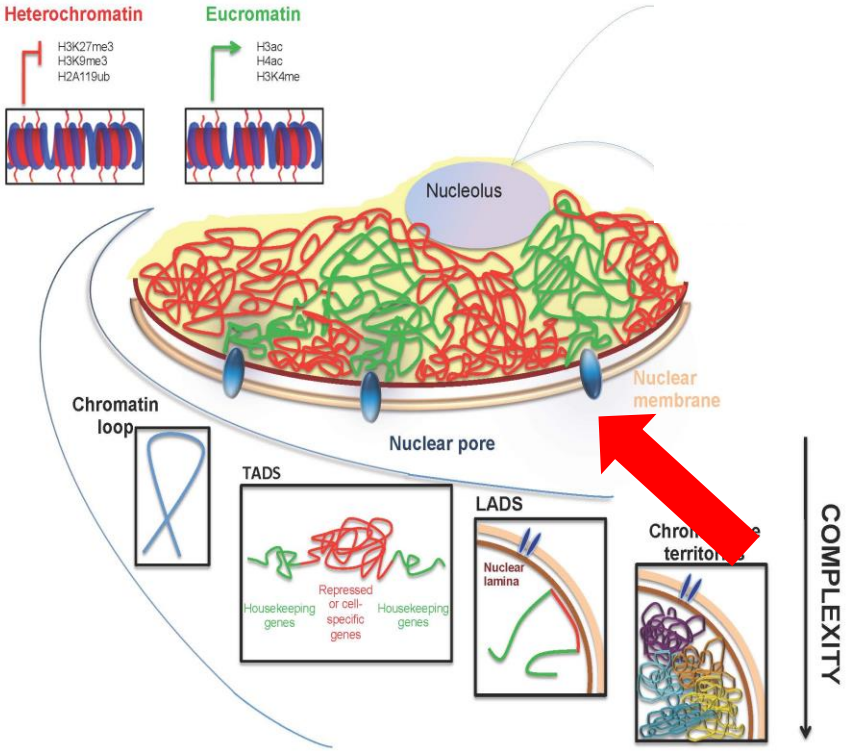
Single nucleotide variants in:

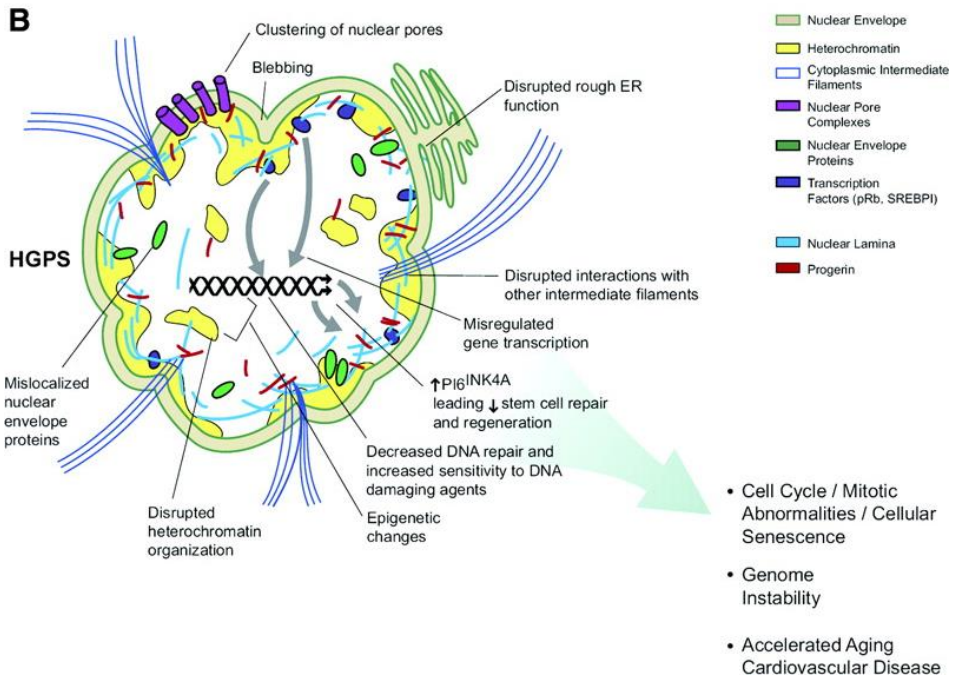
- Splicing sites

- Long non coding transcript

- Single cell approach

SINGLE NUCLEOTIDE VARIATIONS ASSOCIATED WITH PROTEIN IMPORTANT IN CHROMATIN ORGANIZATION, LAMININ, INDUCES LAMINOPATHIES

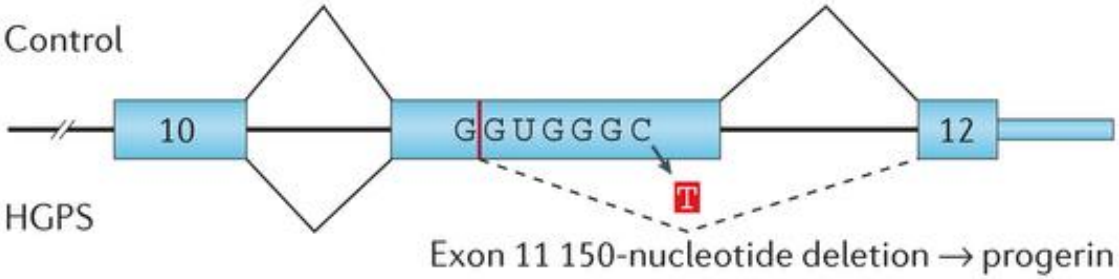


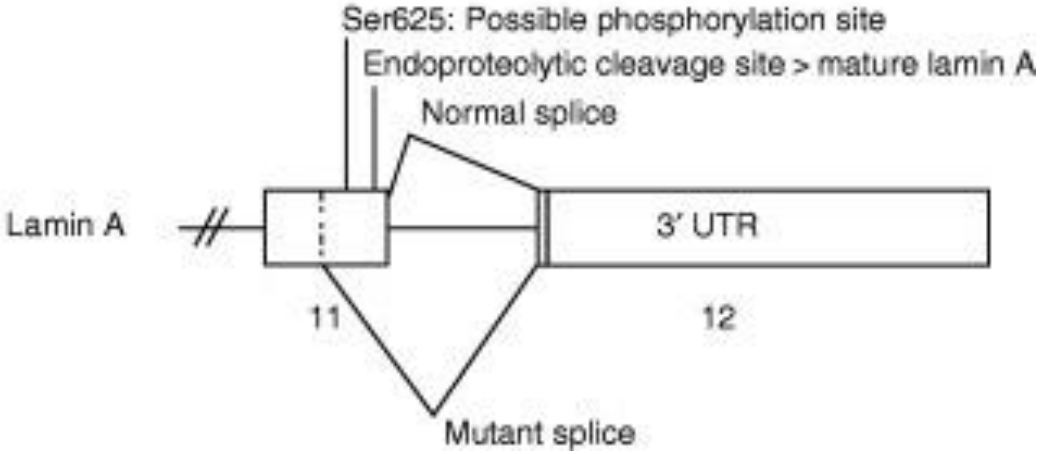


A splicing **defect in exon 11 of the LMNA** gene results in the **150 nucleotides** deletion in exon11 of Lamin A, creating protein lacking 50 amino acids of the carboxy-terminal globular domain.

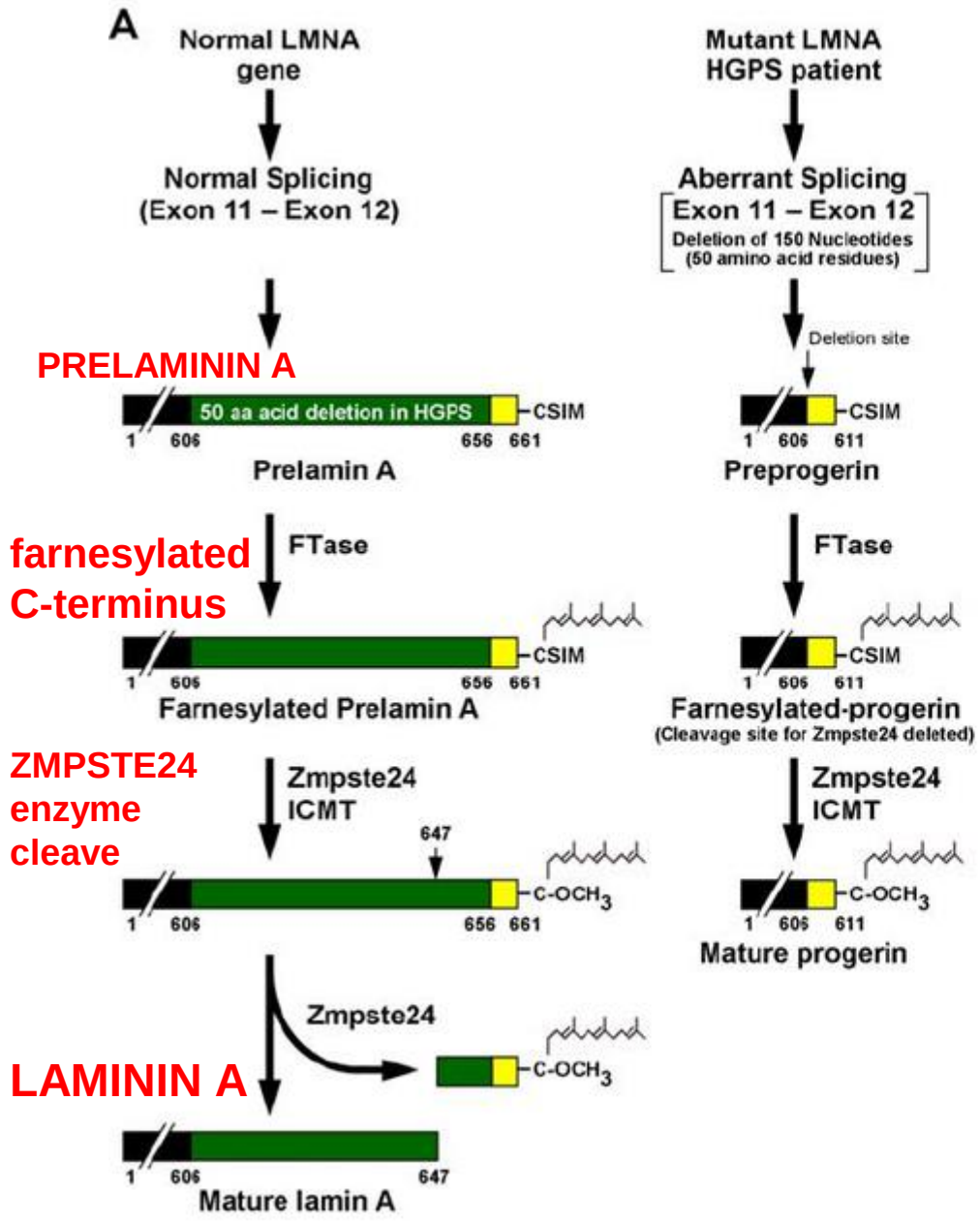
Hutchinson-Gilford progeria syndrome (HGPS)

