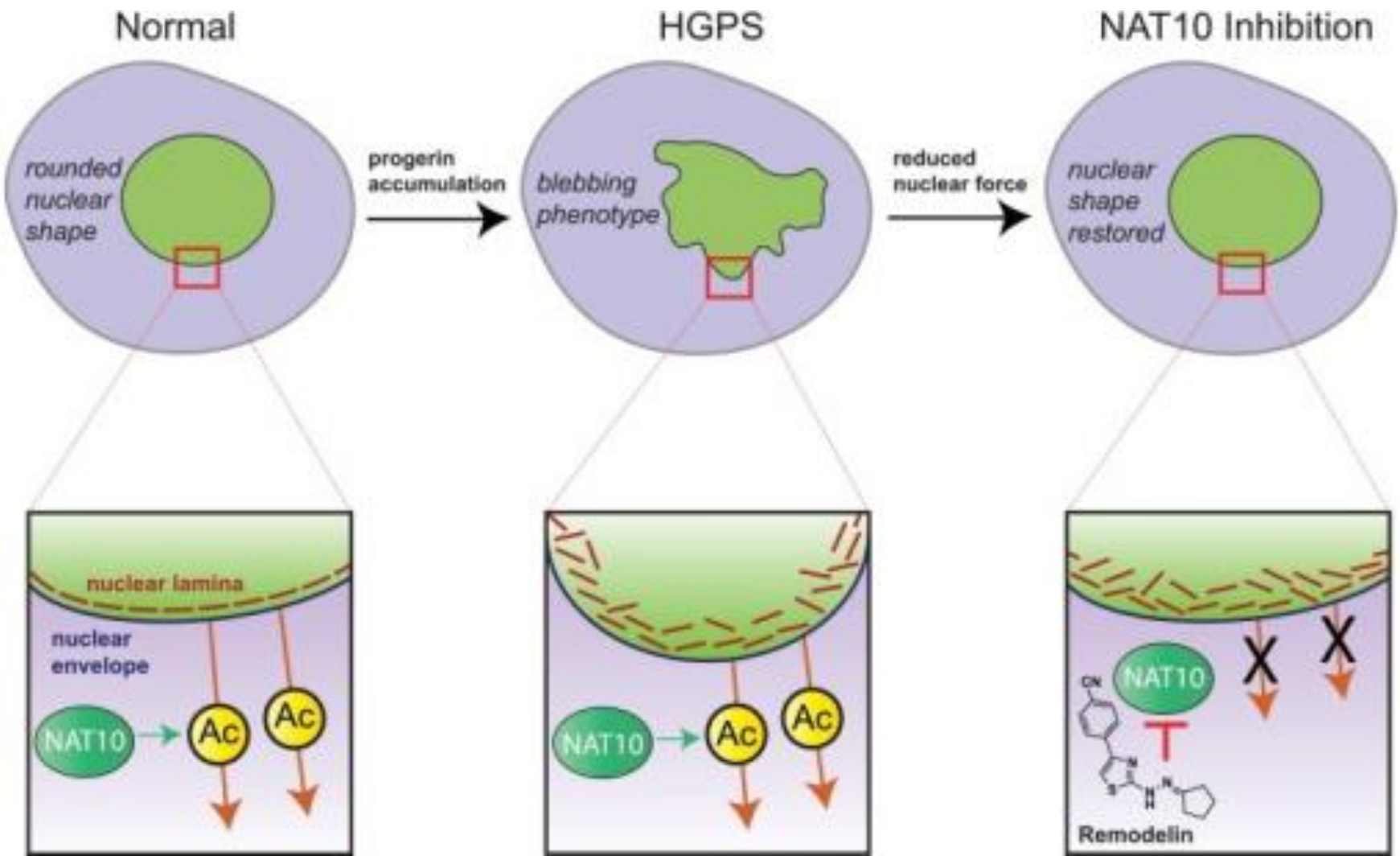


LCS2 effect on genome in several tissues



Progerin reduction in AAV9-sgRNA-LCS2- transduced mice was translated into an increase in their survival, growth and blood glucose level




Normal
HGPS
NAT10 Inhibition
rounded nuclear shape
progerin accumulation
blebbing phenotype
reduced nuclear force
nuclear shape restored
nuclear lamina
nuclear envelope
NAT10
Ac
Ac
NAT10
Ac
Ac
NAT10
X
X
Remodelin

Another approach for progeria theraphy

ARTICLE

DOI: 10.1038/s41467-018-03770-3 OPEN

Targeting of NAT10 enhances healthspan in a mouse model of human accelerated aging syndrome

Gabriel Balmus[1,2], Delphine Larrieu[1,9], Ana C. Barros[1,2], Casey Collins[2], Monica Abrudan[2], Mukerrem Demir[1], Nicola J. Geisler[1,2], Christopher J. Lelliott[2], Jacqueline K. White[2], Natasha A. Karp[2,3], James Atkinson[4], Andrea Kirton[2], Matt Jacobsen[4], Dean Clift[5], Raphael Rodriguez[6,7,8], Sanger Mouse Genetics Project, David J. Adams[2] & Stephen P. Jackson[1]

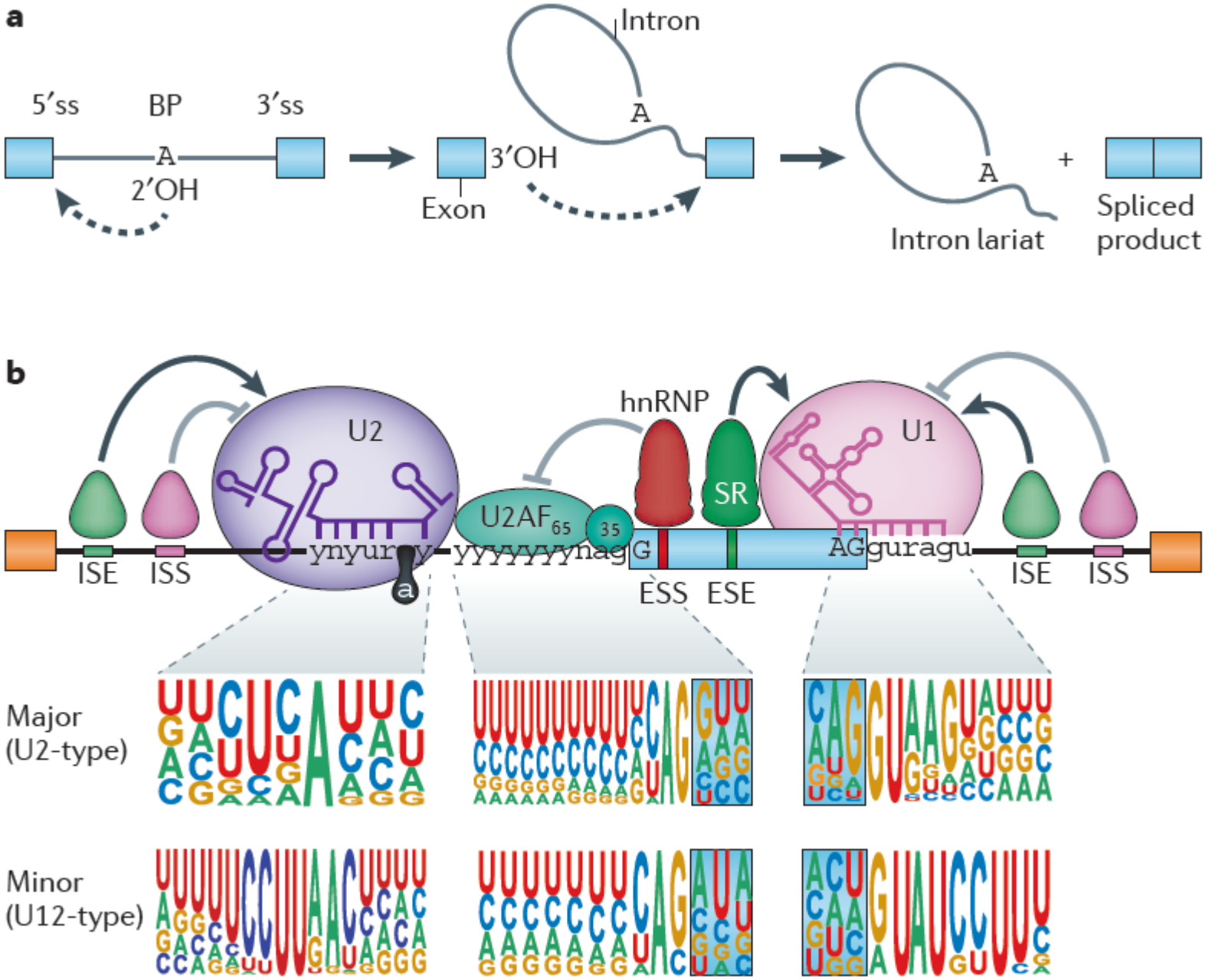
Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare, but devastating genetic disease characterized by segmental premature aging, with cardiovascular disease being the main cause of death. Cells from HGPS patients accumulate progerin, a permanently farnesylated, toxic form of Lamin A, disrupting the nuclear shape and chromatin organization, leading to DNA-damage accumulation and senescence. Therapeutic approaches targeting farnesylation or aiming to reduce progerin levels have provided only partial health improvements. Recently, we identified Remodelin, a small-molecule agent that leads to amelioration of HGPS cellular defects through inhibition of the enzyme N-acetyltransferase 10 (NAT10). Here, we show the preclinical data demonstrating that targeting NAT10 in vivo, either via chemical inhibition or genetic depletion, significantly enhances the healthspan in a *Lmna*^{G609G} HGPS mouse model. Collectively, the data provided here highlights NAT10 as a potential therapeutic target for HGPS.

RNA mis-splicing in disease

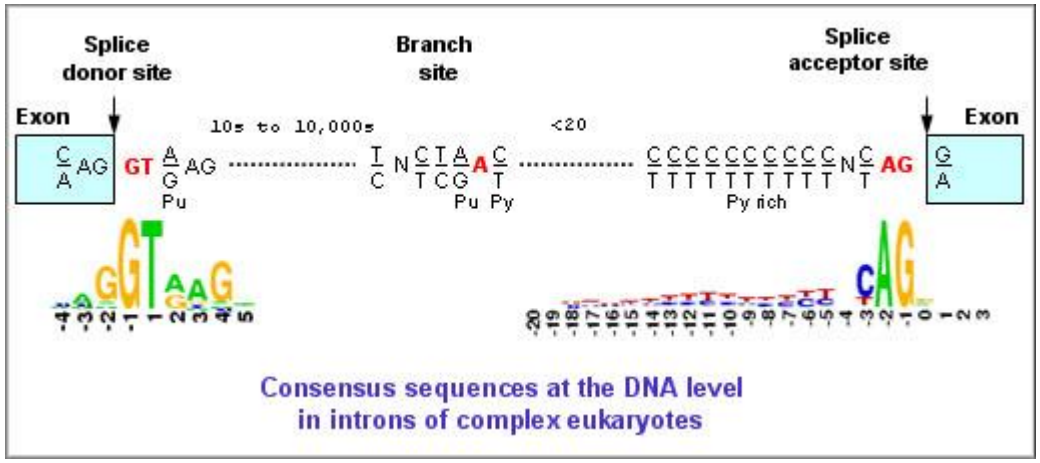
Marina M. Scotti and Maurice S. Swanson

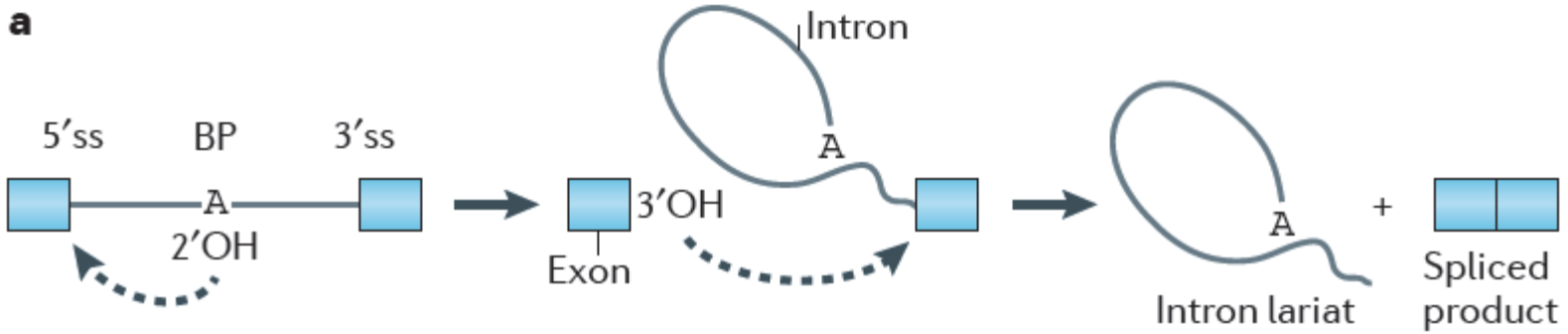
Abstract | The human transcriptome is composed of a vast RNA population that undergoes further diversification by splicing. Detecting specific splice sites in this large sequence pool is the responsibility of the major and minor spliceosomes in collaboration with numerous splicing factors. This complexity makes splicing susceptible to sequence polymorphisms and deleterious mutations. Indeed, RNA mis-splicing underlies a growing number of human diseases with substantial societal consequences. Here, we provide an overview of RNA splicing mechanisms followed by a discussion of disease-associated errors, with an emphasis on recently described mutations that have provided new insights into splicing regulation. We also discuss emerging strategies for splicing-modulating therapy.

RNA SPLICING REGULATION



RNA SPLINCING REGULATION



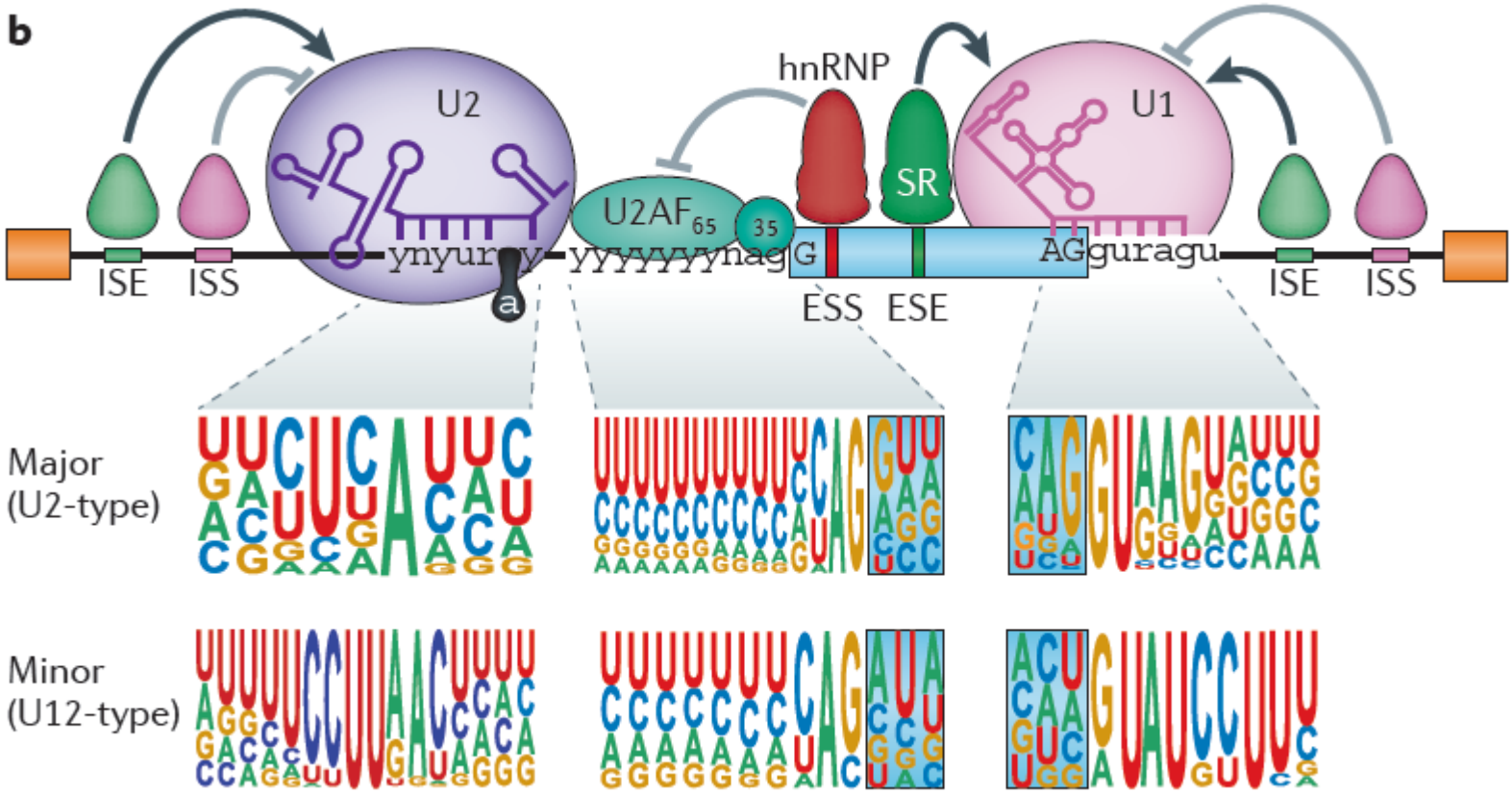


Sequential transesterification reactions are initiated by a **nucleophilic attack of the 5´ splice site (5´ss) by the branch adenosine** (branch point; BP) in the downstream intron resulting in the formation of an intron lariat intermediate with a 2´, **5´-phosphodiester linkage**.

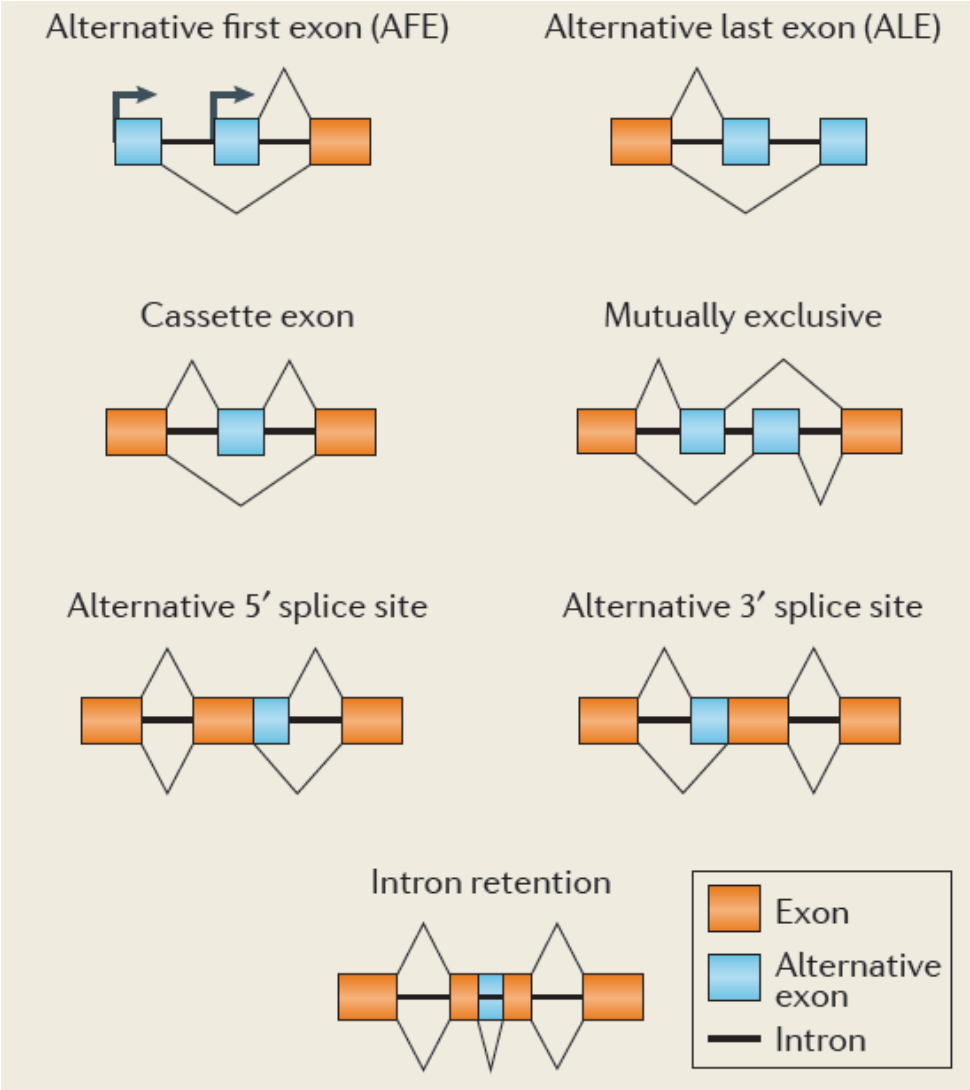
This is followed by a **5´ss-mediated attack on the 3´ss**, leading to the removal of the intron lariat and the formation of the spliced RNA product

RNA SPLINCING REGULATION

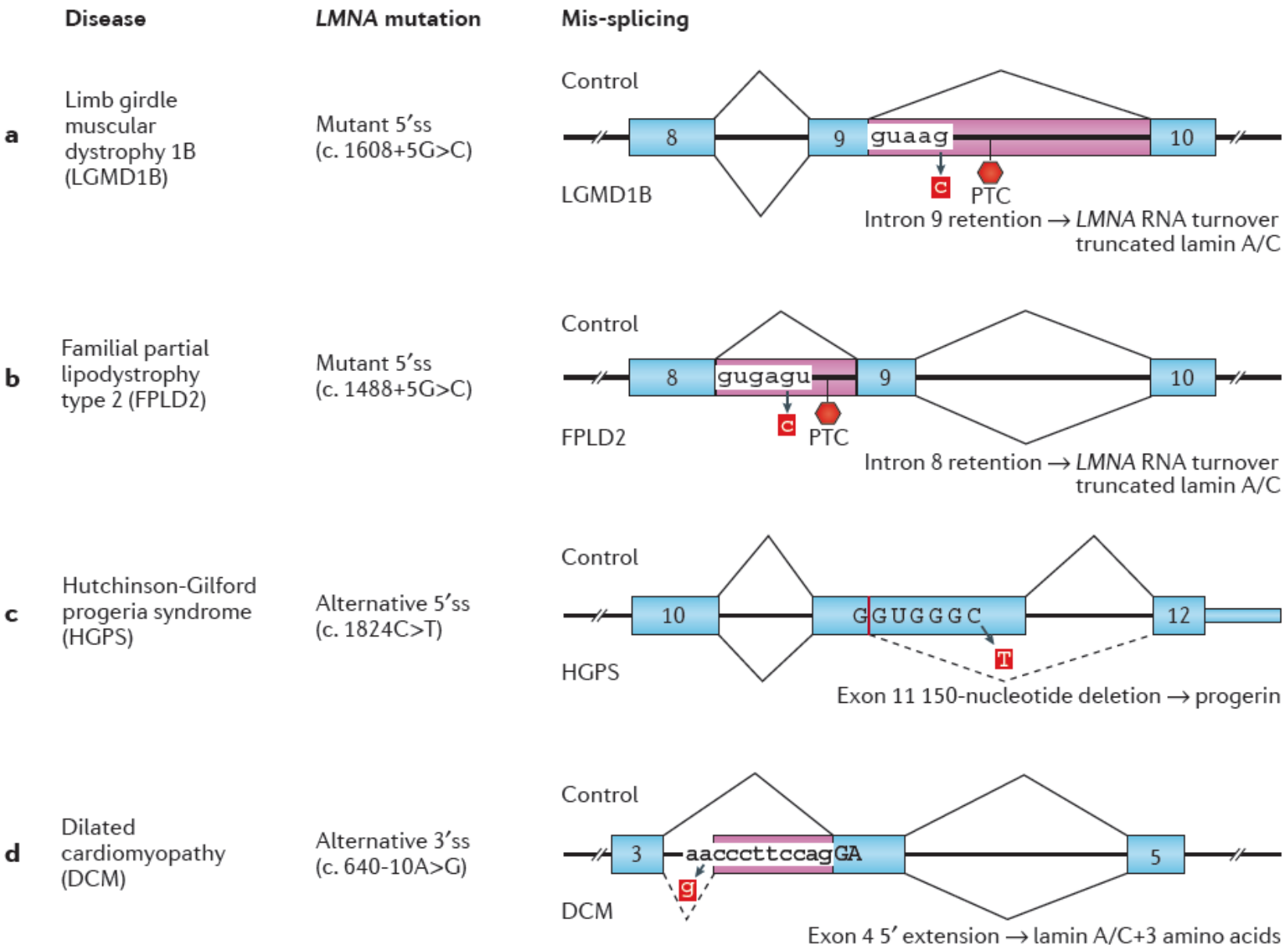
The difficult tasks of splice site identification and regulated splicing is accomplished principally by two exceptionally dynamic macromolecular machines, the major (U2-dependent) and minor (U12-dependent) spliceosomes.



Alternative splicing adds another layer of complexity with multiple and developmentally regulated splicing patterns including the inclusion of alternative first and last exons (AFE and ALE, respectively), retained intron, cassette exon, mutually exclusive cassettes and alternative 5´ and 3´ splice sites



Mis-splicing of a single gene results in different diseases.



Mis-splicing of a single gene results in different diseases.

Aberrant splicing of lamin A (LMNA) pre-mRNA is associated with multiple hereditary disorders. Normal exons are shown in blue, introns are shown as thick black lines, normal splicing is indicated by thin black lines, and disease-associated splicing is indicated in dotted lines or purple boxes (intron retention).

a | **Limb girdle muscular dystrophy type 1B (LGMD1B)** is caused by a G>C 5‘ splice site (5‘ss) mutation that results in intron 9 retention, a premature termination codon (PTC) and nonsense-mediated decay (NMD). **c.1608 + 5 indicates that the mutations occurs 5 nucleotides into the intron that follows coding position** (c) 1608. However, a lamin A/C protein truncated in intron 9 with a unique carboxy-terminal sequence may also be produced.

b | **In familial partial lipodystrophy type 2 (FPLD2**), **a G>C transversion mutation occurs in the exon 8 5‘ss, leading to intron 8 retention**, NMD and potential translation of another truncated lamin A/C with a unique C-terminal region.

c | A common cause of **Hutchinson–Gilford progeria syndrome (HGPS) is a C>T transition in exon 11, which activates a cryptic 5‘ss and results in a 150 nucleotide** deletion that is translated into the ageing-associated protein progerin.

d | For **LMNA-linked dilated cardiomyopathy (DCM),** an **alternative 3‘ss is generated by an A>G mutation upstream of the normal exon 4 3‘ ss** so that nine additional nucleotides are inserted in-frame between exons 3 and 4, resulting in a 3-amino-acid insertion in the resultant protein.

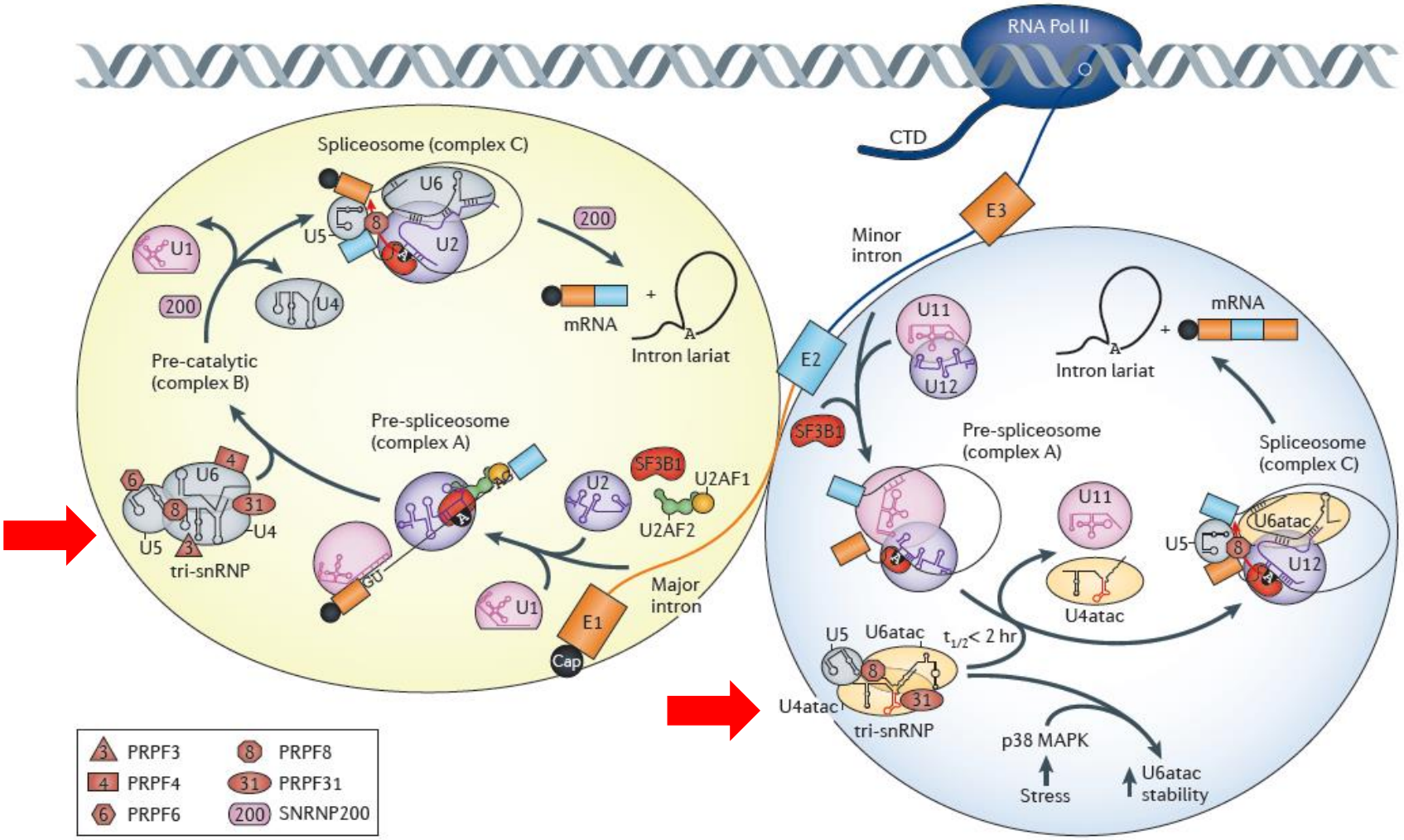
Major and minor spliceosome mutations.

The figure shows the splicing steps and core spliceosomal components of both the major (U2-dependent) and minor (U12-dependent) spliceosomes, including their interactions in the pre-spliceosomal complex (complex A) and spliceosome (complex C).

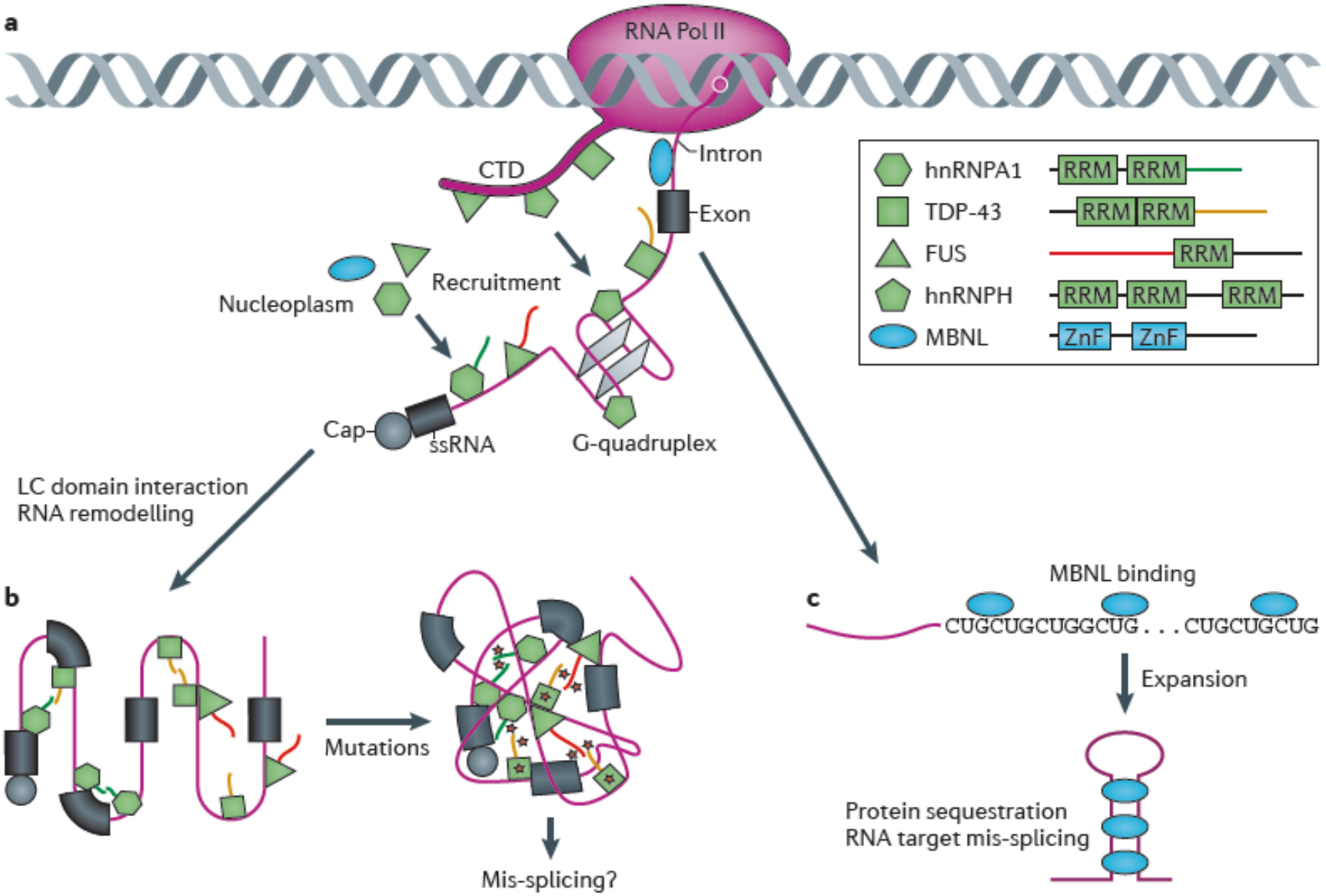
Pre-mRNA processing factor 3 (PRPF3), PRPF4), PRPF6, PRPF8 and PRPF31 components of the U4/U6.U5 tri-small nuclear ribonucleoprotein (tri-snRNP) dysregulated in **autosomal dominant retinitis pigmentosa (adRP)** are shown. Also indicated is the SNRNP200 helicase, which is required at several dissociation steps in the spliceosomal cycle.

Several PRPF components are common to both the U4/U6.U5 tri-snRNP and the U4atac/U6atac.U5 tri-snRNP complexes. Some mutations in the U4atac snRNA 5‘ stem-loop found **in microcephalic osteodysplastic primordial dwarfism type 1 (MOPD I)** are highlighted in red. In addition, stress-induced upregulation of p38 mitogen-activated protein kinase (MAPK) leads to increased stability of U6atac (t1/2 <2 hours).