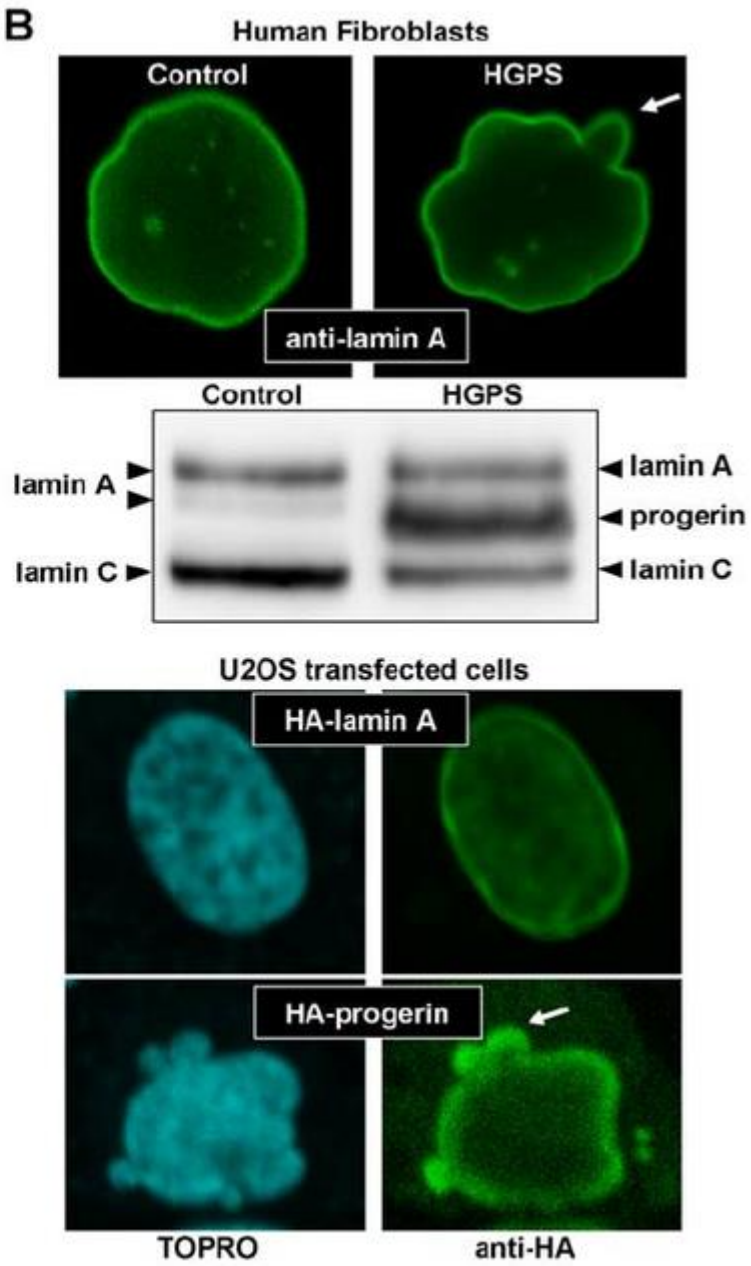
Alternative 5′ss (c. 1824C>T)



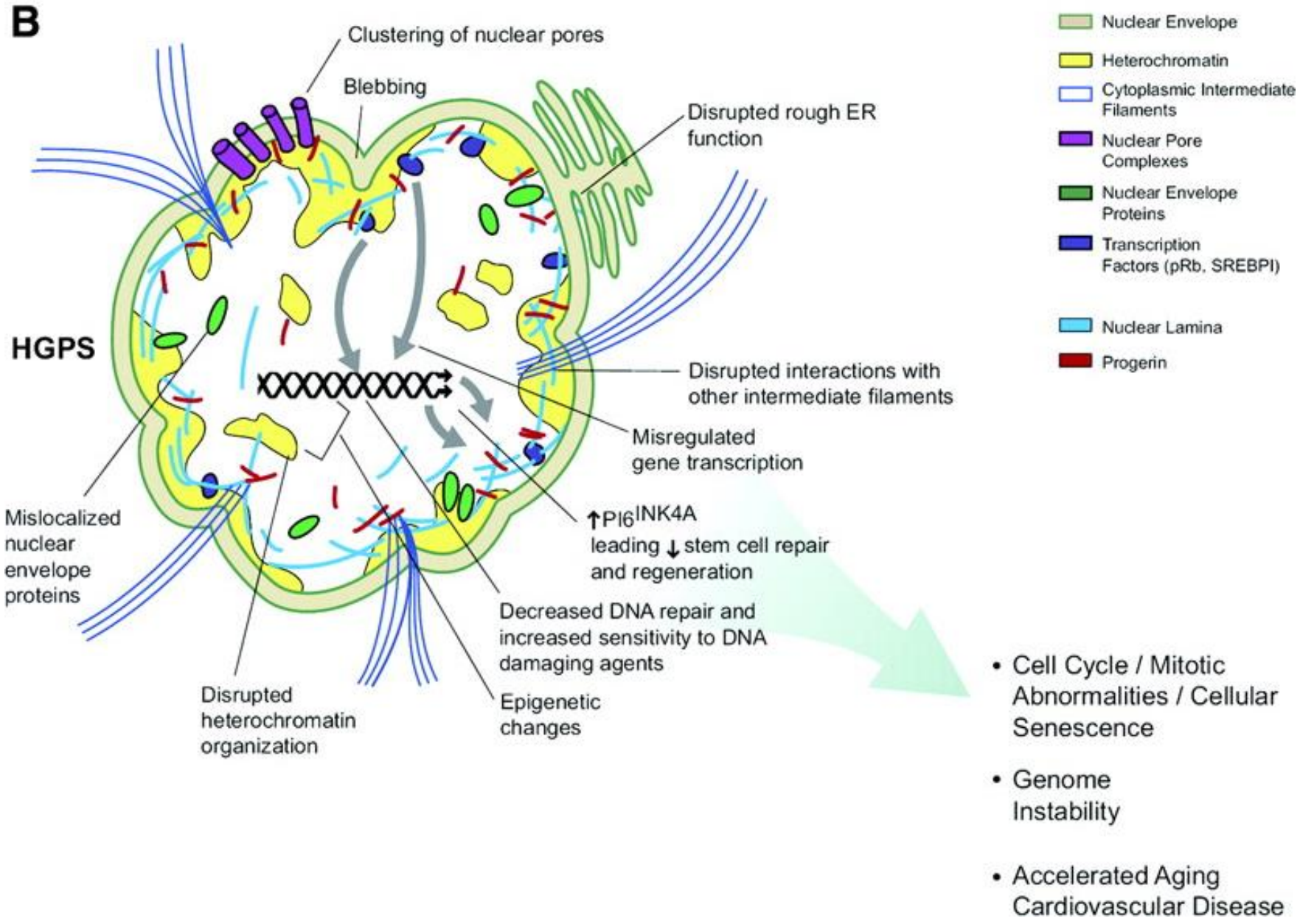


Loss of cleavage site for protease

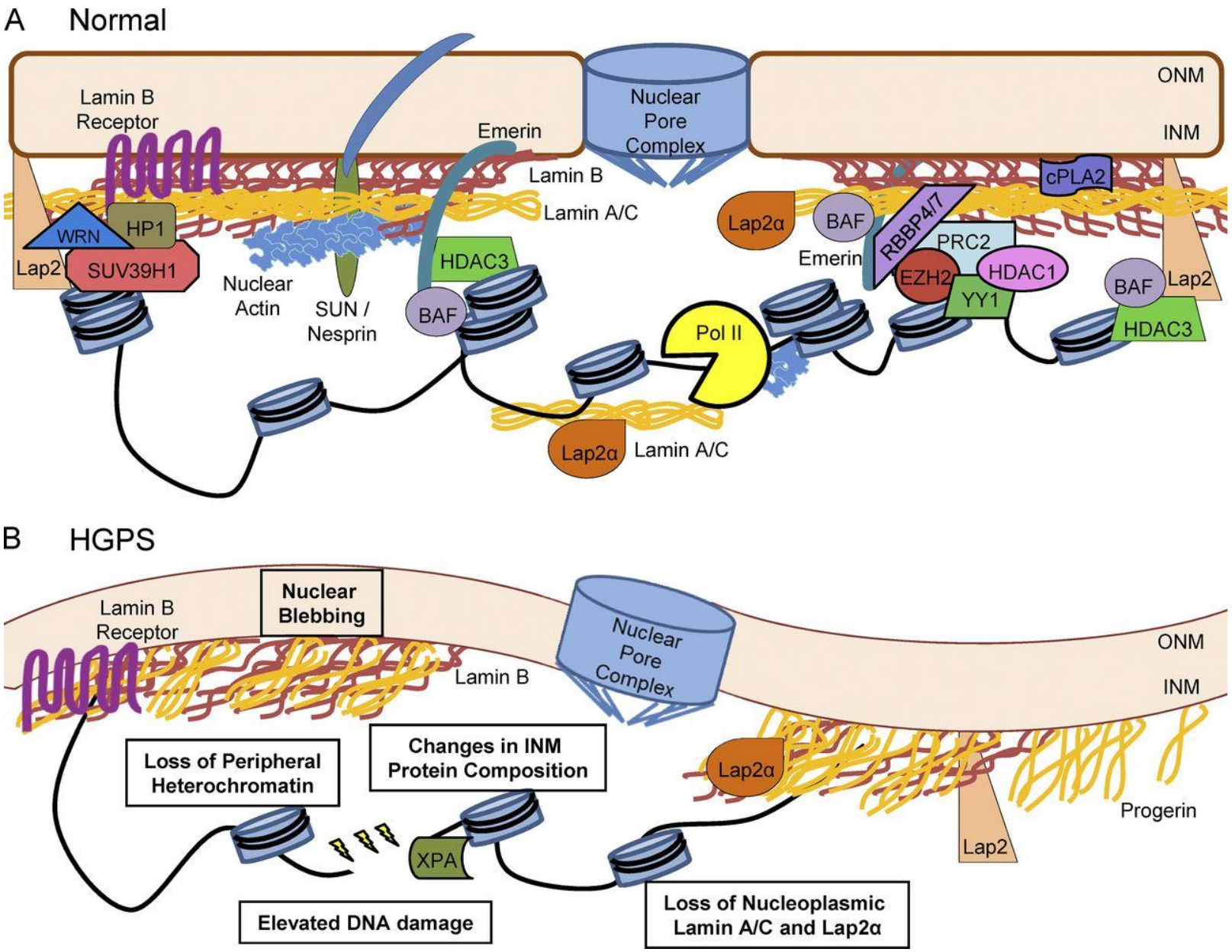

A
Normal LMNA gene
Normal Splicing (Exon 11 – Exon 12)
PRELAMININ A
50 aa acid deletion in HGPS
CSIM
1 606 656 661
Prelamin A
farnesylated C-terminus
FTase
CSIM
1 606 656 661
Farnesylated Prelamin A
ZMPSTE24 enzyme cleave
Zmpste24 ICMT
647
C-OCH3
1 606 656 661
Zmpste24
C-OCH3
LAMININ A
1 606 647
Mature 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