Major and minor spliceosome mutations.



Co-transcriptional splicing factor recruitment and disease mutations



Therapeutic strategies.

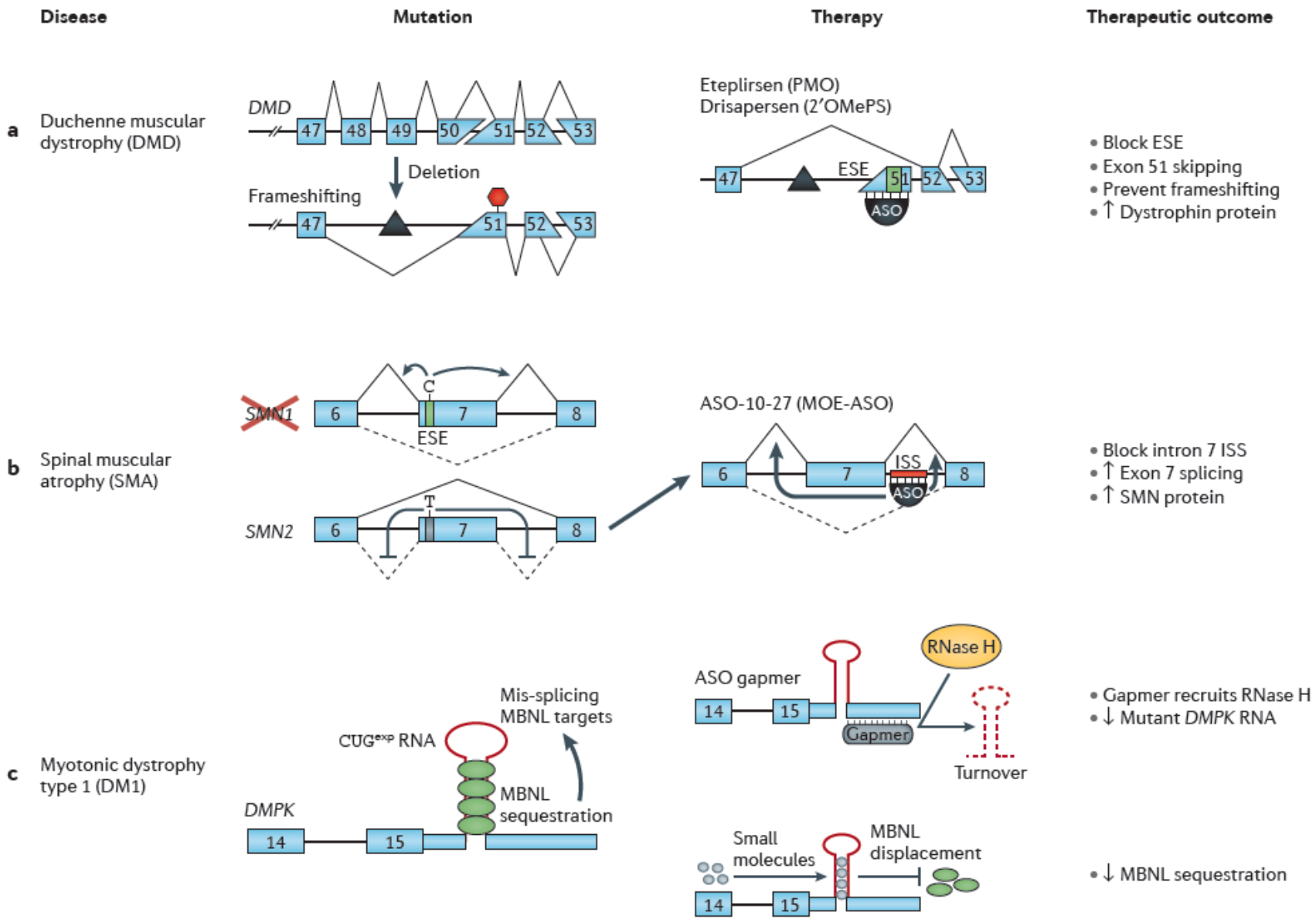
Examples of therapies based on antisense oligonucleotide (ASO) and small molecule approaches.

a | **Duchenne muscular dystrophy** is often caused by chromosomal deletions (black triangle) that remove exons 48–50, loss of dystrophin protein. The red hexagon indicates the **premature stop codon** resulting from frameshifted exon 51. To prevent frameshifting, both **phosphorodiamidate morpholino oligomer (PMO) and 2'OMePS (2'O-methyl-phosphorothioate) ASOs (black semicircle) block an exon 51 exonic splicing enhancer (**ESE; green rectangle) and shift splicing to the in-frame exon 52.

b | **In spinal muscular atrophy**, survival of motor neuron 1 (SMN1), which produces the majority of SMN protein, is either deleted or inactivated by mutations, and the paralogous SMN2 expresses low levels of SMN due to a C>T transition (grey box) that suppresses exon 7 splicing. **ASO-10-27 targets an intronic splicing silencer** (ISS; red bar) and enhances exon 7 splicing to produce stable SMN protein.

c | In **myotonic dystrophy type 1**, CUG expansion (CUGexp) RNA (red hairpin) binds muscleblind-like (MBNL) proteins (green ovals) and causes mis-splicing of MBNL RNA targets. **Mutant MBNL–RNA complexes accumulate in the nucleus, and so ASO gapmers preferentially target mutant RNAs for degradation** (dotted red line). Alternatively, **small molecule compounds bind to mutant CUGexp RNA**, displace MBNL and rescue abnormal splicing. DMPK, DM protein kinase.

Therapeutic strategies.



IN SUMMARY:

- **Morpholino application for recovering the lamin A expression in Progeria disease**
- **Role of Lamin A in chromatin organization and biological function**
- **Mutations in the SPLINCING MACHINERY are involved in disease**

IMMUNE REGULATION

A long noncoding RNA associated with susceptibility to celiac disease

Ainara Castellanos-Rubio,[1] Nora Fernandez-Jimenez,[2] Radomir Kratchmarov,[1] Xiaobing Luo,[3] Govind Bhagat,[4,5] Peter H. R. Green,[5] Robert Schneider,[6] Megerditch Kiledjian,[3] Jose Ramon Bilbao,[2] Sankar Ghosh[1]*

Recent studies have implicated long noncoding RNAs (lncRNAs) as regulators of many important biological processes. Here we report on the identification and characterization of a lncRNA, lnc13, that harbors a celiac disease–associated haplotype block and represses expression of certain inflammatory genes under homeostatic conditions. Lnc13 regulates gene expression by binding to hnRNPD, a member of a family of ubiquitously expressed heterogeneous nuclear ribonucleoproteins (hnRNPs). Upon stimulation, lnc13 levels are reduced, thereby allowing increased expression of the repressed genes. Lnc13 levels are significantly decreased in small intestinal biopsy samples from patients with celiac disease, which suggests that down-regulation of lnc13 may contribute to the inflammation seen in this disease. Furthermore, the lnc13 disease-associated variant binds hnRNPD less efficiently than its wild-type counterpart, thus helping to explain how these single-nucleotide polymorphisms contribute to celiac disease.

Celiac disease (CeD)

is a chronic, immune-mediated intestinal disorder that is caused by intolerance to ingested gluten and develops in genetically susceptible individuals.

The NEW ENGLAND JOURNAL of MEDICINE

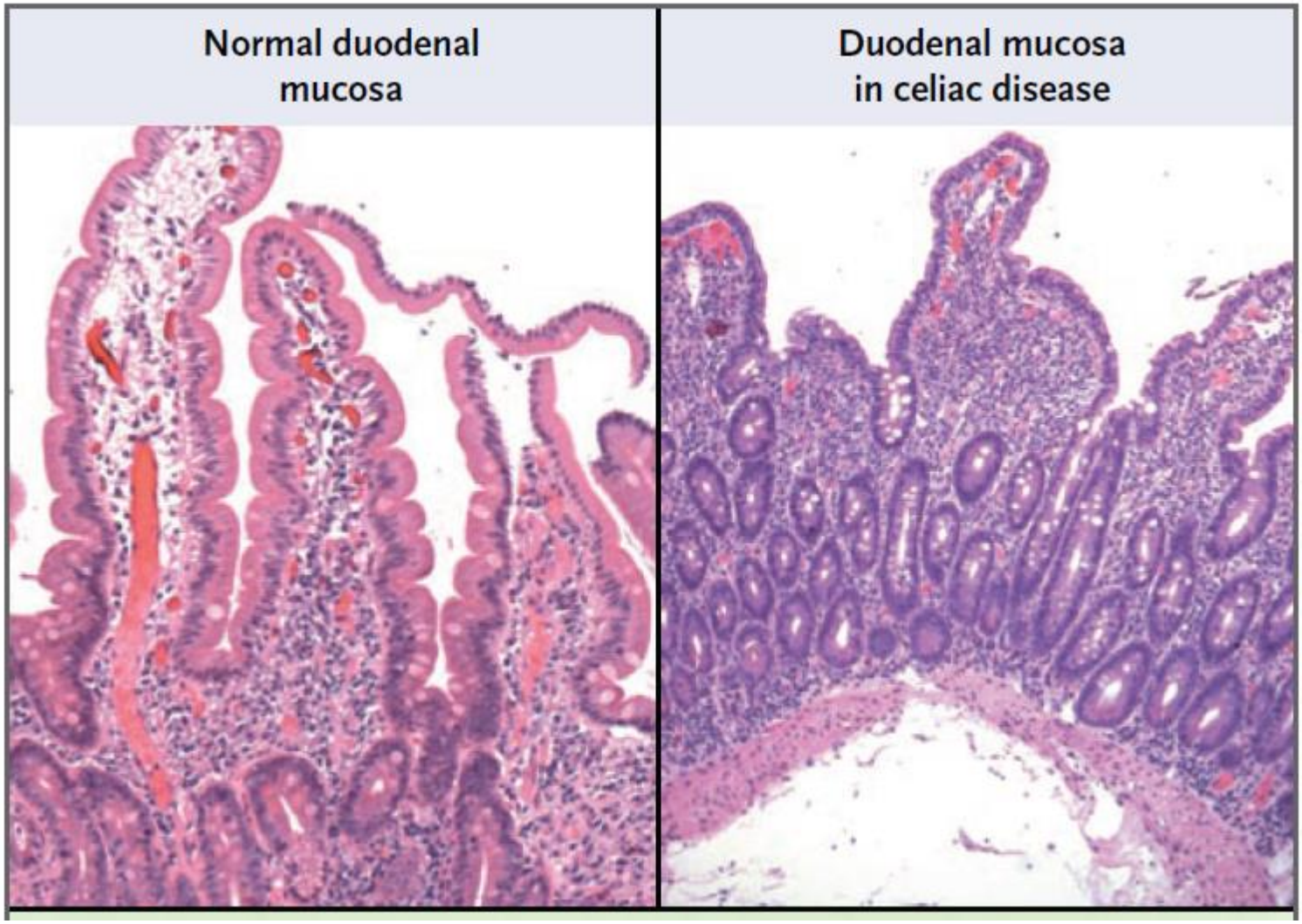
REVIEW ARTICLE

MEDICAL PROGRESS

Celiac Disease

Peter H.R. Green, M.D., and Christophe Cellier, M.D., Ph.D.

Immune system activation from gluten damage duodenal mucosa



Molecular Mechanisms that underpin CELIAC DISEASE

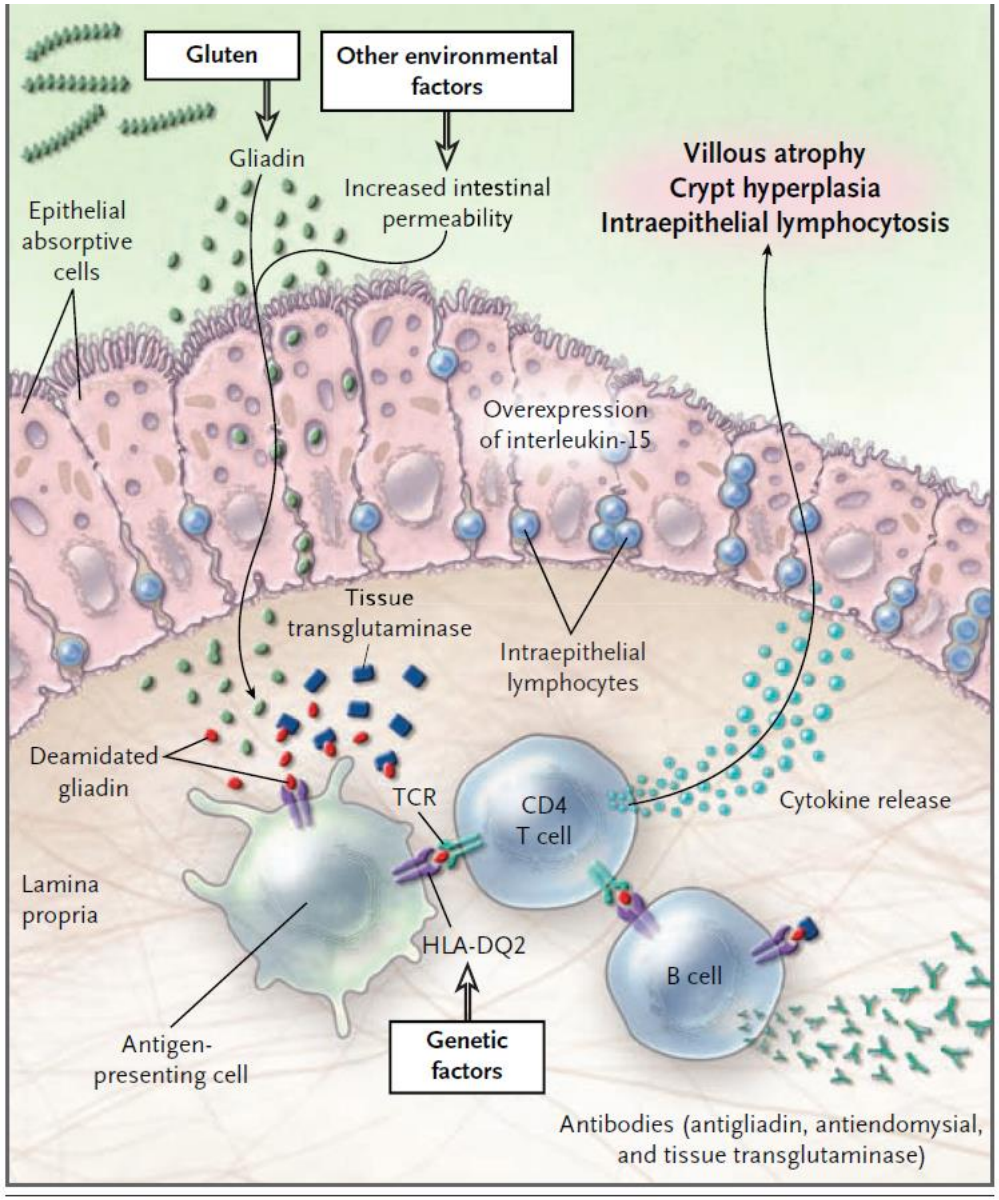


Figure 1. Interaction of Gluten with Environmental, Immune, and Genetic Factors in Celiac Disease.

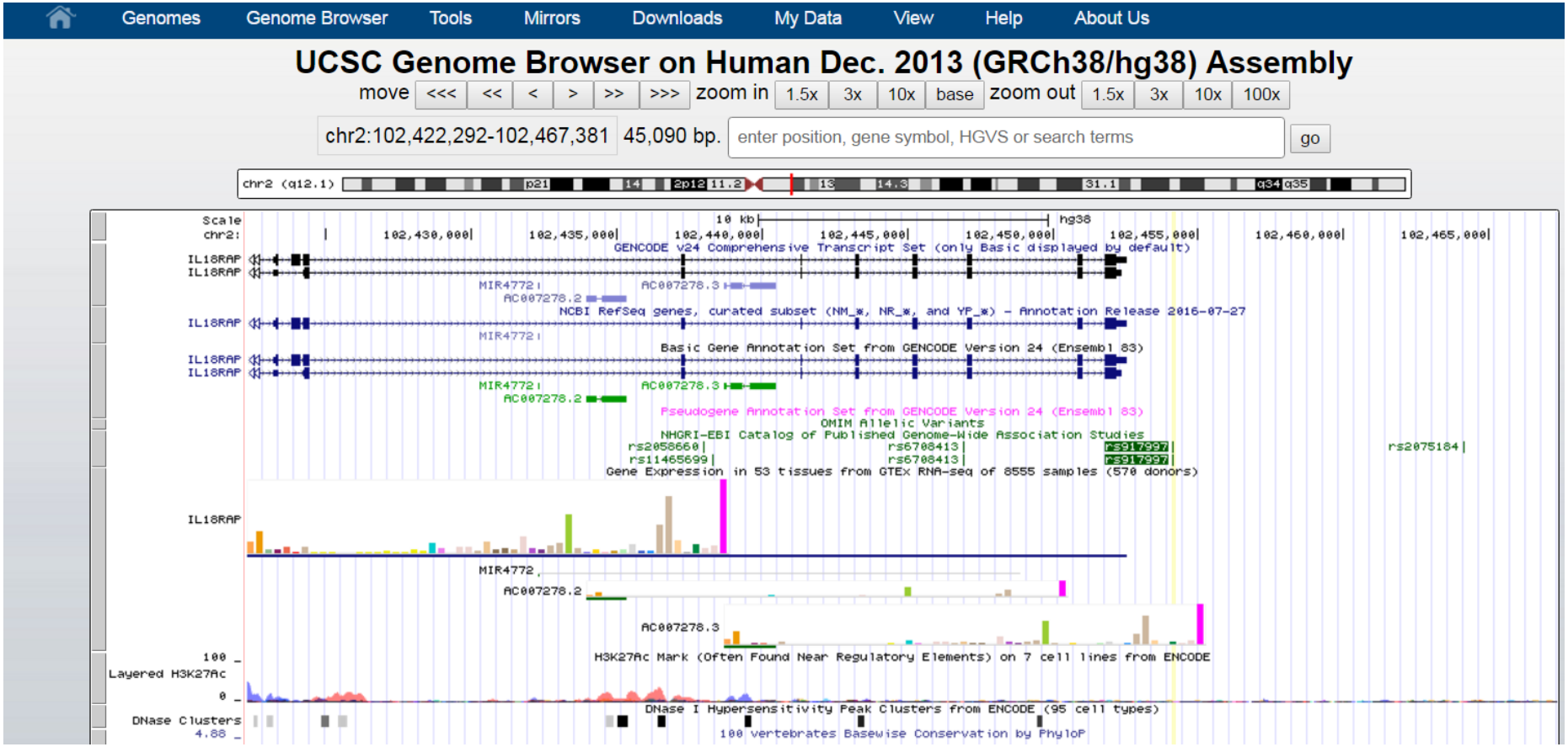
Gluten is digested by luminal and brush-border enzymes into amino acids and peptides. The gliadin peptides induce changes in the epithelium through the innate immune system and, in the lamina propria, through the adaptive immune system. In the epithelium, gliadin damages epithelial cells, resulting in increased expression of interleukin-15, which in turn activates intraepithelial lymphocytes. These lymphocytes become cytotoxic and kill enterocytes that express MIC-A (a stress protein) on their surface. During infections or as the result of permeability changes, gliadin enters the lamina propria, where it is deamidated by tissue transglutaminase, allowing interaction with HLA-DQ2 (or HLA-DQ8) on the surface of antigen-presenting cells. Gliadin is presented to gliadin-reactive CD4+ T cells through a T-cell receptor, resulting in the production of cytokines that cause tissue damage. This leads to villous atrophy and crypt hyperplasia, as well as the activation and expansion of B cells that produce antibodies. Images of mucosa courtesy of Govind Bhagat, M.D.