PROGERIA EFFECTS ON THE BIOLOGICAL FUNCTIONS

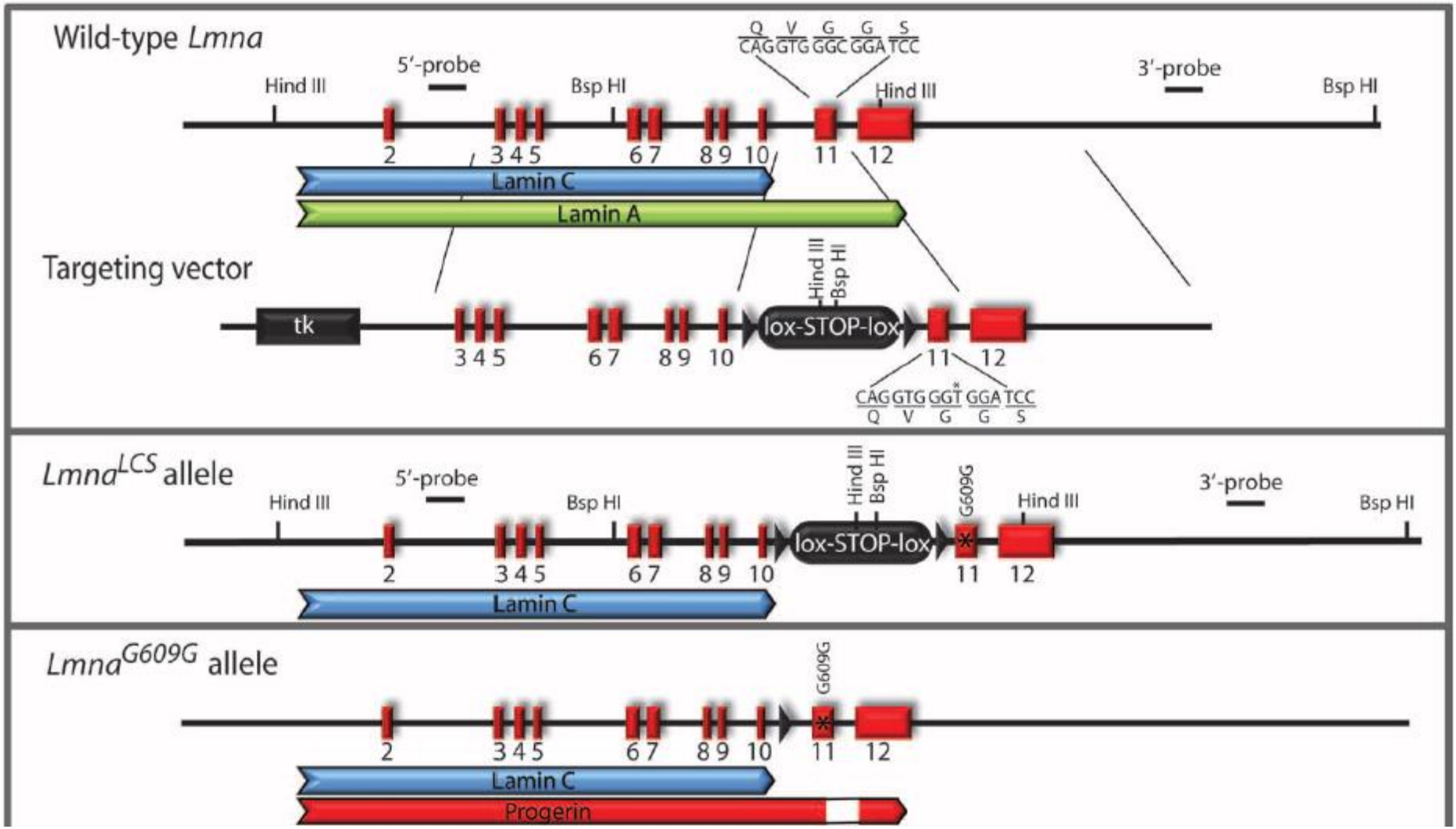


Loss of protein complexes organization in HGPS

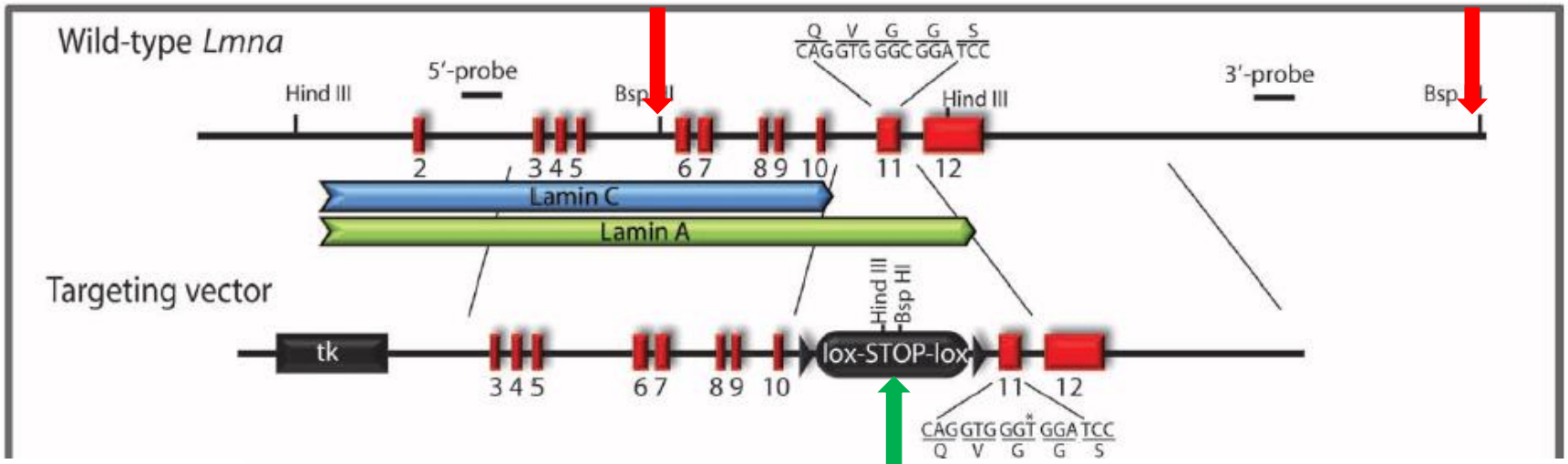


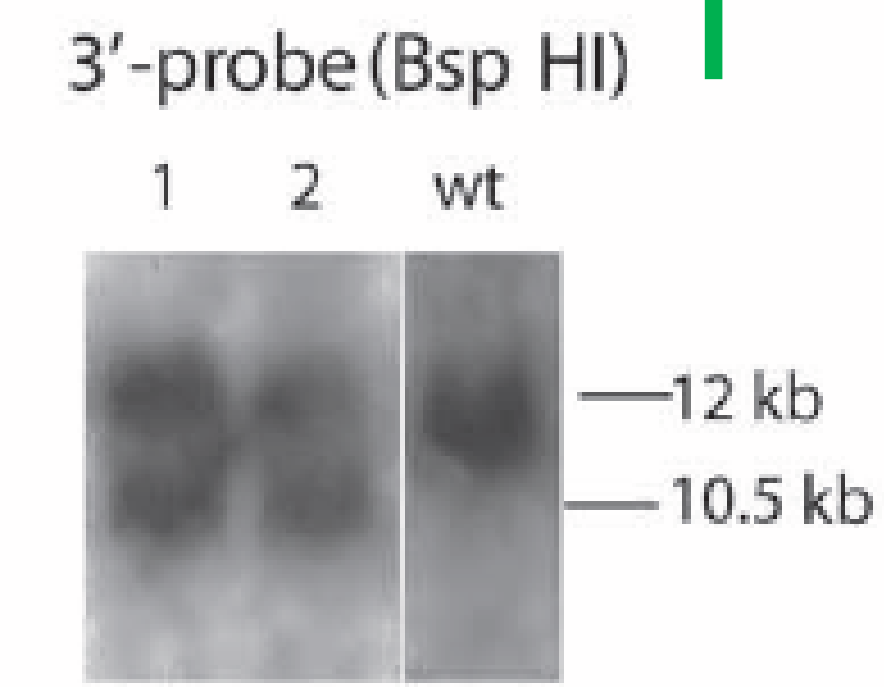
CONDITIONAL TRANSGENIC MICE

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.





In the southern blot, 12 kbp corrispondes with wt sample because there are two restriction sites in this locus. 10.5 kbp derived from an additional site in the lox sites that is used to obtain transgenic mice.

Mouse mutants are created to study the role of progerin mutations. The authors verified the genomic mutations in the trangenic models. Which is the technique that is used and why are there two bands?

This is the scheme of mutant constructs. Bsp H1 is the restriction enzyme that is used for southern blot analysis and 3'-probe marks DNA fragments.

Why do heterozygous samples, 1 and 2, have two bands?

Select one:

a.
3'-probe marks an aspecific band in heterozigous

b.
Three restriction enzymes sites: two at the exon11 flanking regions and one at lox region.

c. 3' probe recognizes one band for exon11 and one band for lox site ✗

d.
Three restriction enzymes sites: two at the exon3 flanking regions and one at exon11 region.

e.
Two restriction enzymes at exon11 regions creates two fragments marked by 3'-probe

Your answer is incorrect.

The correct answer is:

Three restriction enzymes sites: two at the exon11 flanking regions and one at lox region.

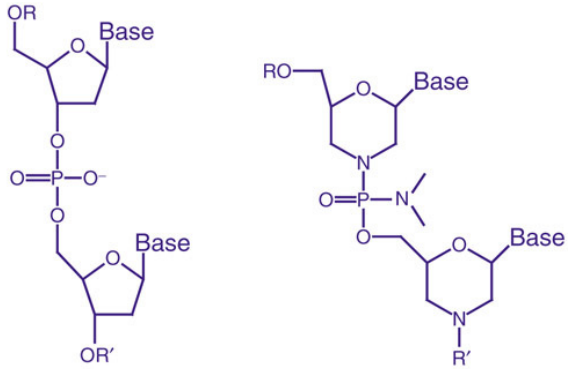
MORFOLINO

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**,

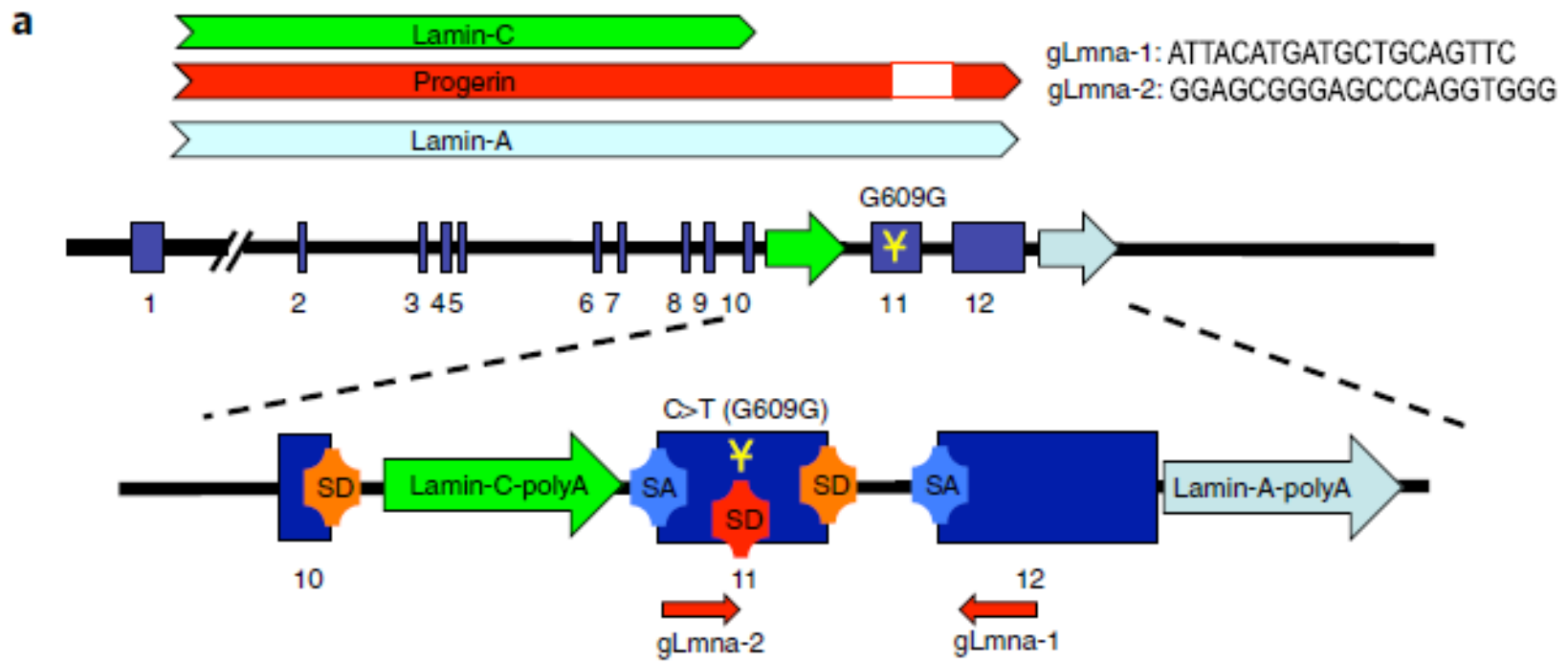
phosphorodiamidate morpholino oligomers (PMOs or Morpholinos) do not result in degradation of their target RNA: not recognized by cellular nucleases.