Steps for the identification of the role for SNP associated with Celiac Disease, rs917997

- **Annotation of SNP on the reference genome for the localization in the specific locus**

- **Searching in the Genome-wide database for epigenetic features that describe SNP-associated locus for cell type or tissues**

rs917997 is 1.5 kb apart from the 3′ end of the IL18RAP coding gene in chromosome 2



Human lnc13 genomic location is predicted based on homology with mouse lnc13 (TransMap alignment 66.5% identity). Conserved regions are represented as thicker lines on mouse lnc13 (AK161196)

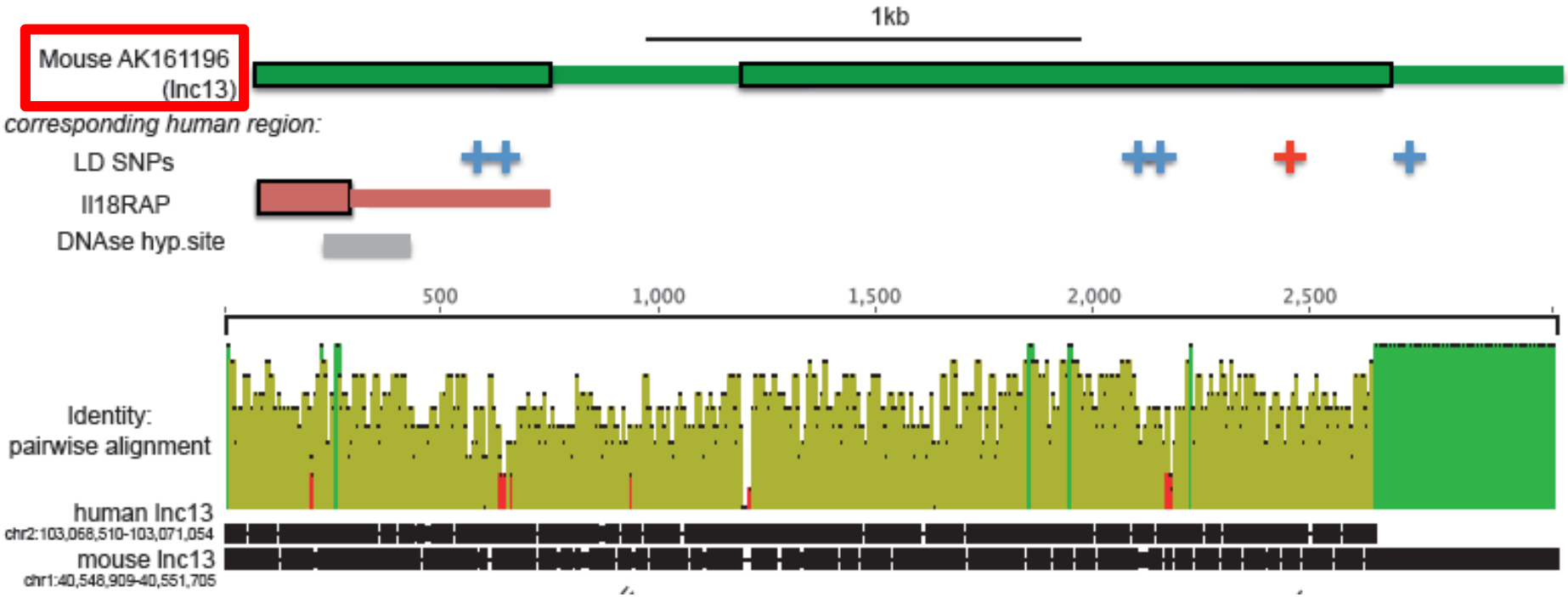
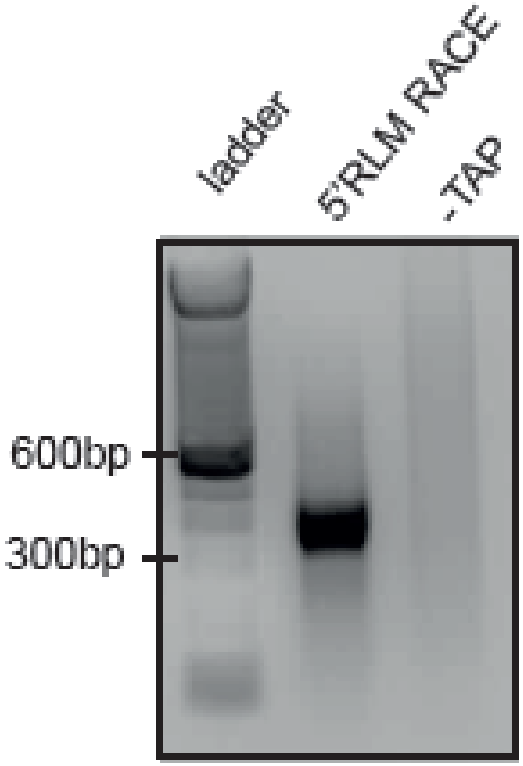


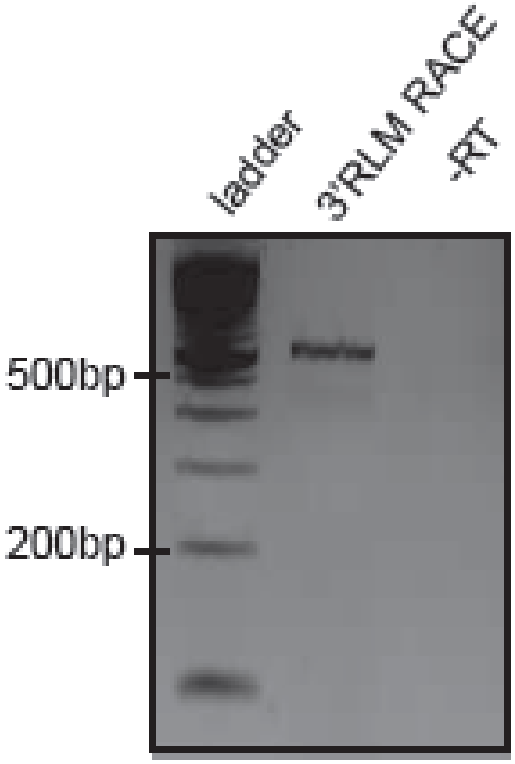
fig. S1A

Steps for the chacterization of Lnc13

- Cloning of Lnc13 using RACE-PCR
- Northen blot to identify Lnc13 expression
- Lnc 13 expression in cell lines and tissues using RNAscope and PCR

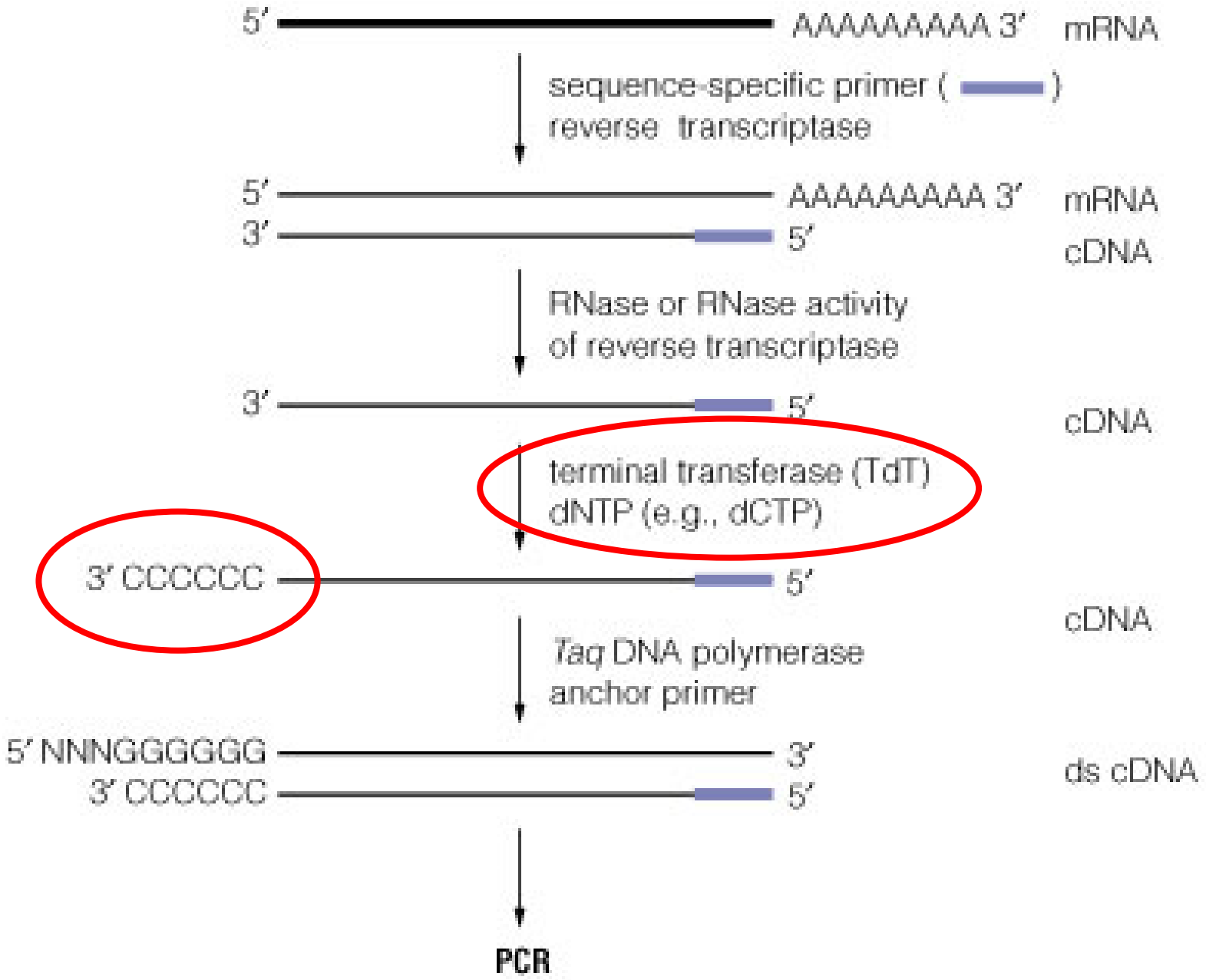
RACE-PCR is used for cloning Lnc13

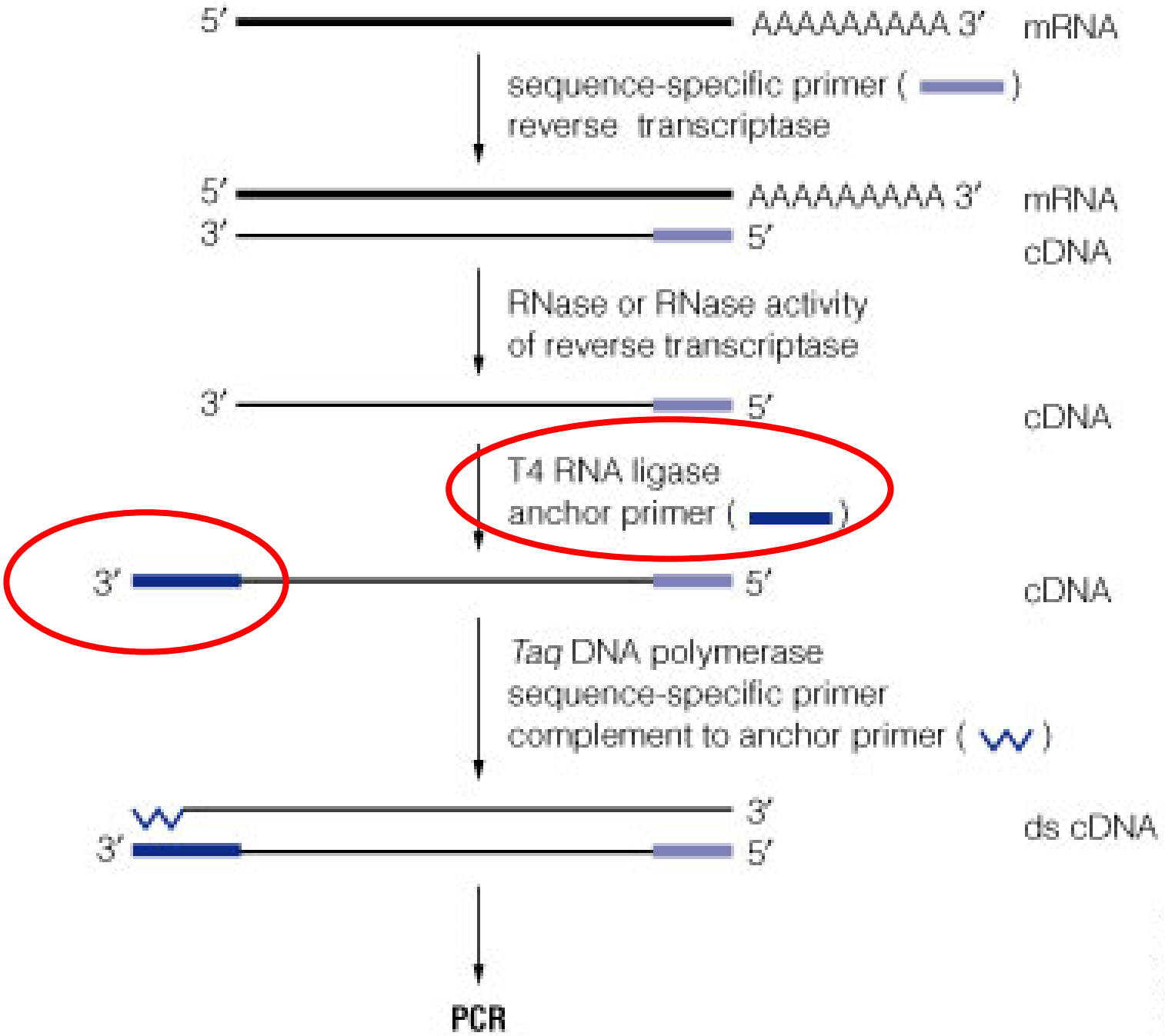




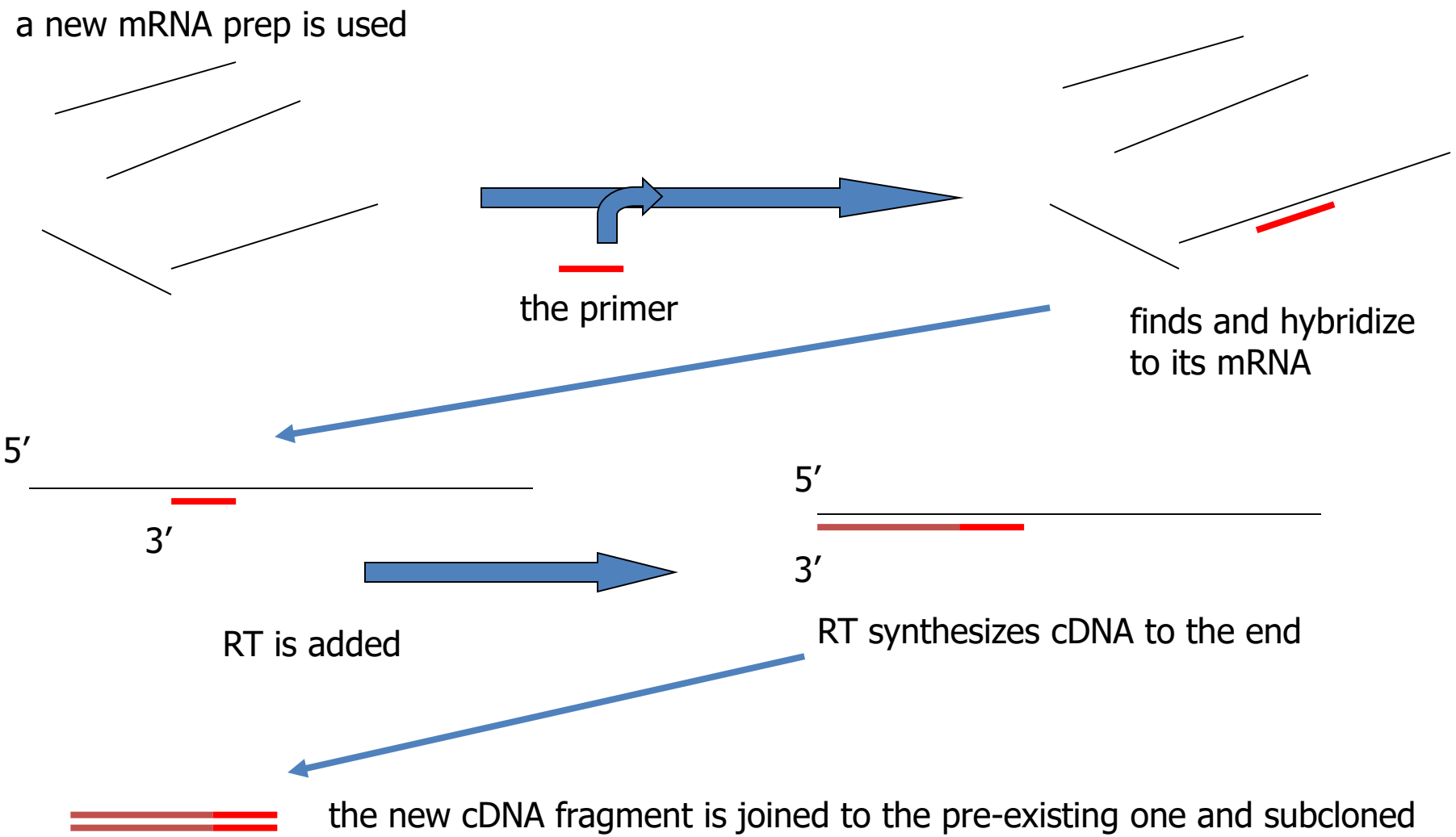
RACE

(rapid amplification of cDNA ends)