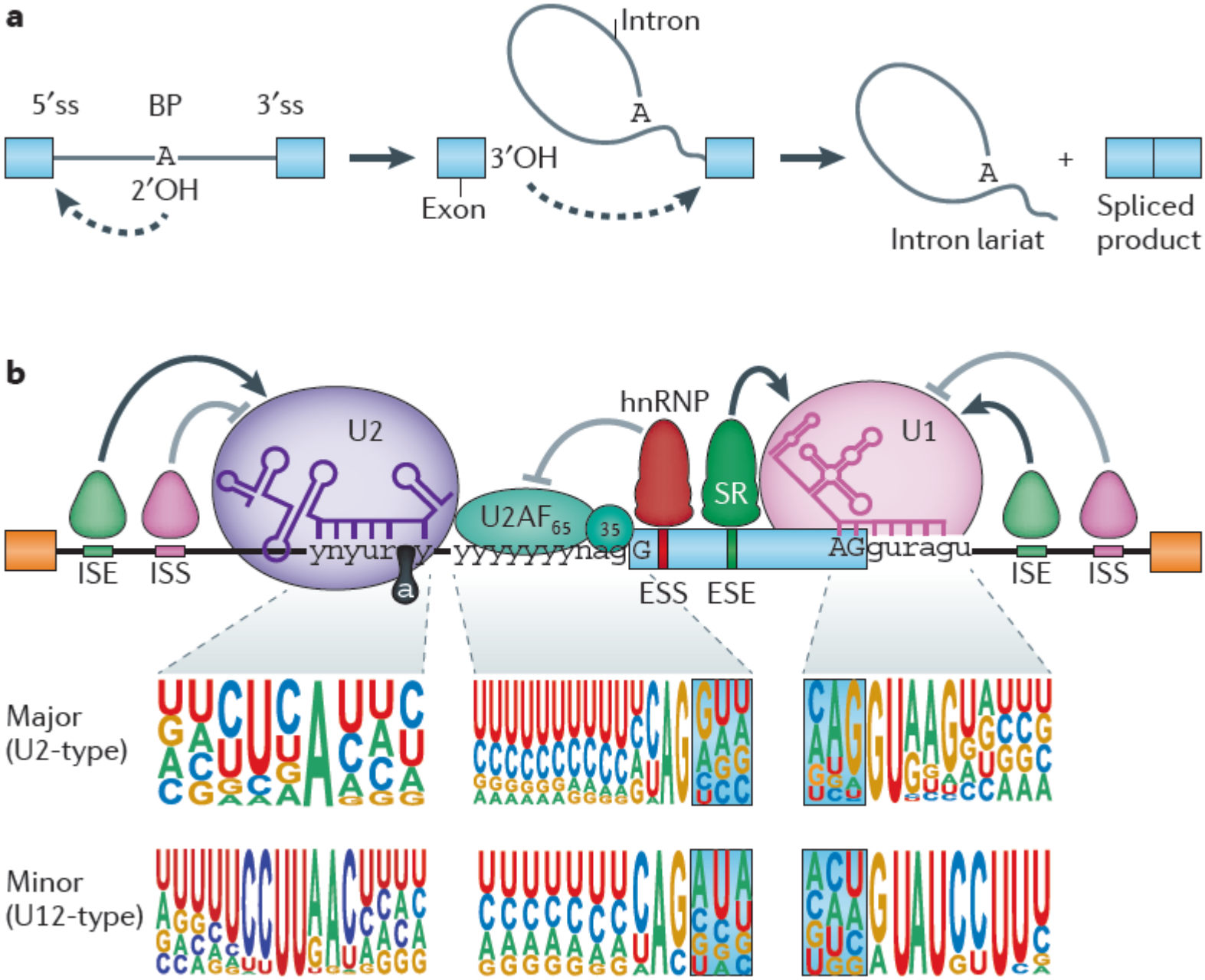
Phosphodiester DNA

Morpholino

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.**



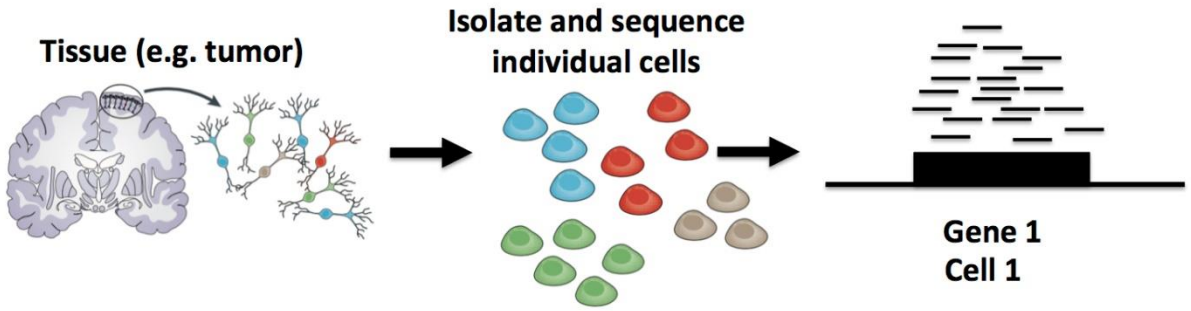
RNA SPLICING REGULATION



Single-cell genomics

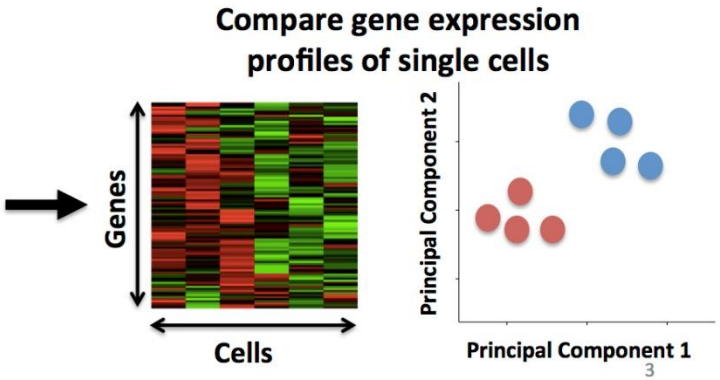
is opening up new ways to tackle these questions
by combining the **comprehensive nature of genomics** with the microscopic resolution that is required to describe complex multicellular systems.

Single-cell RNA-Seq (scRNA-Seq)

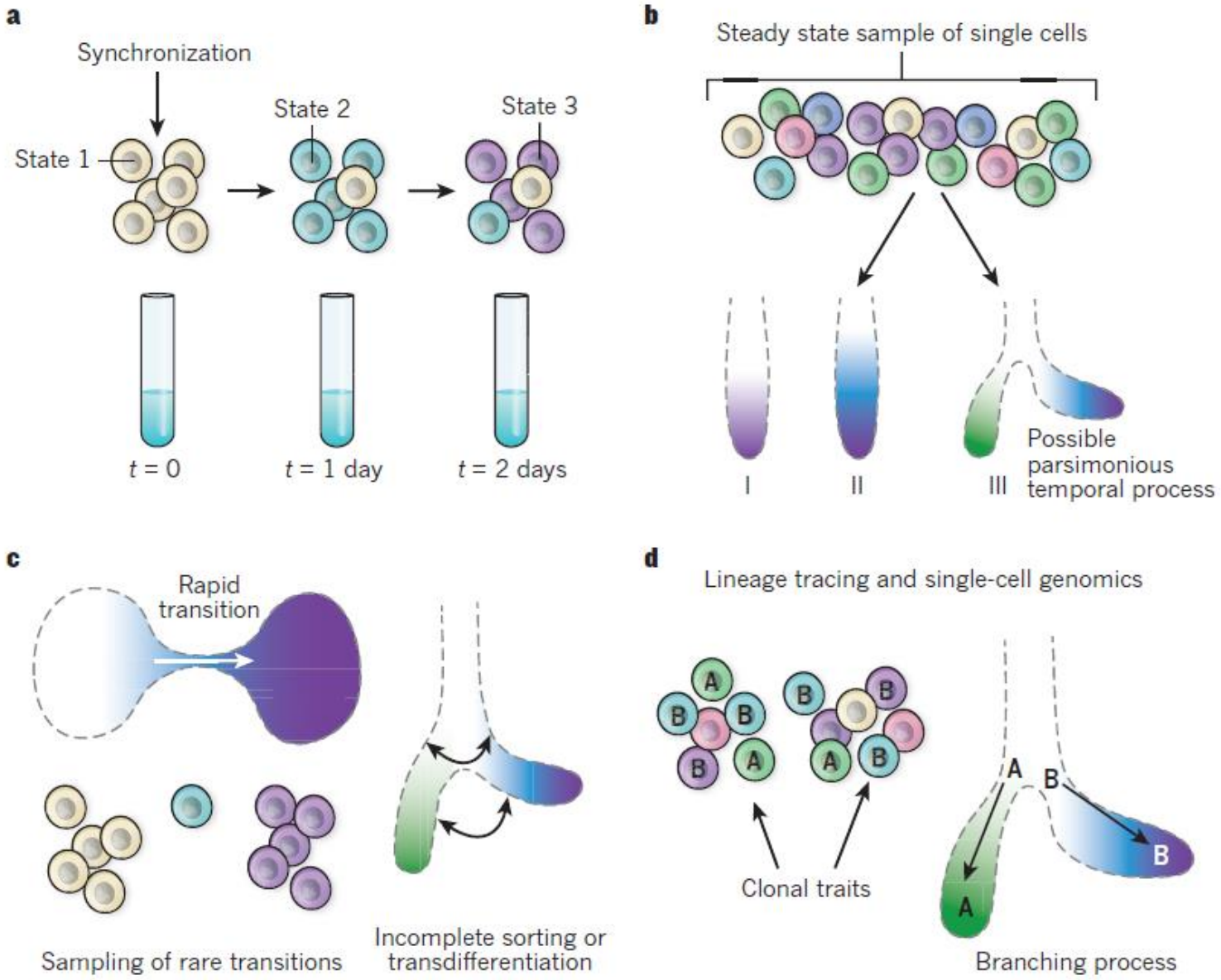


Read Counts

	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

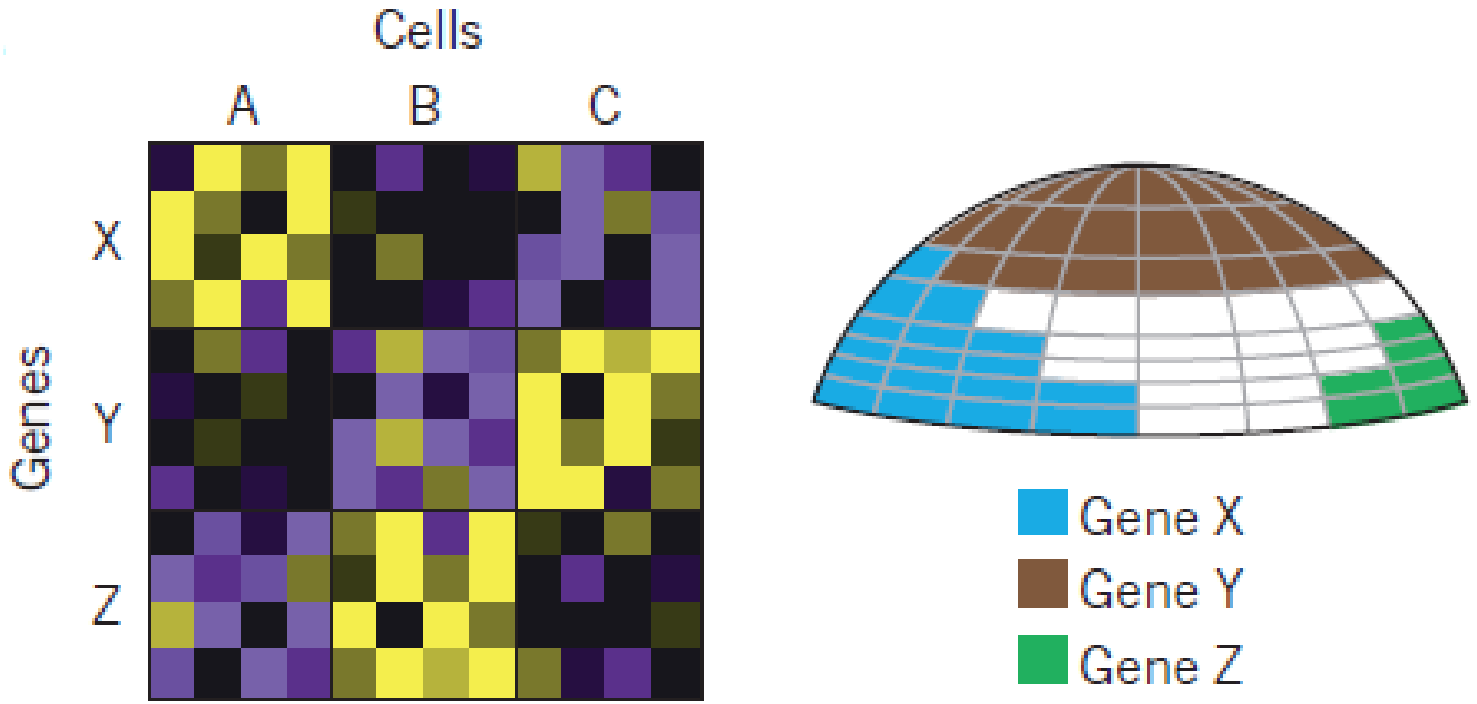


TEMPORAL AXIS



SPATIAL AXIS

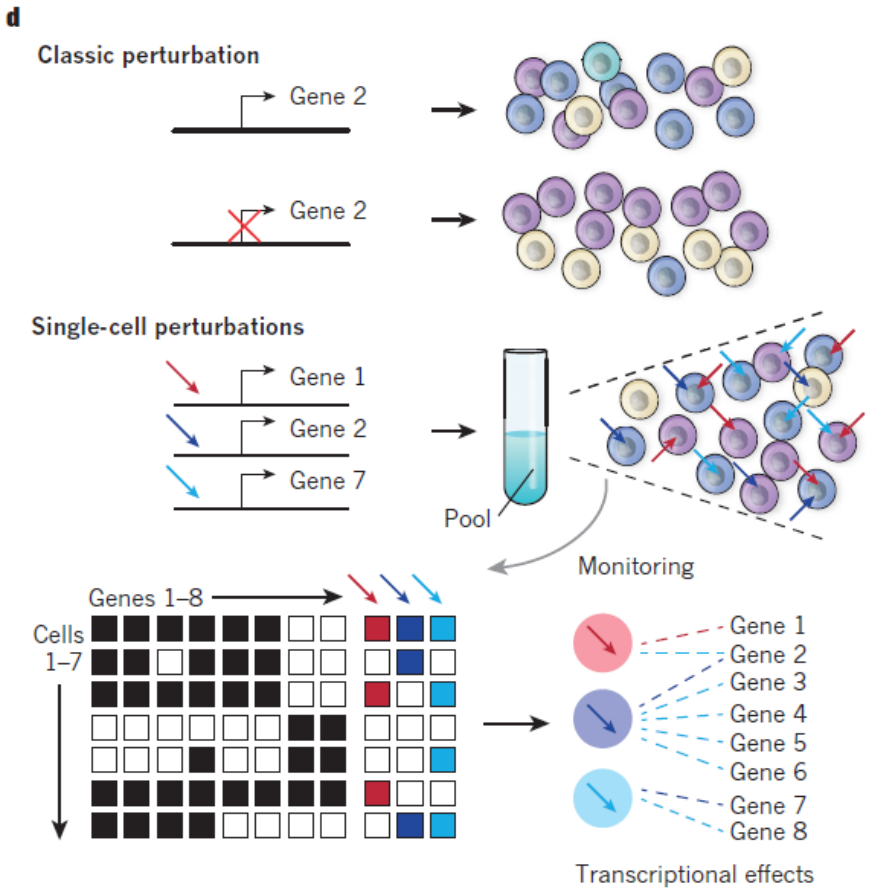
Spatial mapping uses single-cell gene-expression profiles and a reference map of the spatial expression patterns of a small number of landmark genes as input. The expression profile of the landmark genes in a cell is used to determine its spatial position. **(Yellow indicates induced expression**, **purple shows repressed expression** and **black indicates no change in expression**.)