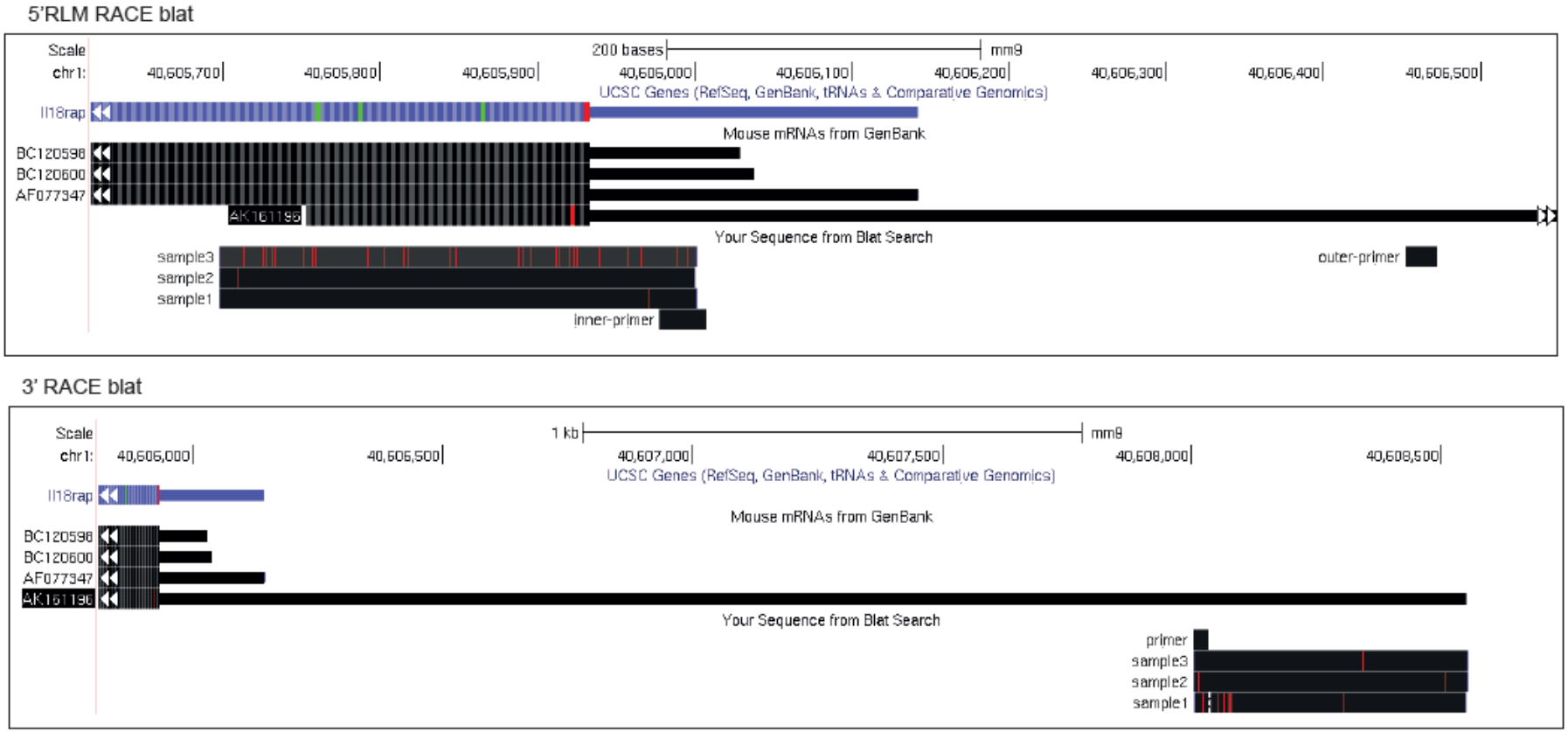

5′
AAAAAAAAA 3′
mRNA
sequence-specific primer ( )
reverse transcriptase
5′
AAAAAAAAA 3′
mRNA
3′
5′
cDNA
RNase or RNase activity
of reverse transcriptase
3′
5′
cDNA
terminal transferase (TdT)
dNTP (e.g., dCTP)
3′ CCCCCC
5′
cDNA
Taq DNA polymerase
anchor primer
5′ NNNGGGGGG
3′
3′ CCCCCC
5′
ds cDNA
PCR


5′
AAAAAAAAA 3′
mRNA
sequence-specific primer ( )
reverse transcriptase
5′
AAAAAAAAA 3′
mRNA
3′
5′
cDNA
RNase or RNase activity
of reverse transcriptase
3′
5′
cDNA
T4 RNA ligase
anchor primer ( )
3′
5′
cDNA
Taq DNA polymerase
sequence-specific primer
complement to anchor primer ( )
3′
3′
5′
ds cDNA
PCR
14401MA04_6A

An oligonucleotide primer is then prepared to match the very 5′ sequence of the cDNA, as determined by dideoxy-sequencing

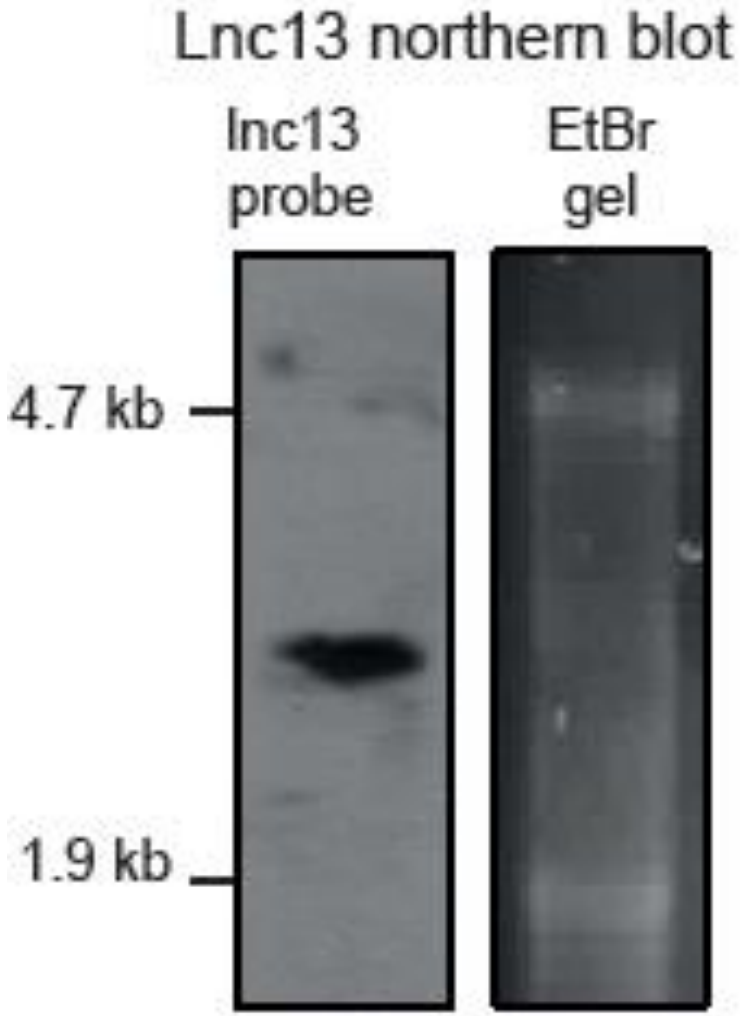


PCR bands were cloned, sequenced and blated against the mouse genome using the UCSC genome browser. AK161196 (in a black box) is the annotated mouse lnc13

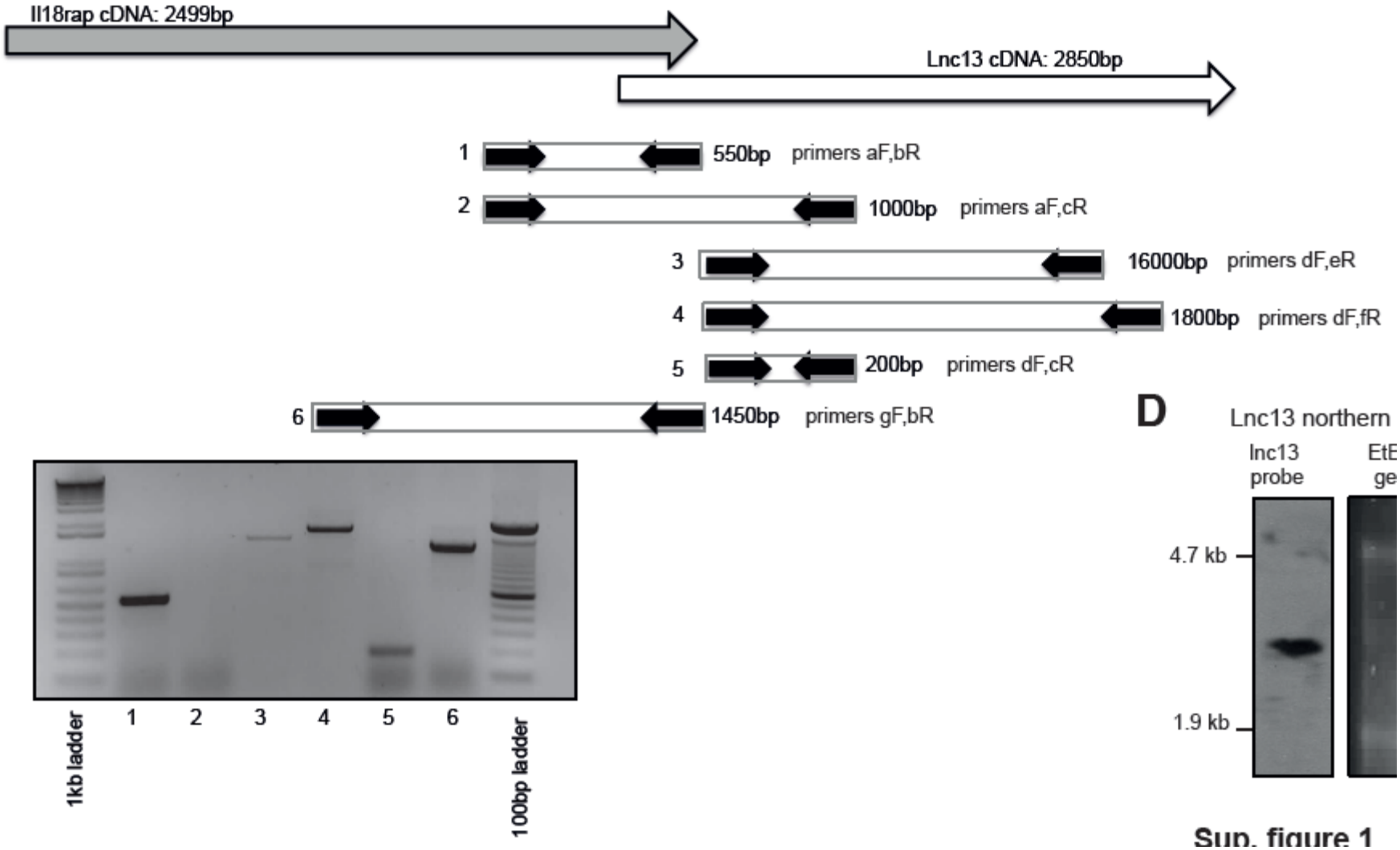


(fig. S1B)

Northern blot of lnc13 expression in iBMM cells



IL18rap and Lnc13 transcripts are indipendent, two different transcripts



Sup. figure 1

fig. S1C

Data from small intestine sample showing the human region corresponding to mouse lnc13 (AK161196).
H3K4me3, H3K4me1, H3K27ac and H3K36me3 peaks denote areas actively transcribed. The chromatin signature points to enhancers (yellow) and actively transcribed chromatin (green). The region encompassing lnc13 is indicated by the line designated as AK161146.

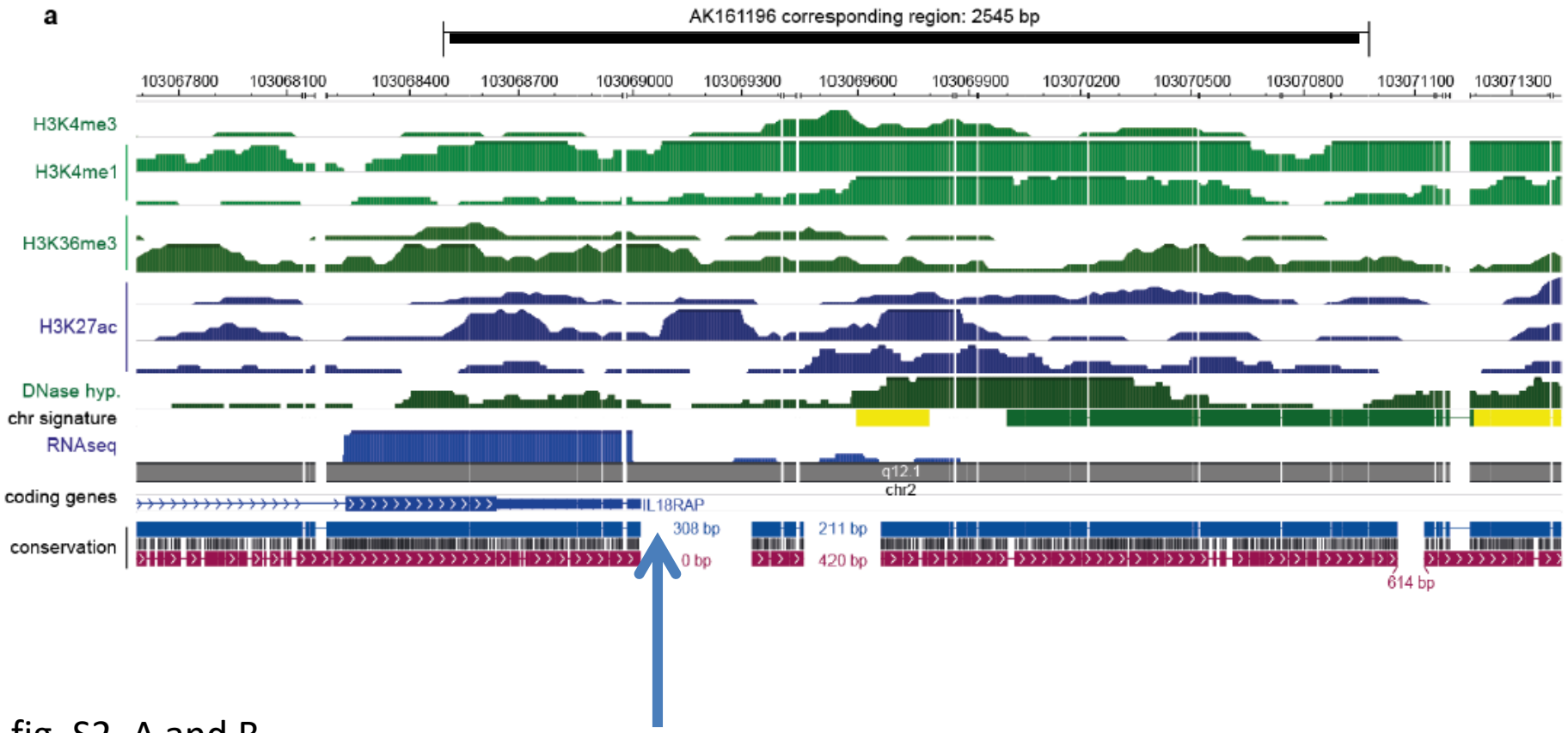
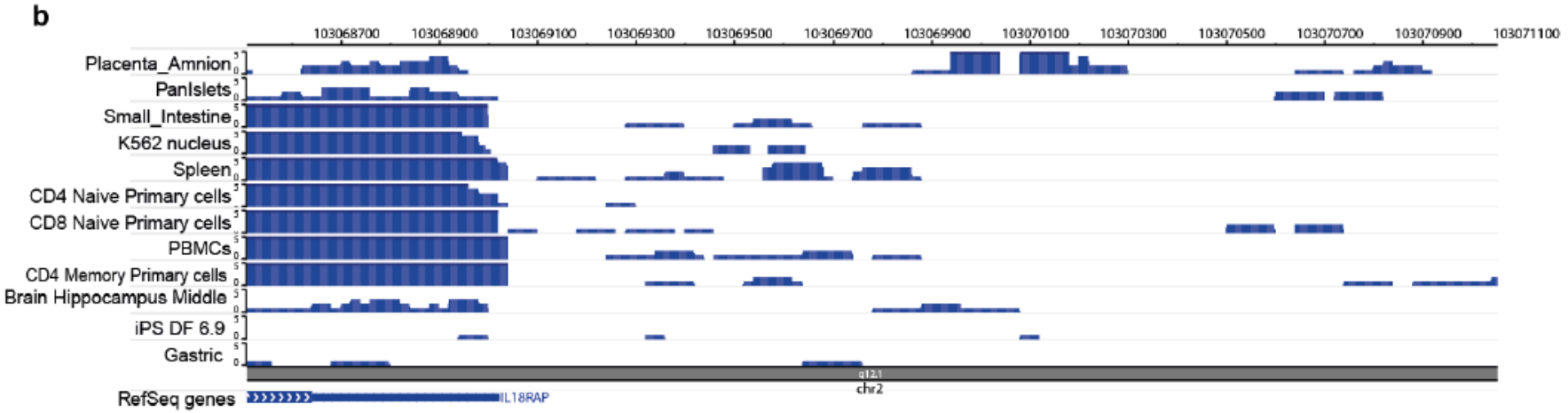
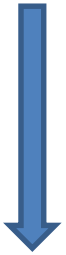


fig. S2, A and B

RNAseq signals in different tissues to the presence of a transcribed region.



The poor **Kozak consensus sequence** in the predicted open reading frame, and the absence of Pfam domains as assessed by the ATG pr and European Molecular Biology Laboratory–European Bioinformatics Institute InterPro prediction programs



lnc13 is unlikely to encode a protein product

Lnc13 expression in human intestinal lamina propria was detected by RNAscope technology.

Biopsies from CeD patients appeared to have substantially lower amounts of lnc13 compared with controls

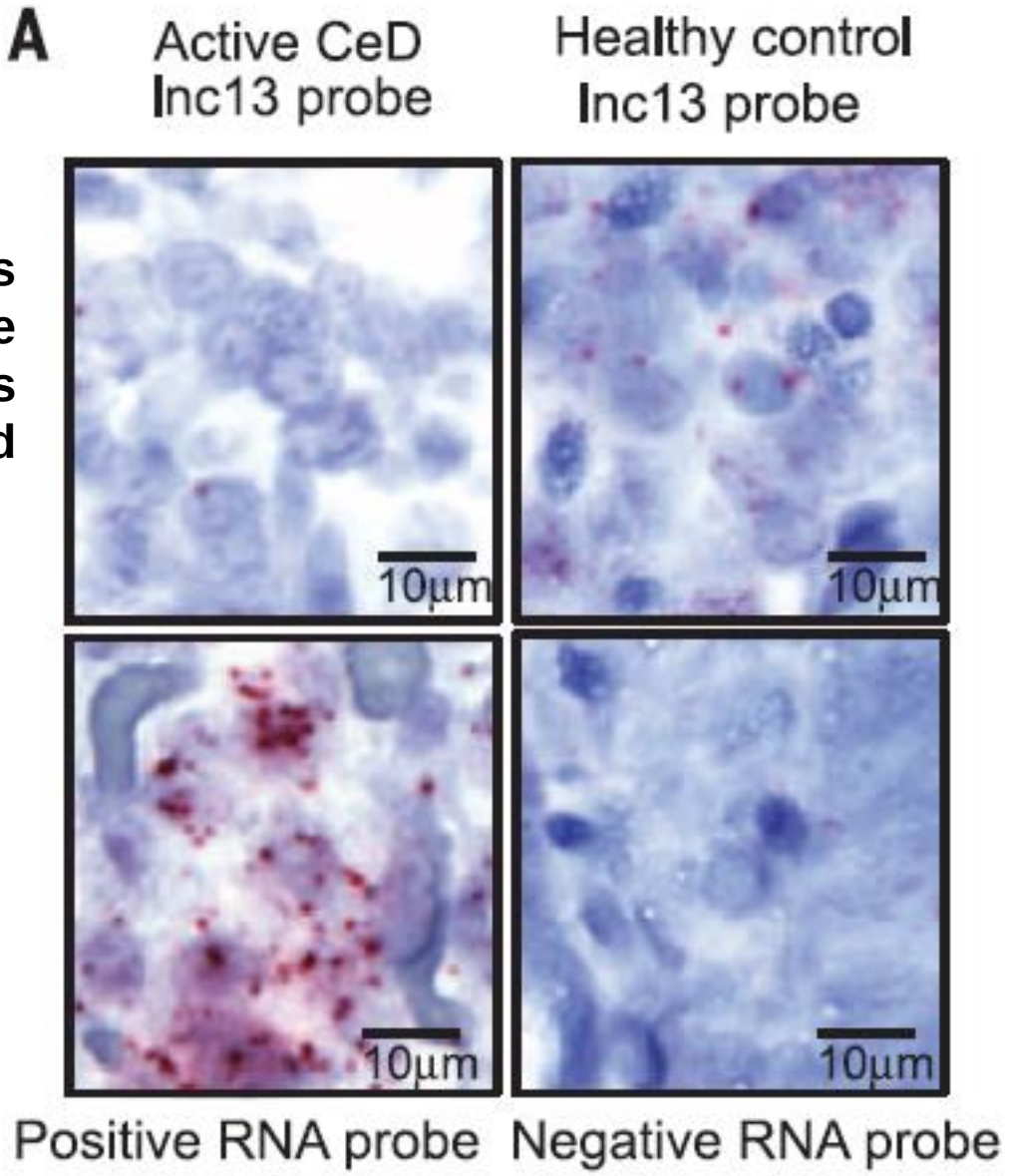


Fig. 1A

Lnc13 expression in human small intestinal biopsies was detected by RNAscope technology.

Biopsies from CeD patients appeared to have substantially lower amounts of lnc13 compared with controls

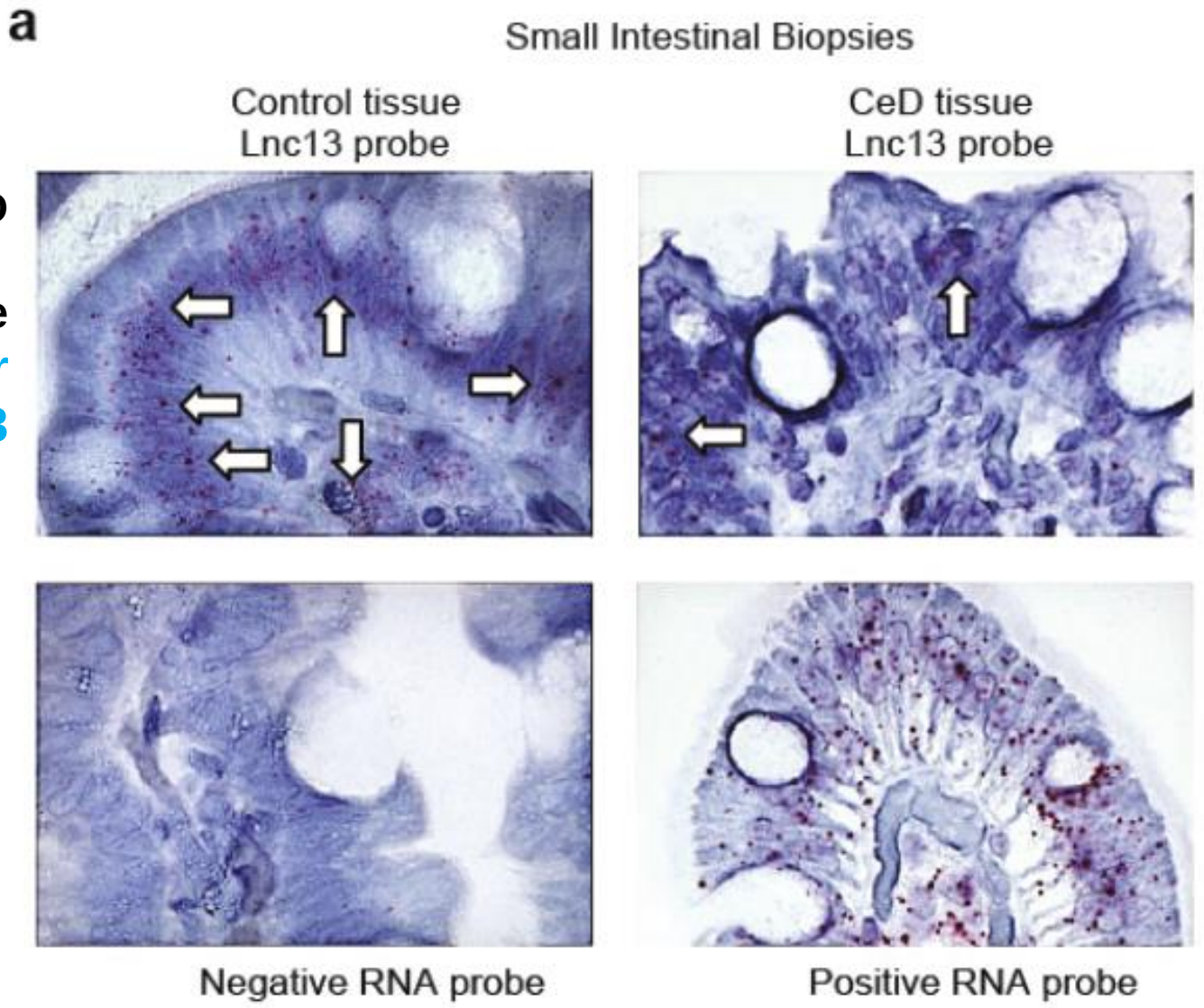


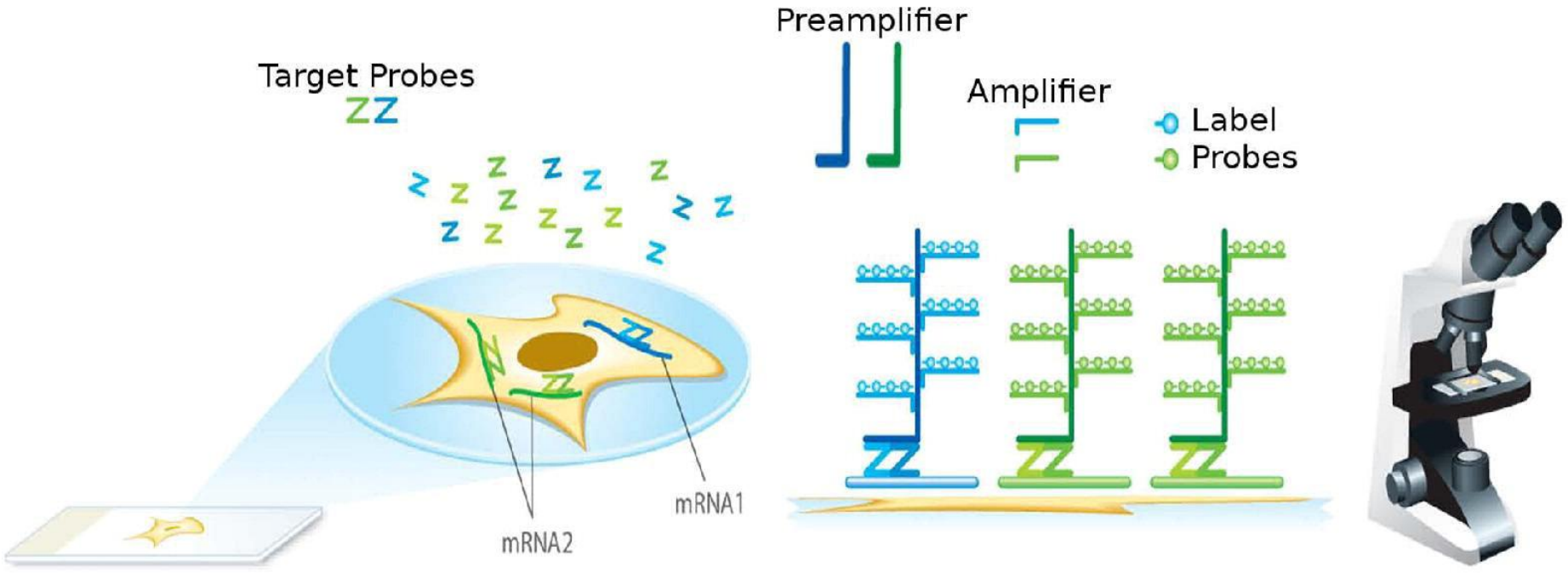
fig. S4A

RNA-SCOPE IS A MODIFIED METHOD OF FISH (IN SITU IBRIDIZATION RNA)

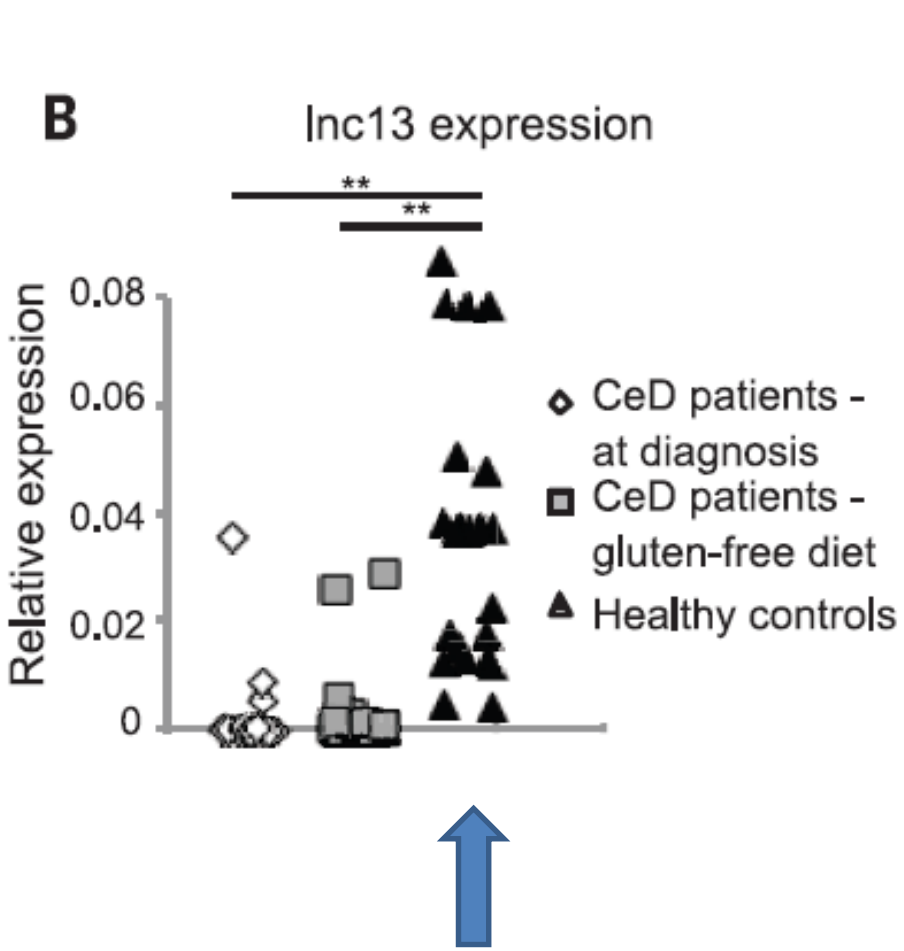
There are **two unlabeled tandem probes**. These probes contain a short complementary region (18-25 bases), a spacer sequence and a 14-base tail sequence.

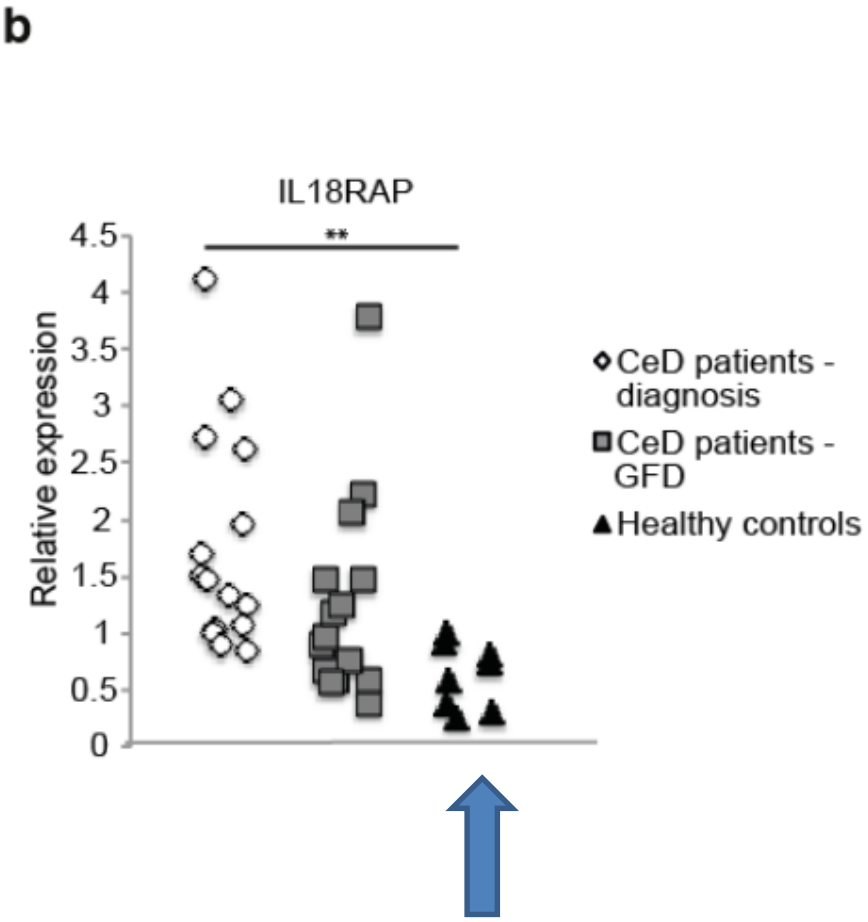
After hybridization with the target probes, comes a **second hybridization step with a pre-amplifier probe**. This is a long probe that contains a complementary sequence to the **28 bases of the two target probes tails** (14+14). So, only when the two target tails hybridize one next to the other the pre-amplifier will hybridize. The **pre-amplifier contains 20 binding sites for an amplifier probe** which in turn contains 20 binding sites for the labeled probe. Thus, for each target probe pair, we get 20×20=400 labeled probes.