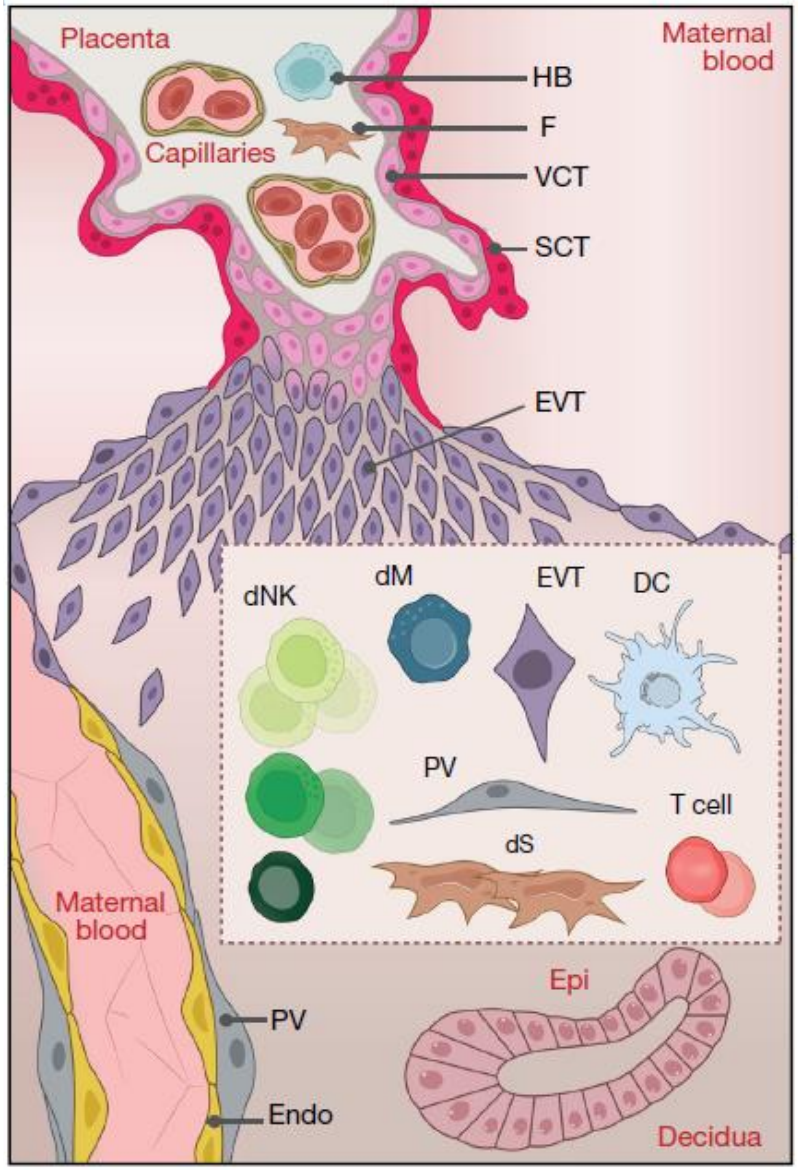
MECHANISM AXIS

Combining single-cell genomics with experimental perturbations of the system of interest provides the most direct avenue for causal inference.

Modern high-throughput perturbation methods, especially those based on clustered regularly interspaced short palindromic repeat **(CRISPR) technology**, can be combined with single-cell genomics to perform causal analysis at an unprecedented scale and resolution.



Identification of cell types at the maternal–fetal interface.

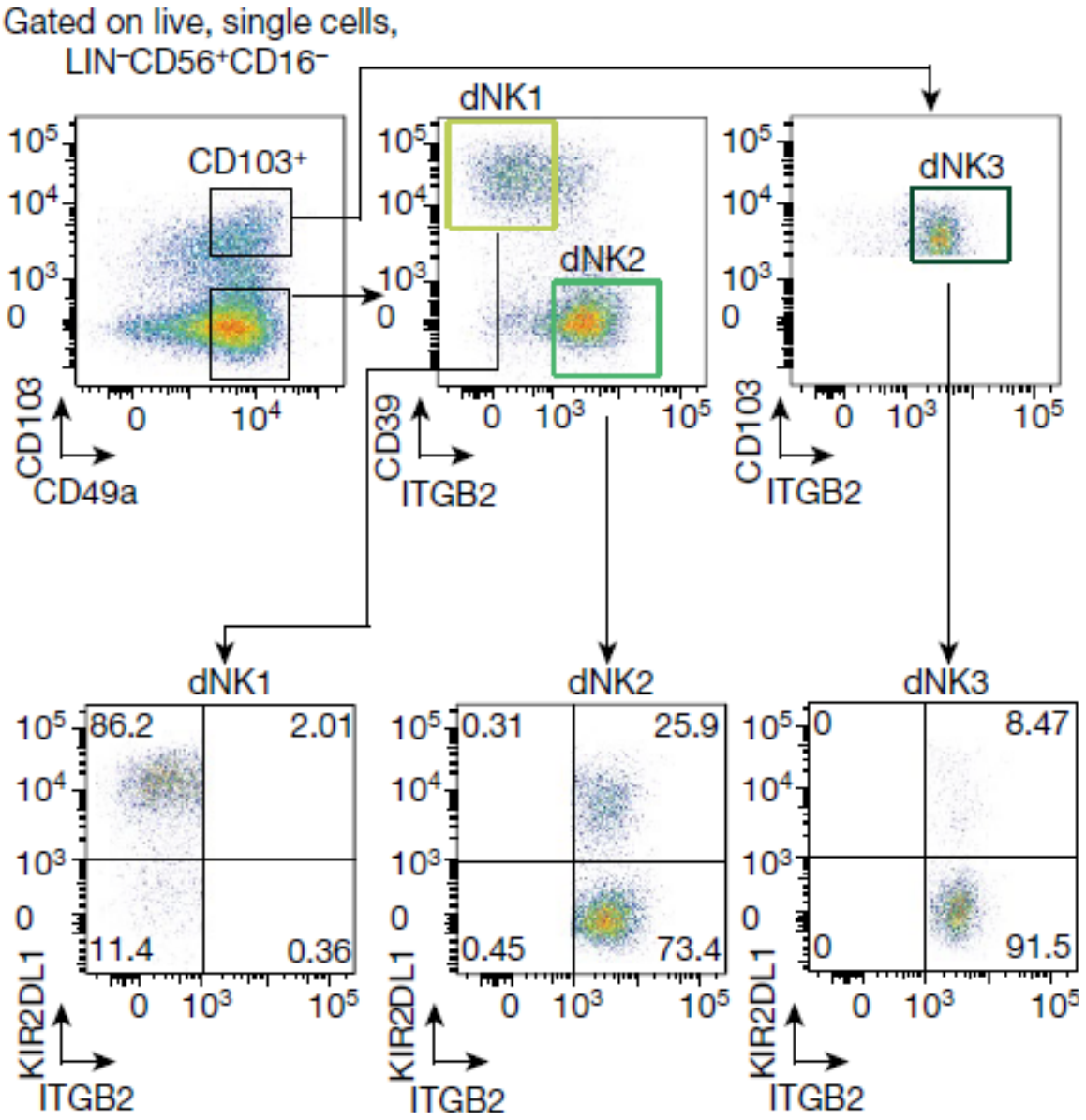


villous cytotrophoblast (VCT) cells, which line placental structures called villi;

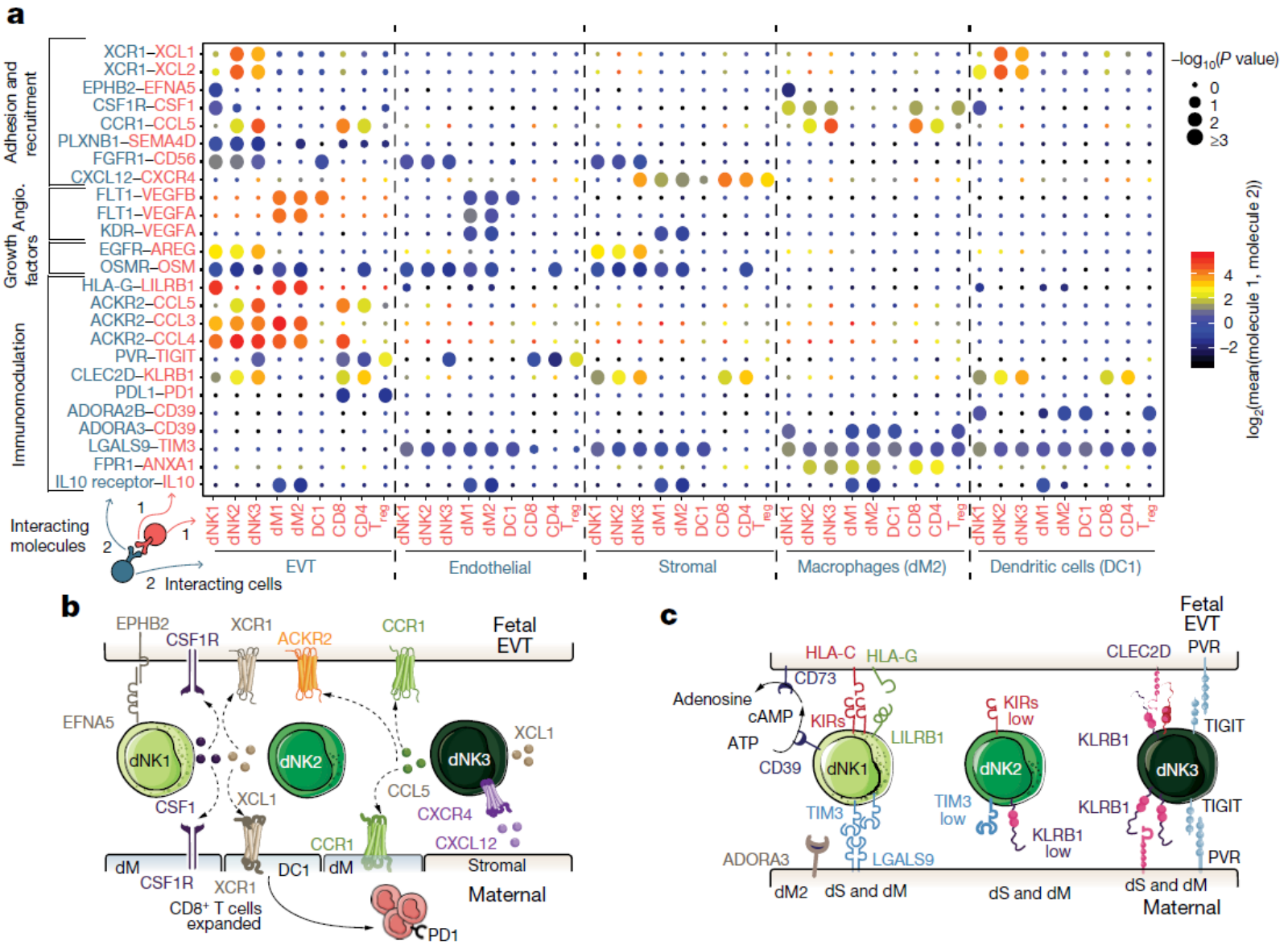
syncytiotrophoblast (SCT) cells that cover the villus surface;