RNA-SCOPE IS A MODIFIED METHOD OF FISH (IN SITU IBRIDIZATION RNA)

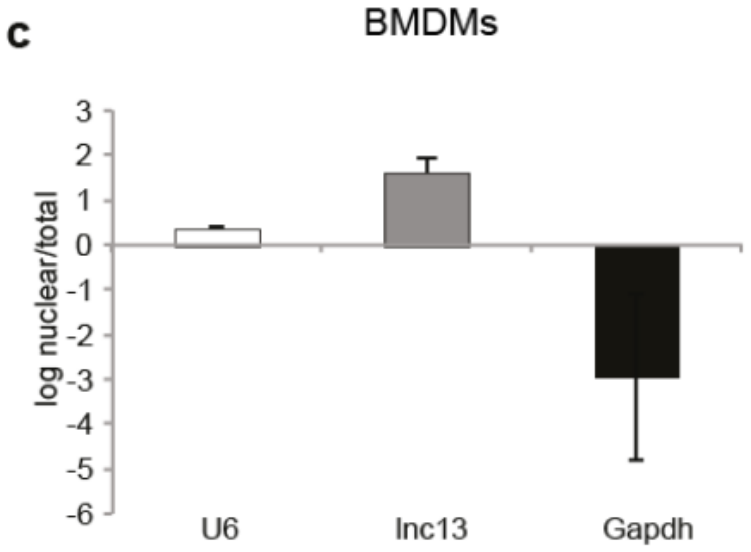


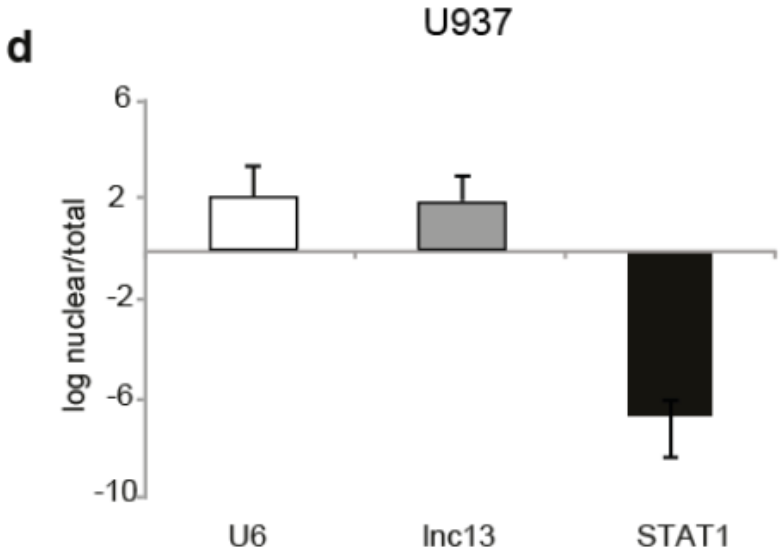
Lnc13 expression is higher in healthy donor respect to Celiac patients, while IL18RAP is low





Subcellular localization of lnc13 is primarily nuclear in both mouse and human macrophages





mouse macrophages

human macrophages

In situ hybridization on small intestinal biopsies showed nuclear localization of lnc13 in the mononuclear cells of the lamina propria

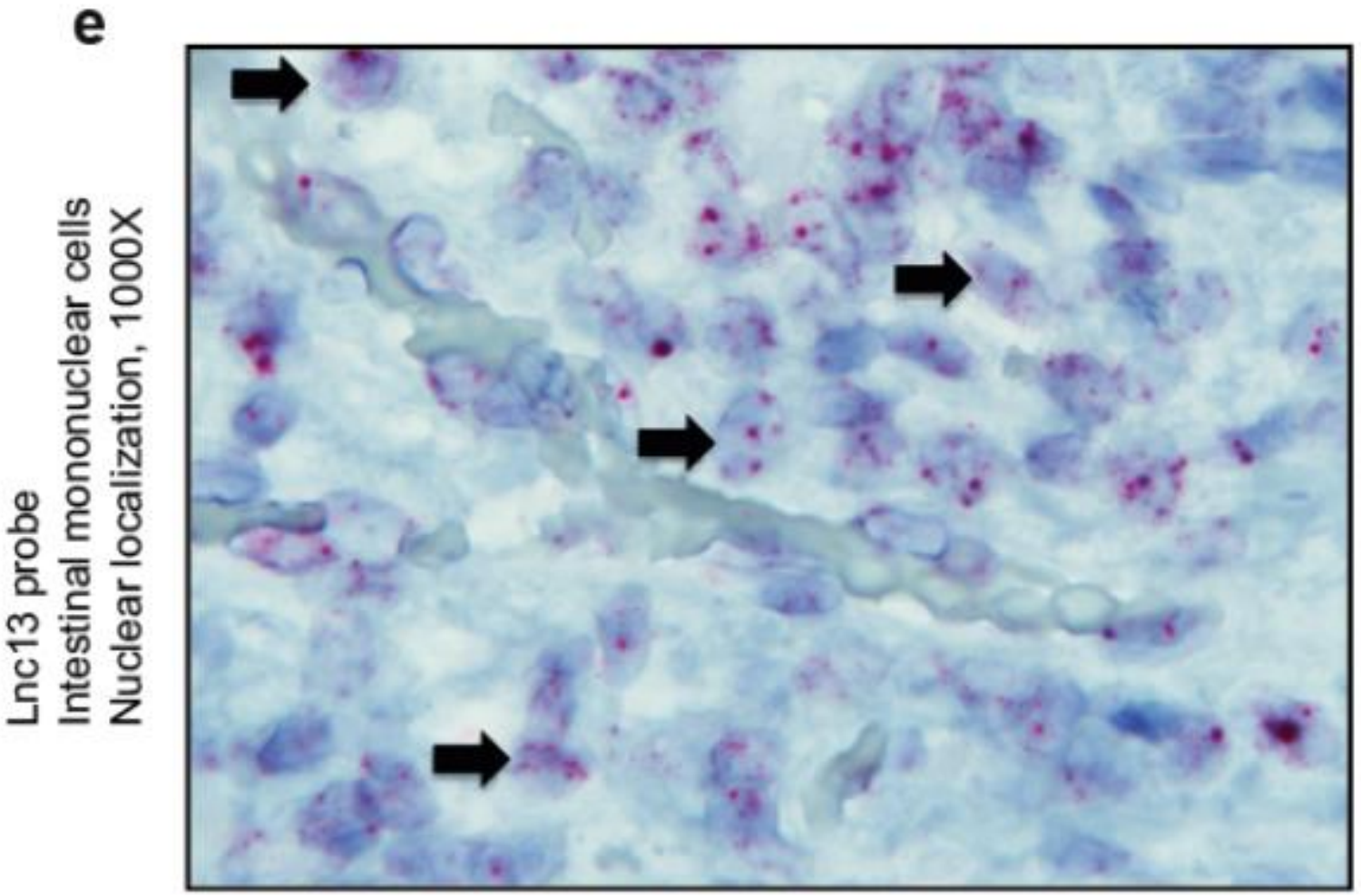
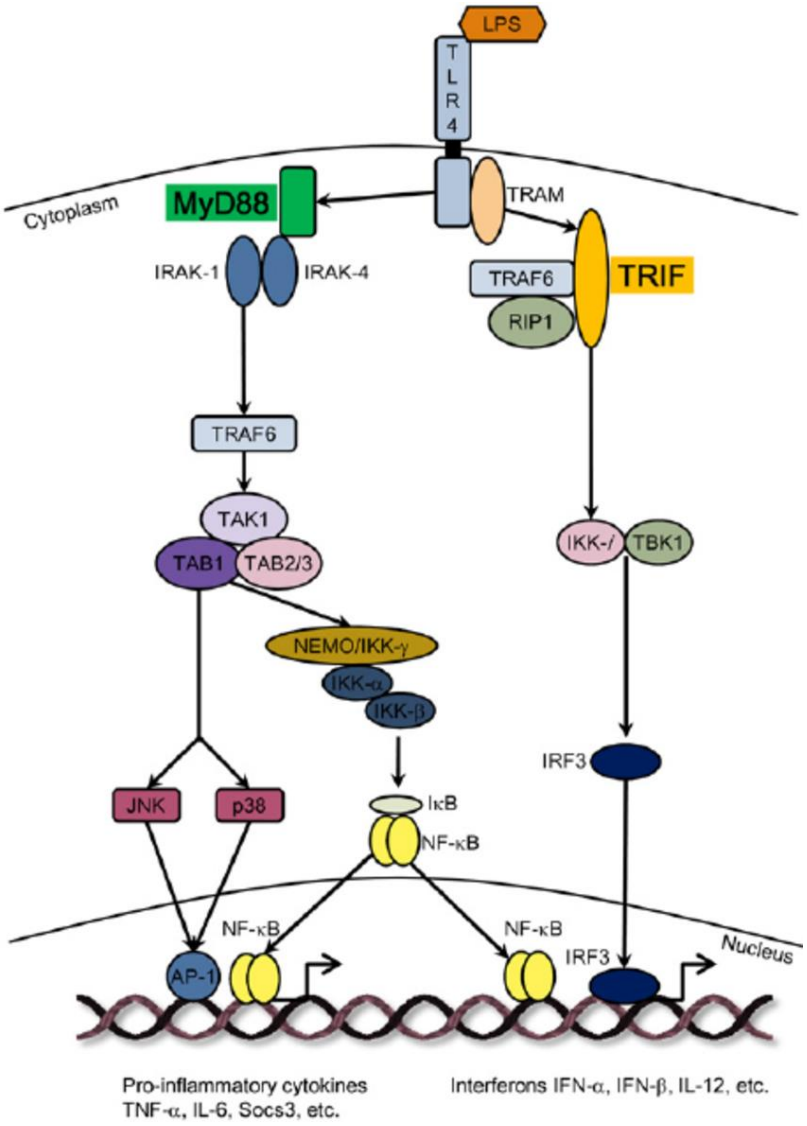


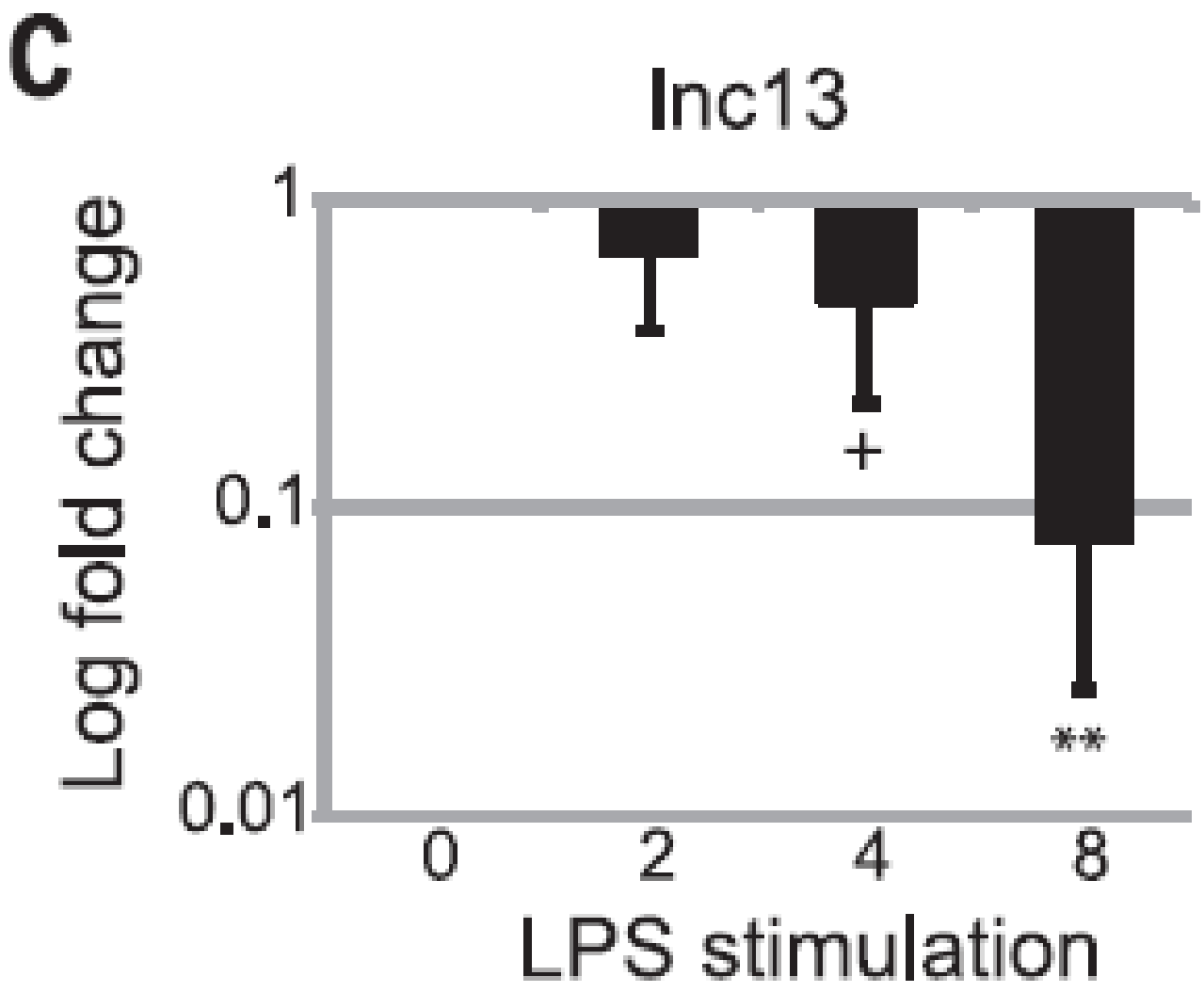
fig. S4E

How lnc13 is modulated in the inflammatory pathway

LPS pathway induces NF-kB activation via MyD88



The level of lnc13 decreased significantly upon LPS stimulation



Myd88 silencing increased lnc13 expression upon LPS treatment, LPS-induced IL12 is inhibited by siMyd88

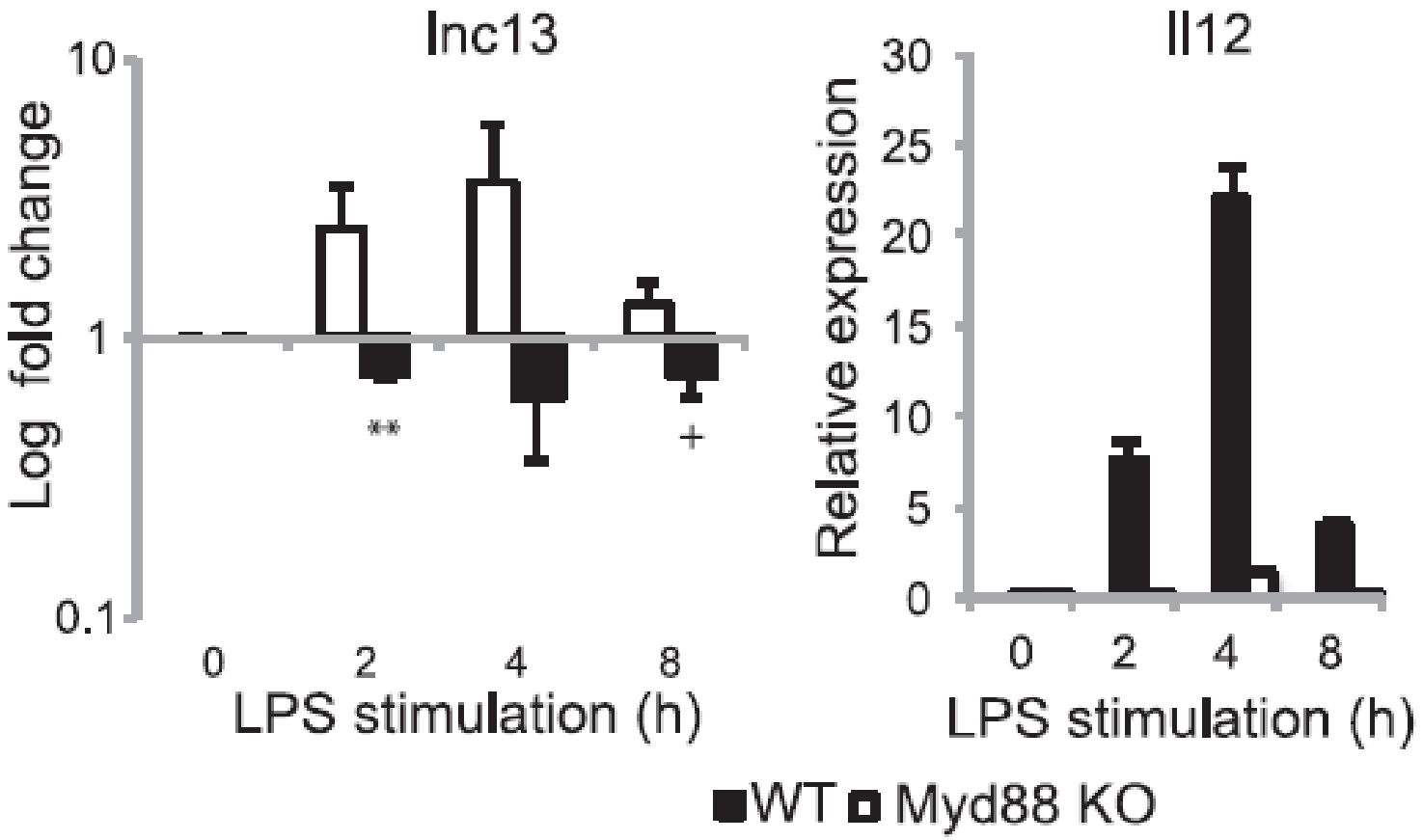
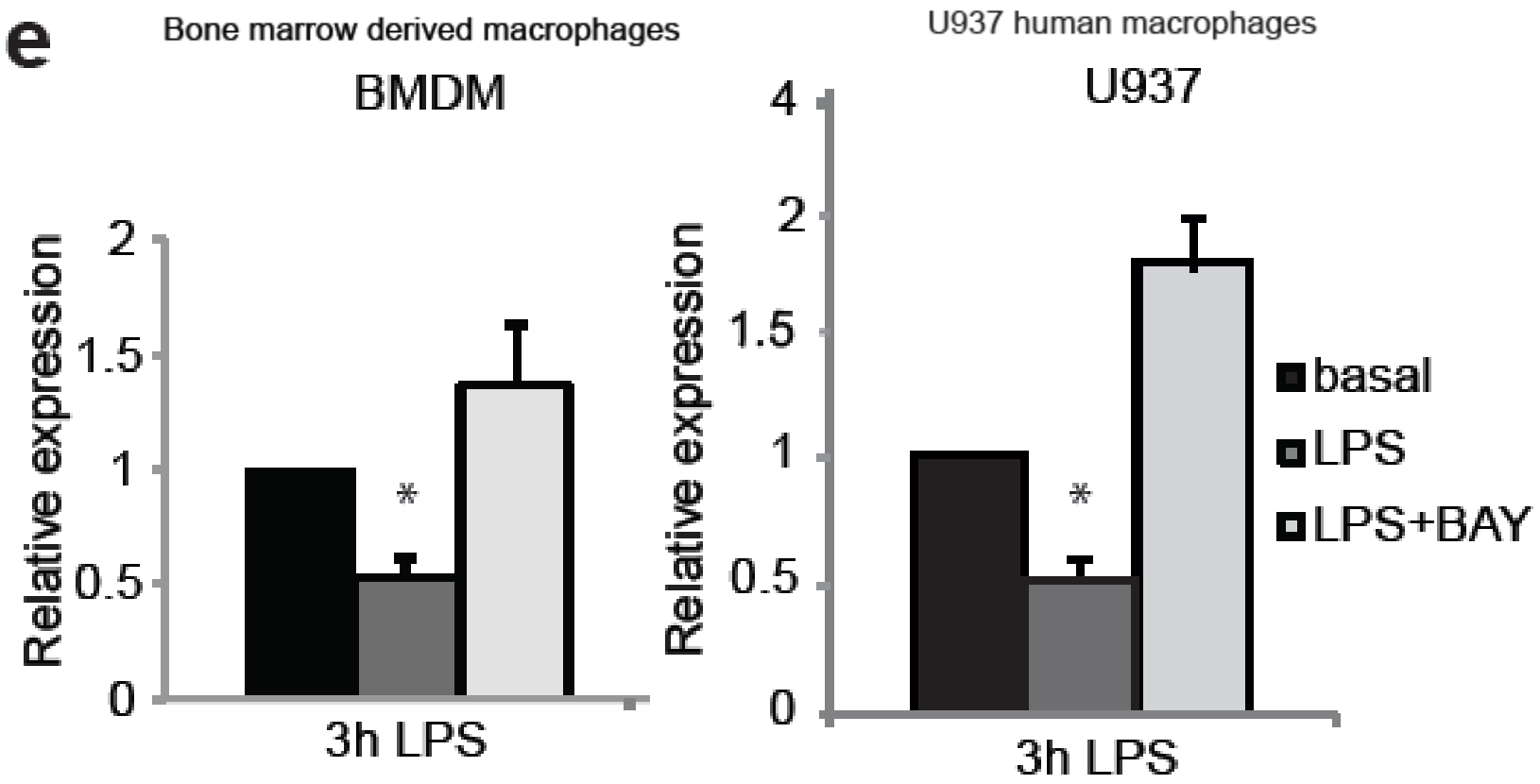


Fig. 1D

LPS induced lnc13 downregulation is blocked after NF-κB inhibition by BAY-11-7082 in mouse and human macrophages.



To identify the Lnc13 TARGET GENES,

gene expression analysis upon LPS stimulation

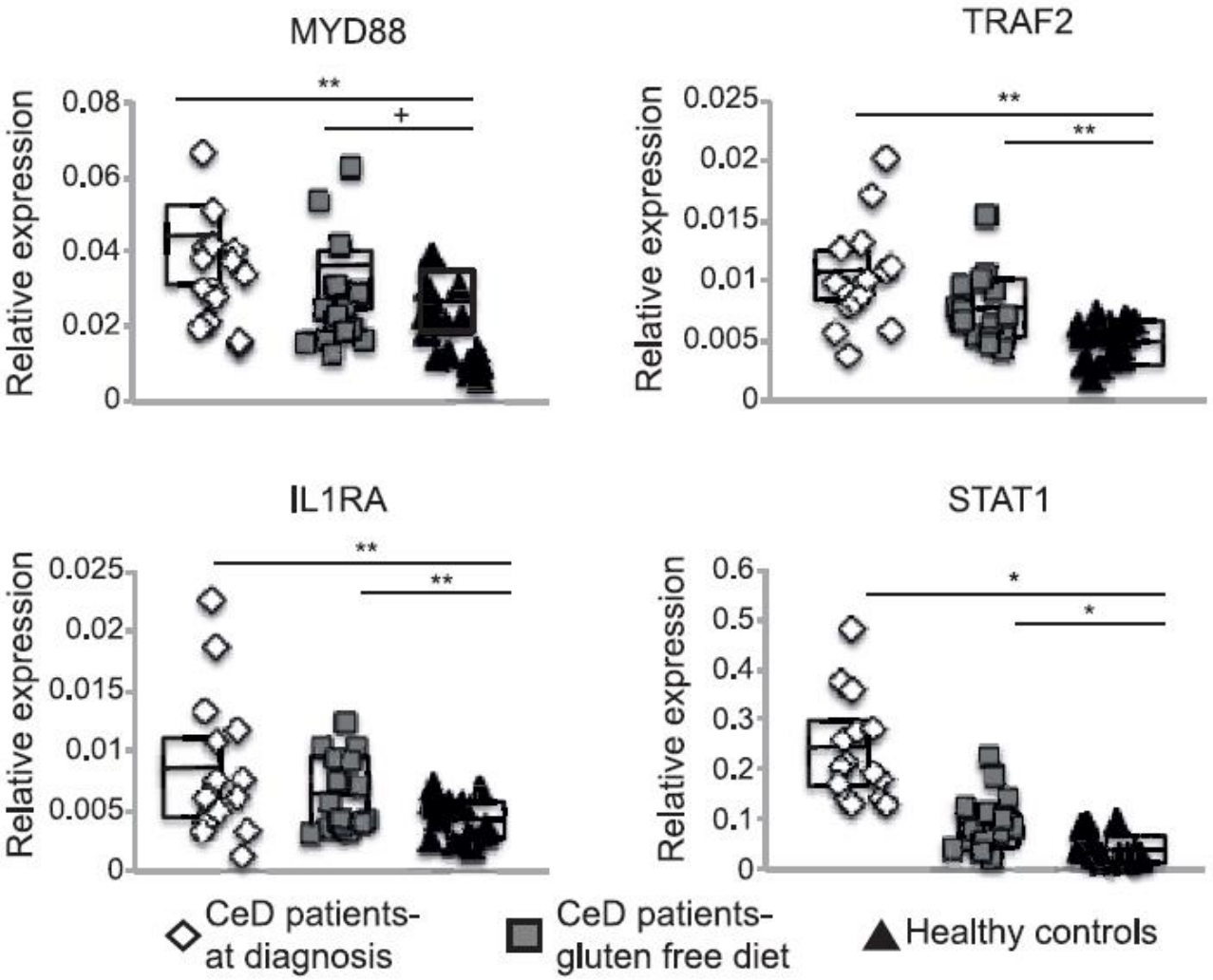
and selection of inflammatory pathway genes

The expression pattern of the Traf2, Stat1, Stat3, Tnfsf10, Il2ra, Ccl12, Myd88, Csf3, and Il1ra genes inversely correlated with changes in lnc13

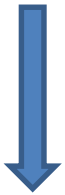
Heat map of the differential expression of a cluster of NF-kB–regulated genes that exhibit expression kinetics highly correlated with lnc13 expression kinetics in response to LPS stimulation (correlation coefficient R > 0.8) of BMDMs.

Fig. 2A

Expression of four of these genes (TRAF2, STAT1, IL1RA, and MYD88) was significantly increased in biopsies from CeD patients



Lnc13 affects the expression of genes involved in inflammatory pathway TRAF2, STAT1, IL1RA, and MYD88



In order to demonstrate that Lnc13 is a modulator of genes TRAF2, STAT1, IL1RA, and MYD88 is used two approaches:

a) **Overexpression**
b) **Downregulation**

Increased levels of lnc13 reduced the expression of the predicted targets

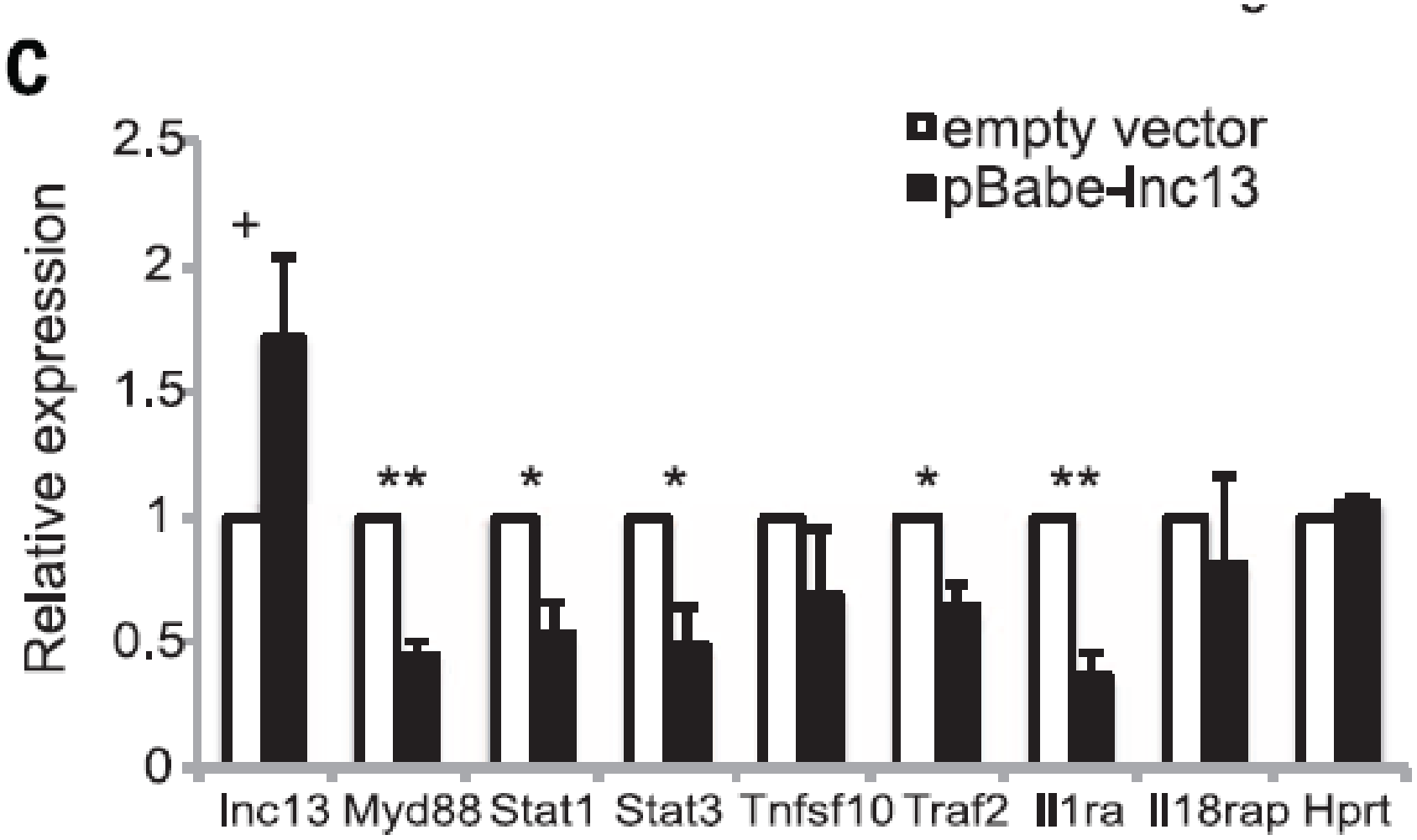


Fig. 2C

Silencing of lnc13 increased the expression of the predicted targets

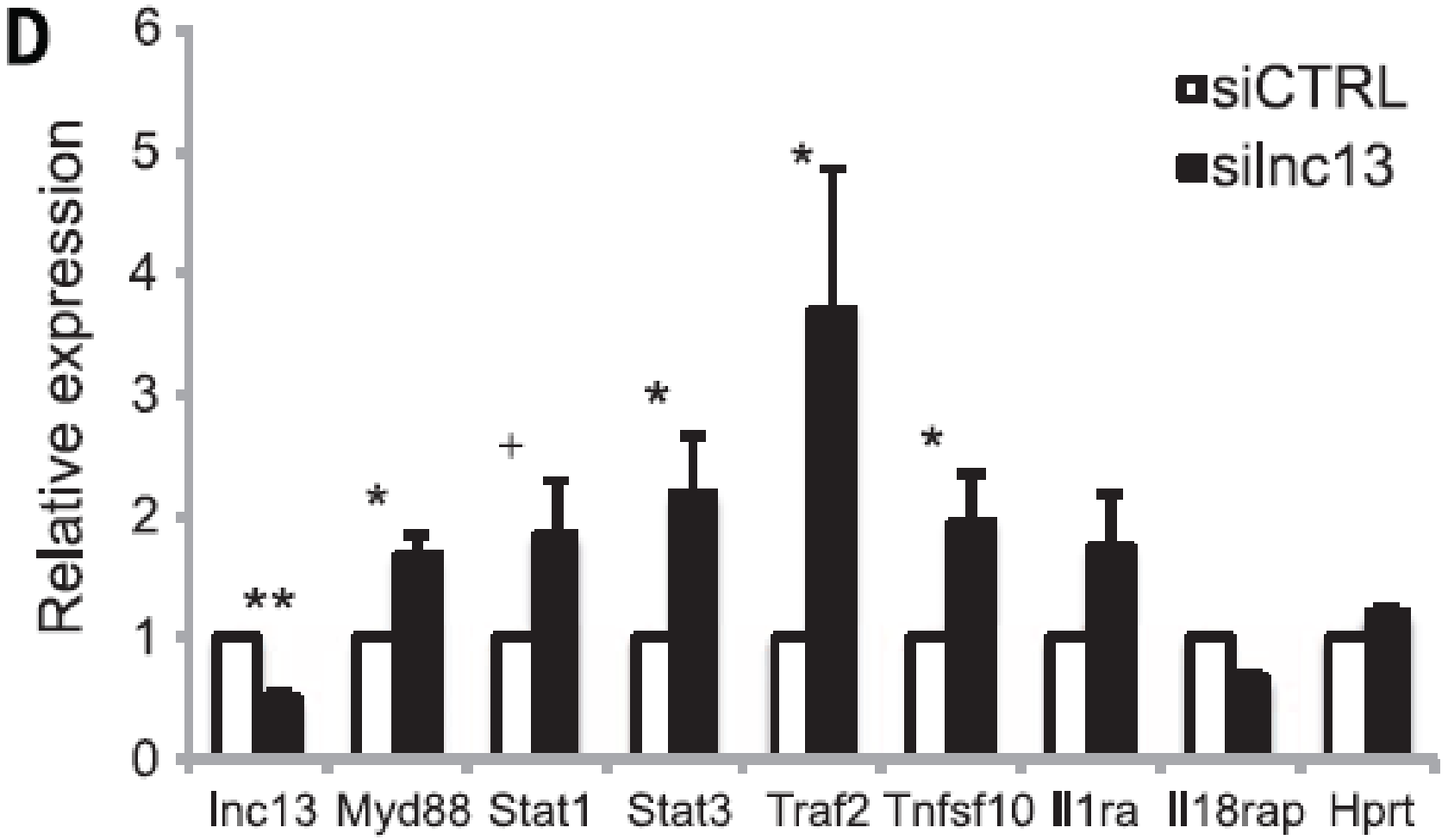


Fig. 2D

IN SUMMARY

- **Lnc13 is a long non coding RNA associated with Celiac Disease**

- **Lnc13 decreased upon inflammatory pathway activation**

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**