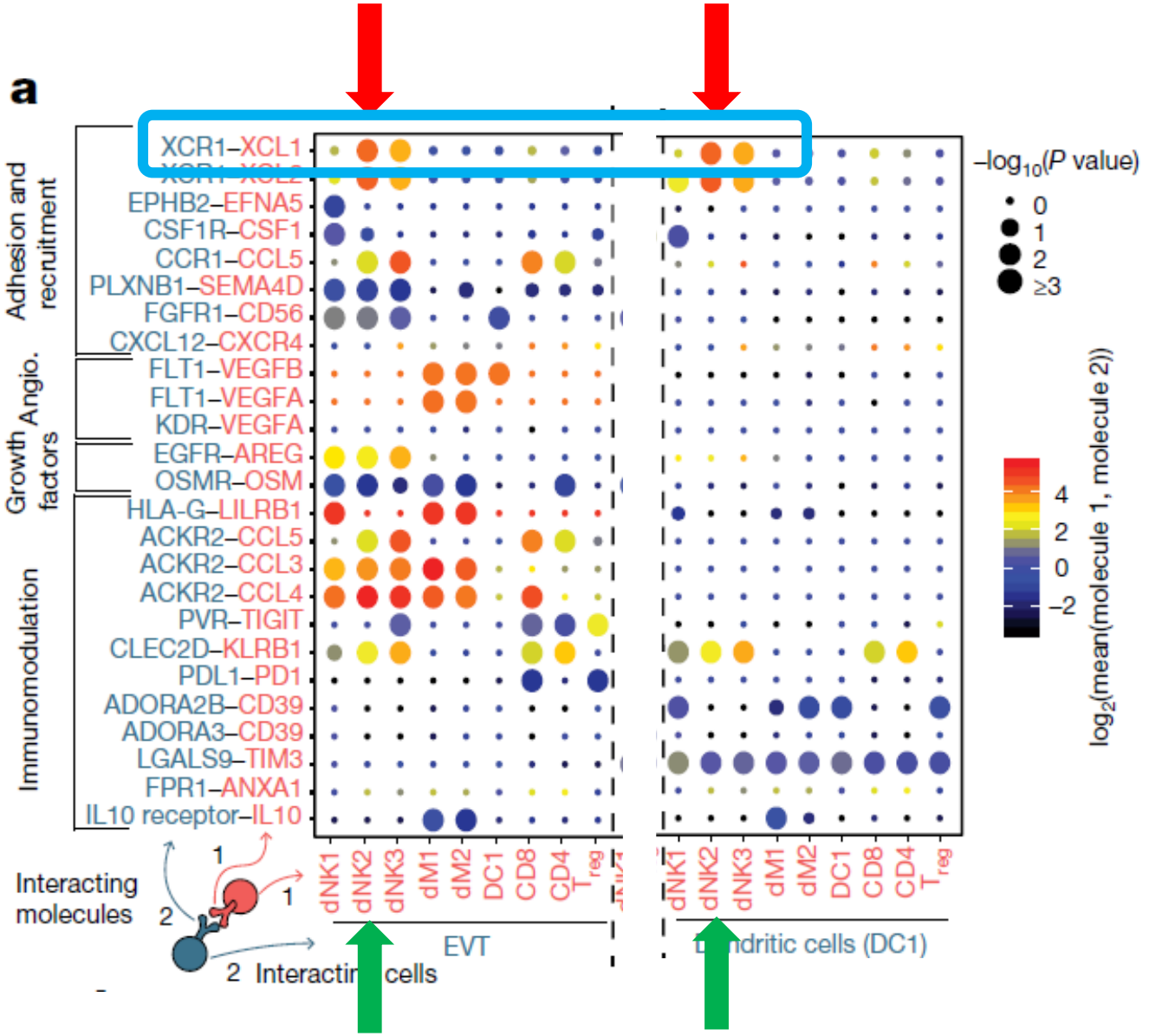
extravillous trophoblast (EVT) cells, which line the maternal blood vessels and intermingle with maternal cells in the decidua.

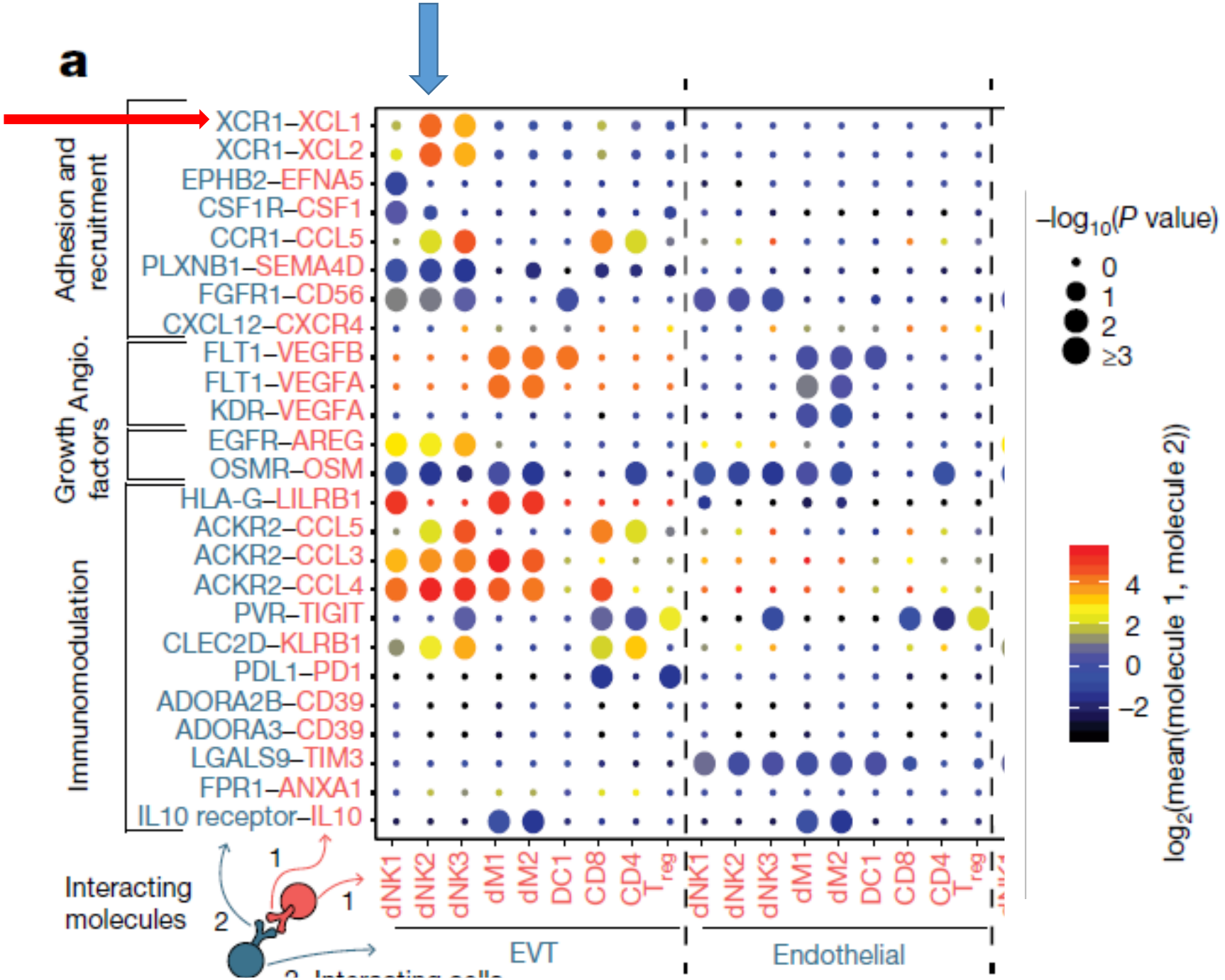
What is cell subtype with high percentage of ITGB2- overexpressed cells?

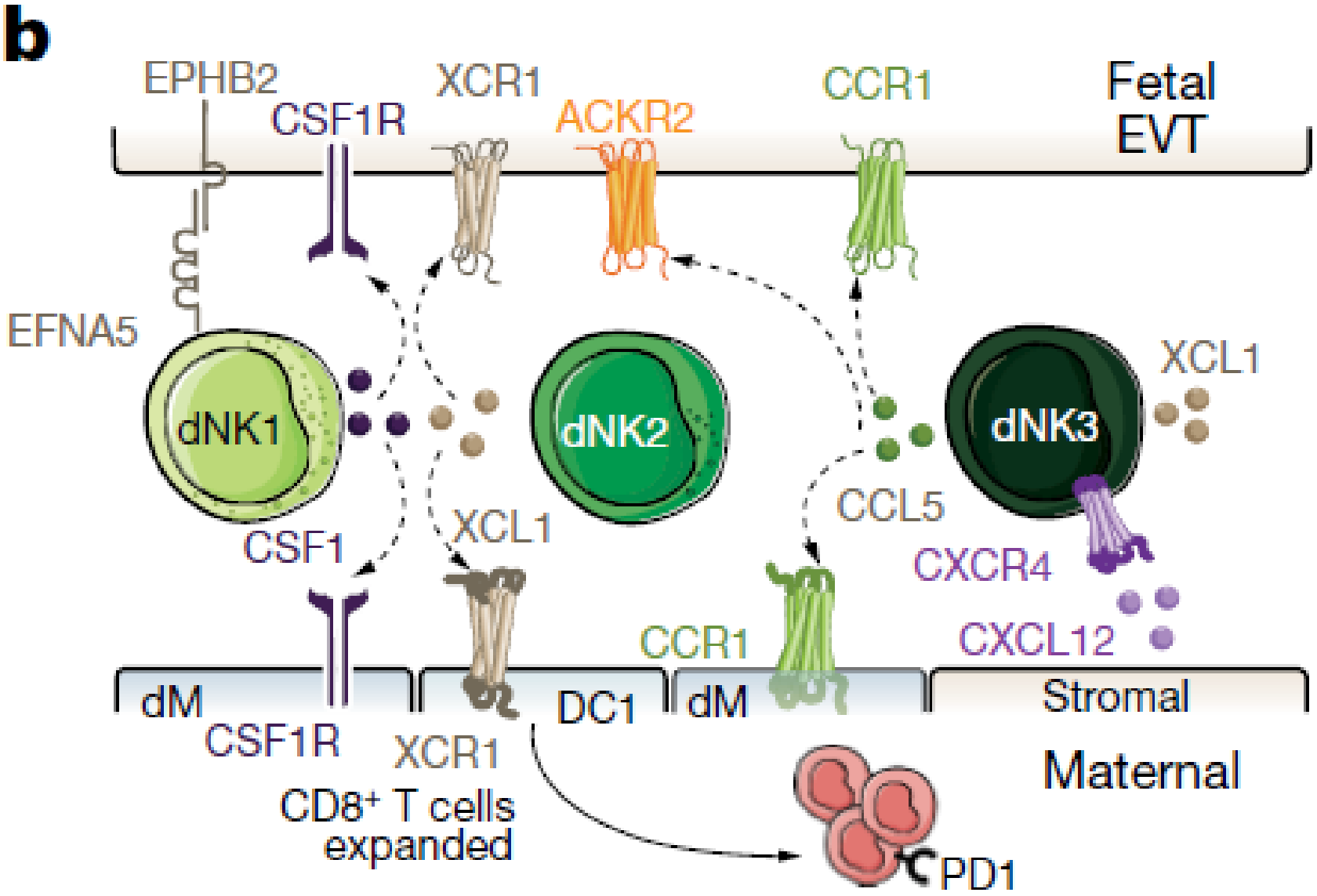


Multiple regulatory immune responses at the site of placentation. Interaction between Natural killer cells and placenta cell subsets




a
Adhesion and recruitment
Growth factors
Angio.
Immunomodulation
XCR1–XCL1
EPHB2–EFNA5
CSF1R–CSF1
CCR1–CCL5
PLXNB1–SEMA4D
FGFR1–CD56
CXCL12–CXCR4
FLT1–VEGFB
FLT1–VEGFA
KDR–VEGFA
EGFR–AREG
OSMR–OSM
HLA-G–LILRB1
ACKR2–CCL5
ACKR2–CCL3
ACKR2–CCL4
PVR–TIGIT
CLEC2D–KLRB1
PDL1–PD1
ADORA2B–CD39
ADORA3–CD39
LGALS9–TIM3
FPR1–ANXA1
IL10 receptor–IL10
dNK1
dNK2
dNK3
dM1
dM2
DC1
CD8
CD4
Treg
EVT
dendritic cells (DC1)
Interacting molecules
1
2
Interacting cells
–log10(P value)
0
1
2
≥3
log2(mean(molecule 1, molecule 2))
4
2
0
–2


a
Adhesion and recruitment
Growth factors
Angio.
Immunomodulation
XCR1–XCL1
XCR1–XCL2
EPHB2–EFNA5
CSF1R–CSF1
CCR1–CCL5
PLXNB1–SEMA4D
FGFR1–CD56
CXCL12–CXCR4
FLT1–VEGFB
FLT1–VEGFA
KDR–VEGFA
EGFR–AREG
OSMR–OSM
HLA-G–LILRB1
ACKR2–CCL5
ACKR2–CCL3
ACKR2–CCL4
PVR–TIGIT
CLEC2D–KLRB1
PDL1–PD1
ADORA2B–CD39
ADORA3–CD39
LGALS9–TIM3
FPR1–ANXA1
IL10 receptor–IL10
dNK1
dNK2
dNK3
dM1
dM2
DC1
CD8
CD4 Treg
EVT
Endothelial
Interacting molecules
1
2
–log10(P value)
0
1
2
≥3
log2(mean(molecule 1, molecule 2))
4
2
0
–2


b
EPHB2
CSF1R
XCR1
ACKR2
CCR1
Fetal
EVT
EFNA5
dNK1
dNK2
dNK3
XCL1
CCL5
CSF1
XCL1
CXCR4
CXCL12
CCR1
dM
CSF1R
XCR1
DC1
dM
Stromal
Maternal
CD8+ T cells
expanded
PD1

The complex between XCL1 and XCR1 is higly expressed in dNK2 both in maternal dendritic cells (DC1) and in fetal EVT.

The expression pattern of the XCL1–XCR1 ligand–receptor complex suggests functional interactions between dNK2 and EVT and DC1. Increase of DC1 cells possibly leads to the expansion of decidual CD8+ T cells and co-expression of PD1.

This type of microenvironment, where are present several types of maternal immune cell, including T cells and three subsets of decidual natural killer (dNK) cell, provide structural support for the decidua.

When the ligand XCL1 produced by dNK2 cells binds the receptors XCR1 present in both fetal EVT and maternal T cells, it induces immunotolerance in the body of the mother in order to not-react against the fetal antigens. This is done because the XCR1 receptor, after the ligand binding, activates a signaling cascade that lead to the stimulation of PD-1 receptor (programmed death receptor) on the surface of immune cells of the mother. The stimulation of PD-1 and the binding with its ligand will generate an inhibitory signal that will allow to develop and maintain tolerance and will avoid the activation of the immune response of the mother against the antigens of the fetus (it can limit effector T-cell response and protect the fetus from immune-mediated tissue damage).

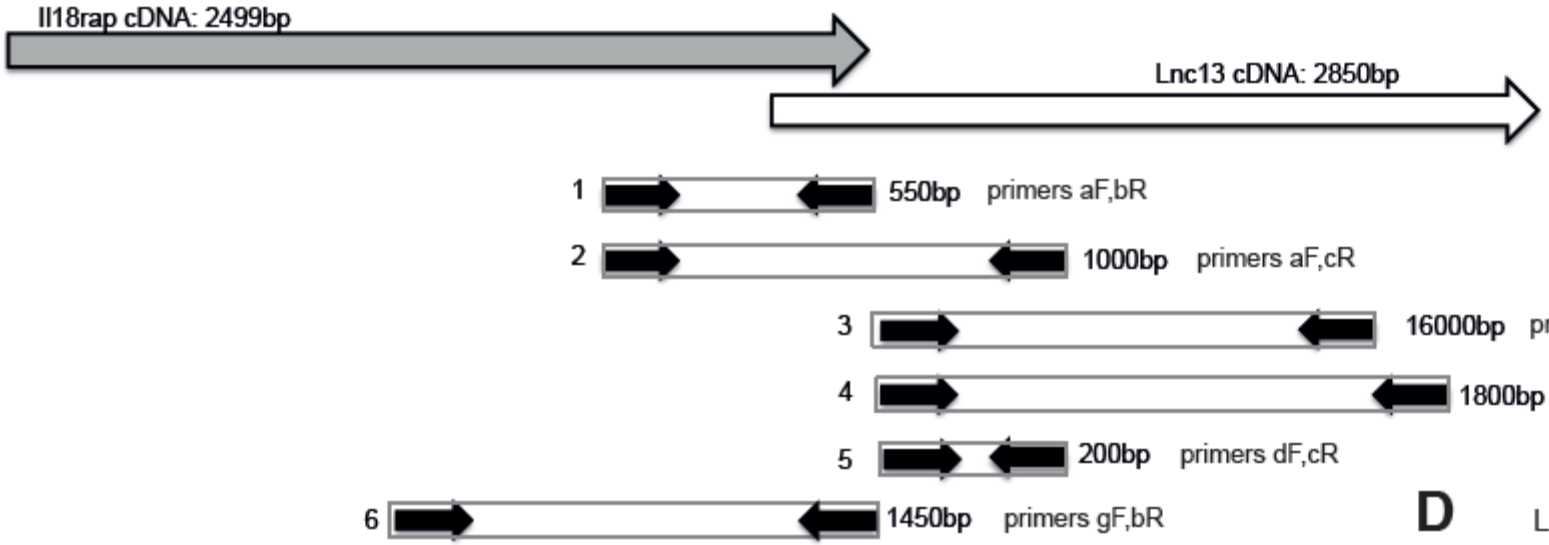
IMMUNE REGULATION

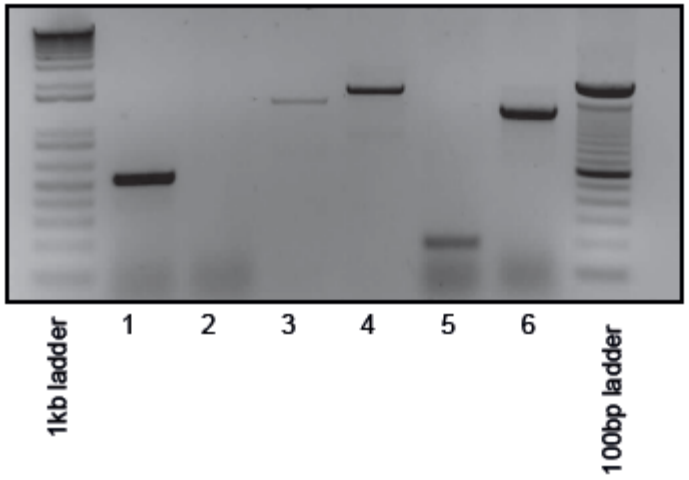
A long noncoding RNA associated with susceptibility to celiac disease

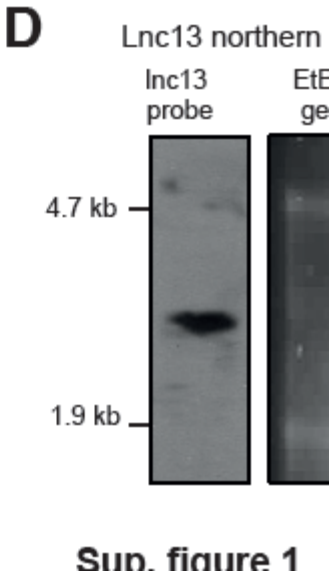
Ainara Castellanos-Rubio,[1] Nora Fernandez-Jimenez,[2] Radomir Kratchmarov,[1] Xiaobing Luo,[3] Govind Bhagat,[4,5] Peter H. R. Green,[5] Robert Schneider,[6] Megerditch Kiledjian,[3] Jose Ramon Bilbao,[2] Sankar Ghosh[1]*

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.

IL18rap and Lnc13 transcripts are indipendent, two different transcripts



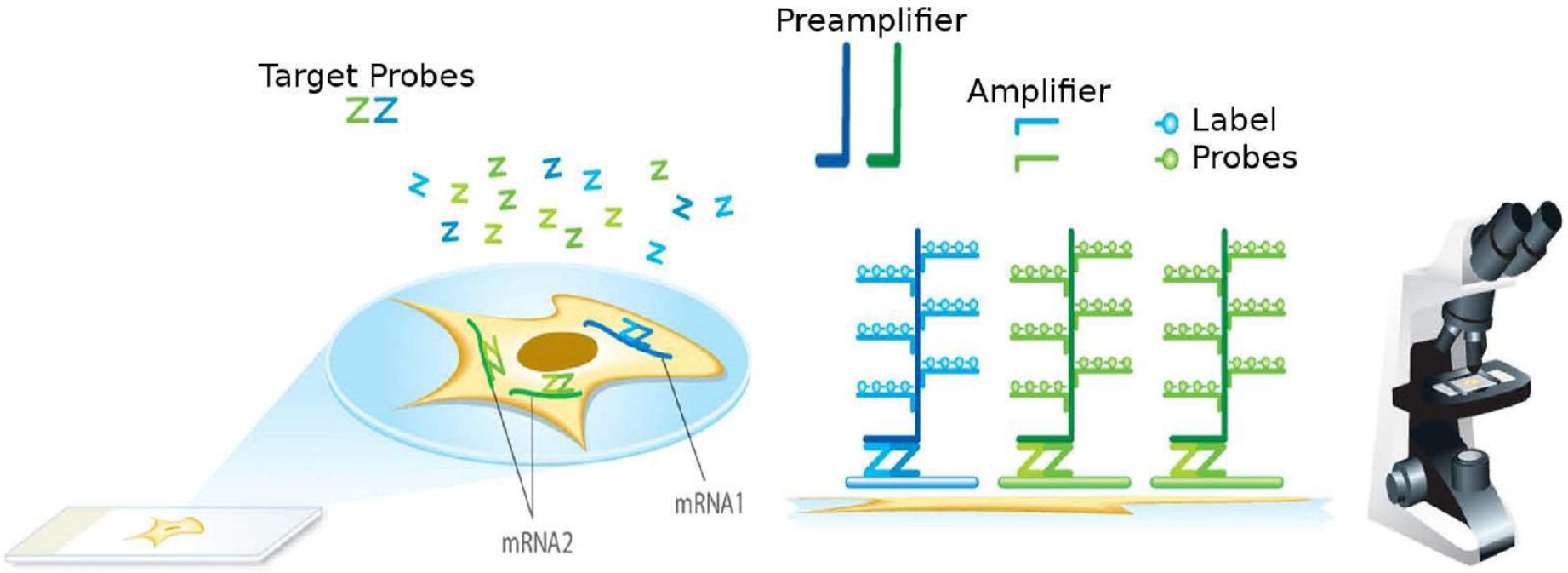




Sup. figure 1

fig. S1C

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



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

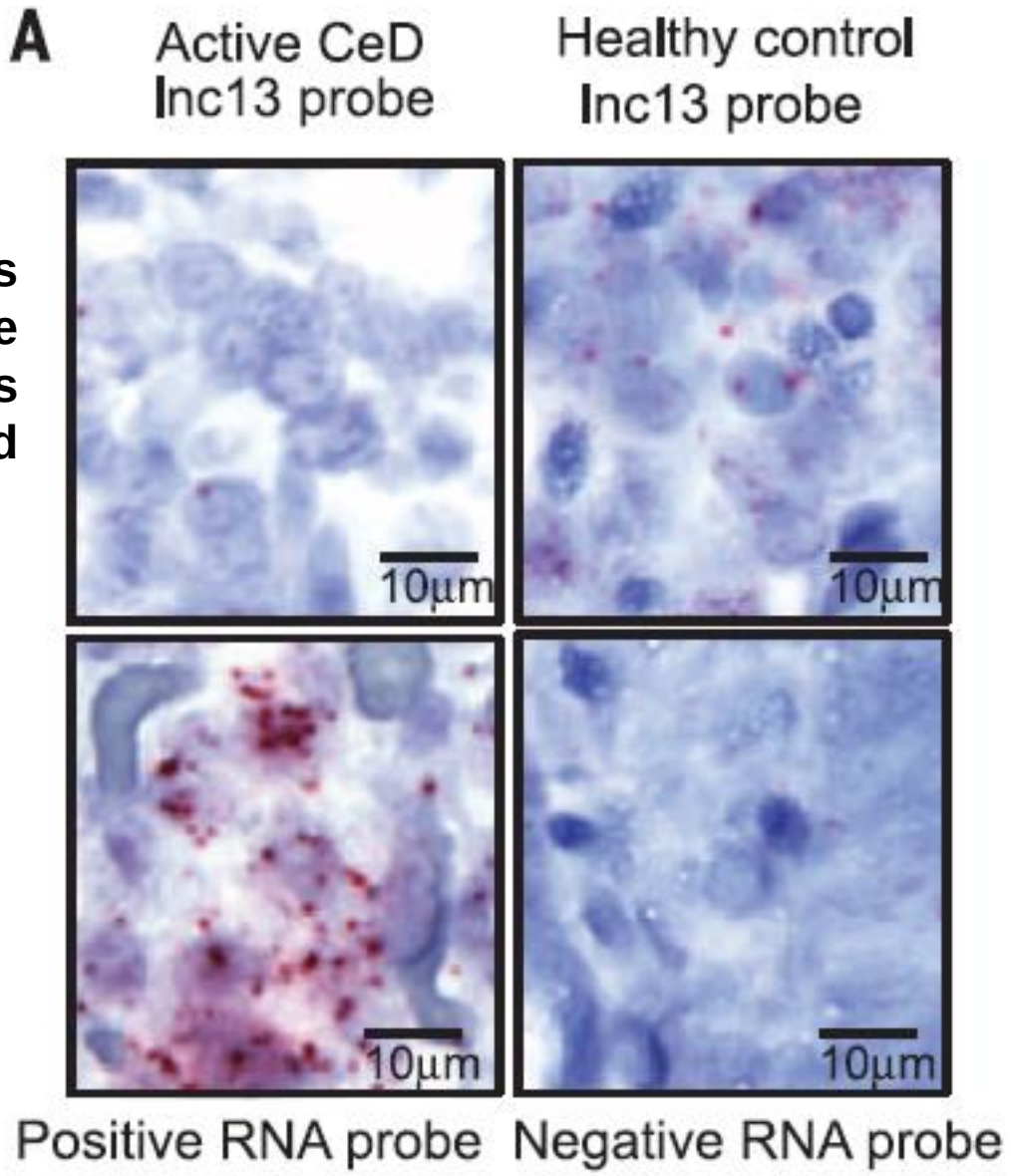


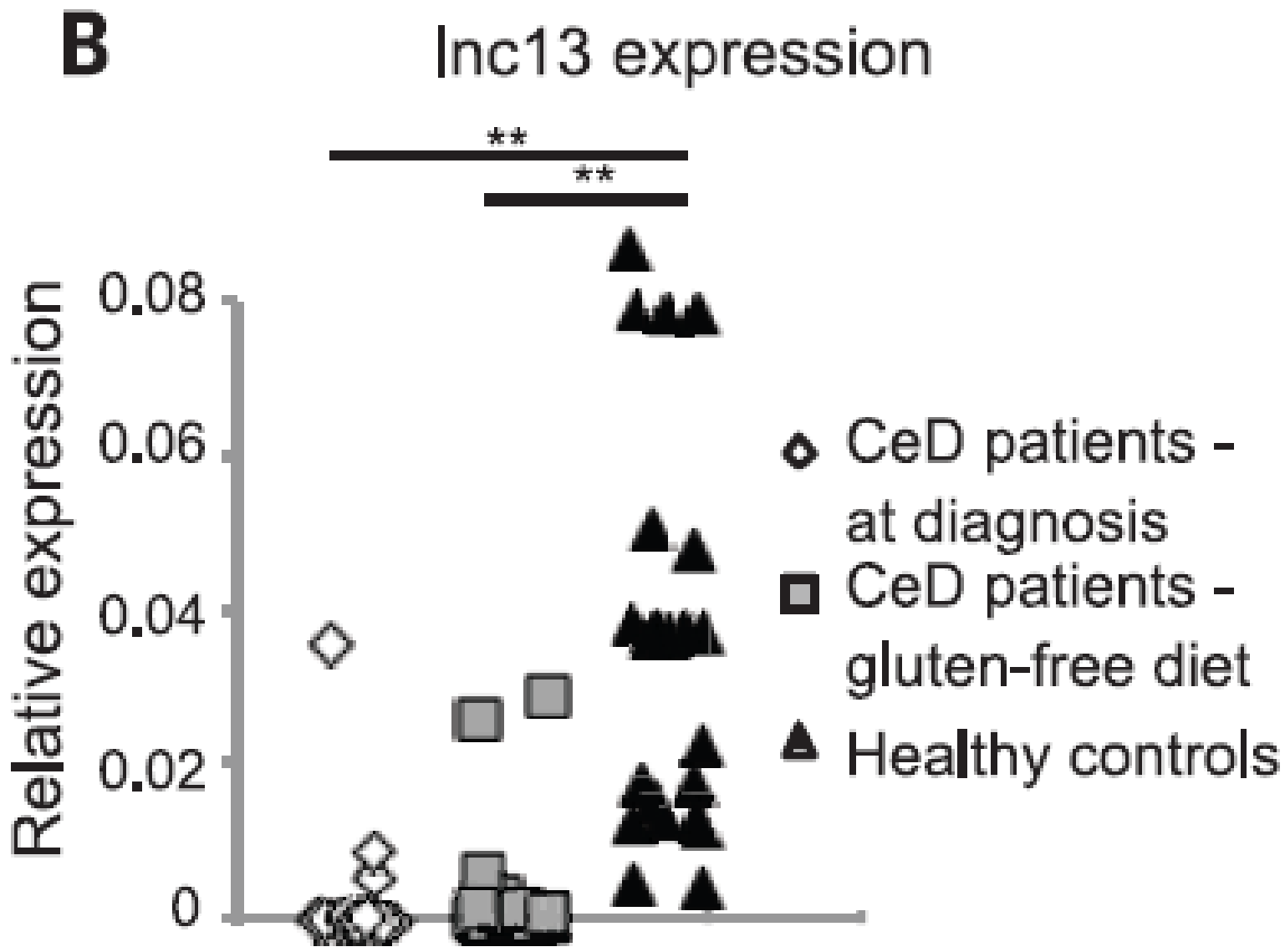
Fig. 1A

Select one:

- a. RNA- probe recognized non coding genomic sequence active in this cell type ✗
- b. RNA- probe recognized a protein that is overexpressed in this cell type
- c. RNA- probe recognized transcript that is overexpressed in this cell type
- d. RNA- probe recognized a transcipt at low expression in this cell type
- e. DNA- probe recognized a protein that is overexpressed in this cell type

Your answer is incorrect.

The correct answer is: RNA- probe recognized transcript that is overexpressed in this cell type


B
lnc13 expression
**
**
Relative expression
0.08
0.06
0.04
0.02
0
CeD patients - at diagnosis
CeD patients - gluten-free diet
Healthy controls

Select one:

- a. Lnc13 expression decreased respect to healthy donor
- b. Lnc13 expression increased respect to patients that did not change diet
- c. Lnc13 remained the same of patients that did not change diet
- d. Lnc13 expression decreased respect to patients that did not change diet ✗
- e. Lnc13 expression increased respect to healthy donor

Your answer is incorrect.

The correct answer is: Lnc13 remained the same of patients that did not change diet

Silencing of lnc13 increased the expression of the predicted targets

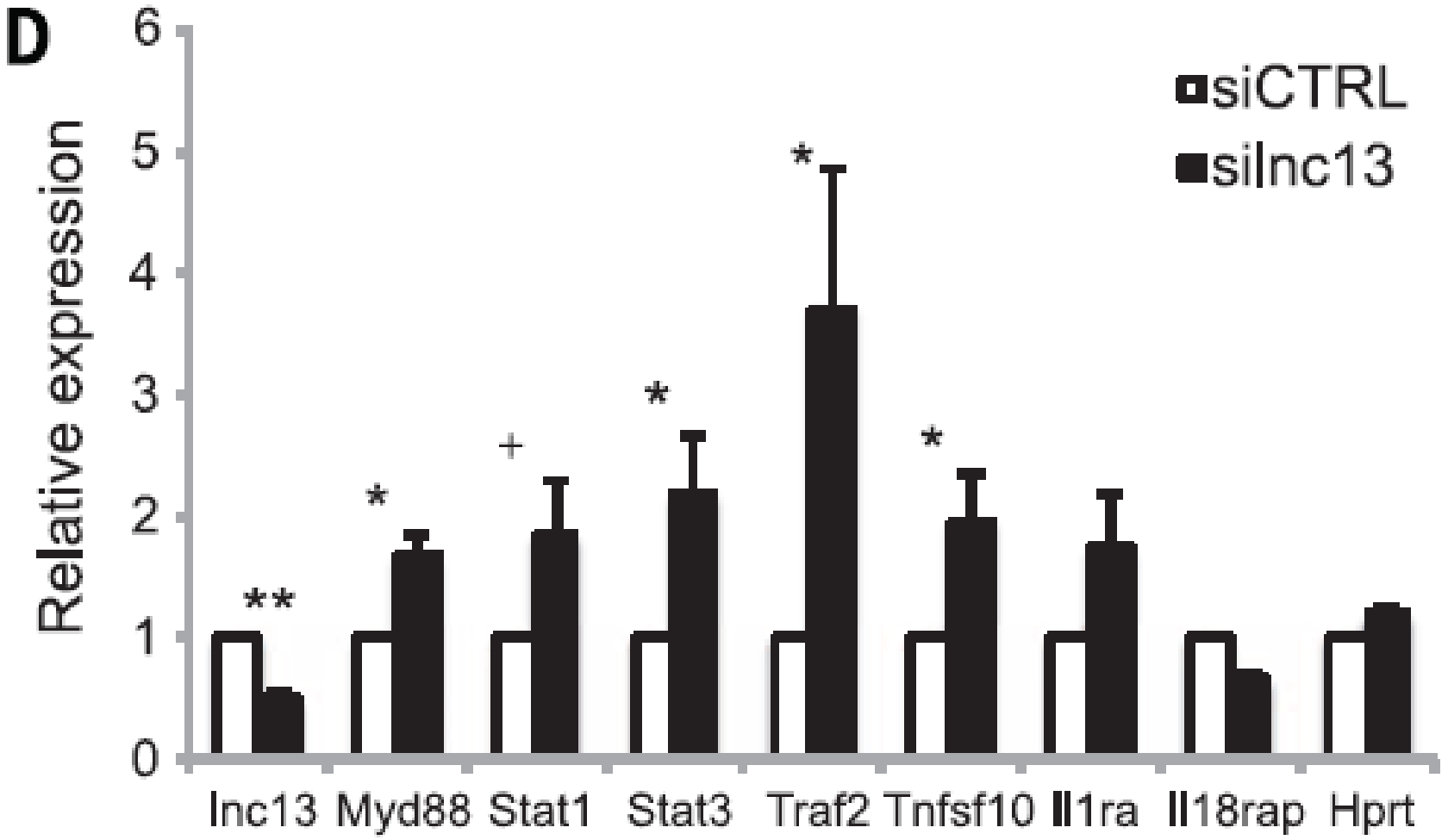


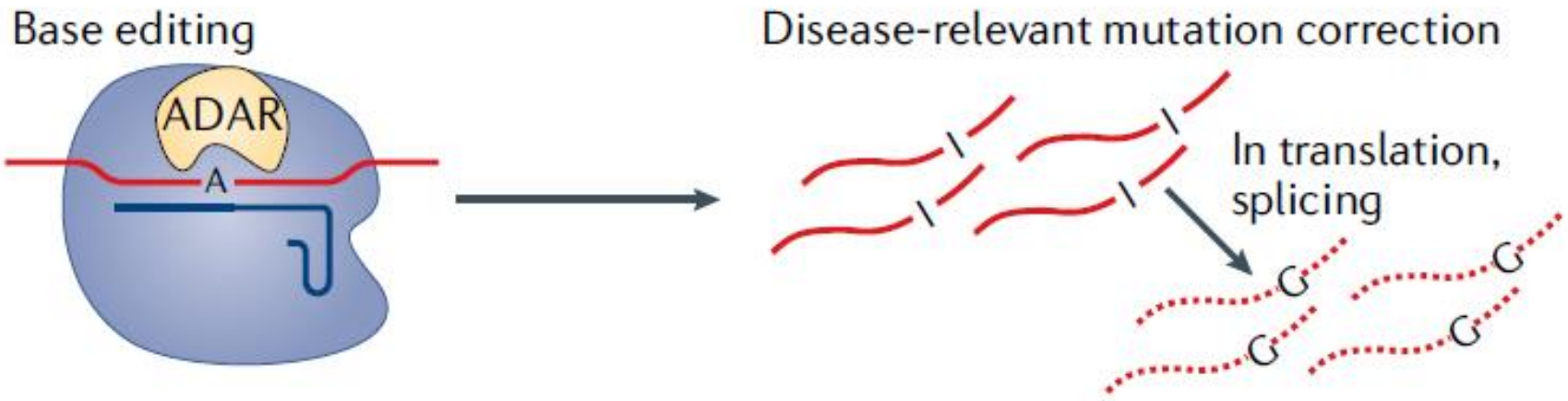
Fig. 2D

Describe how you can modify a single nucleotide variant in the lnc13 sequence.

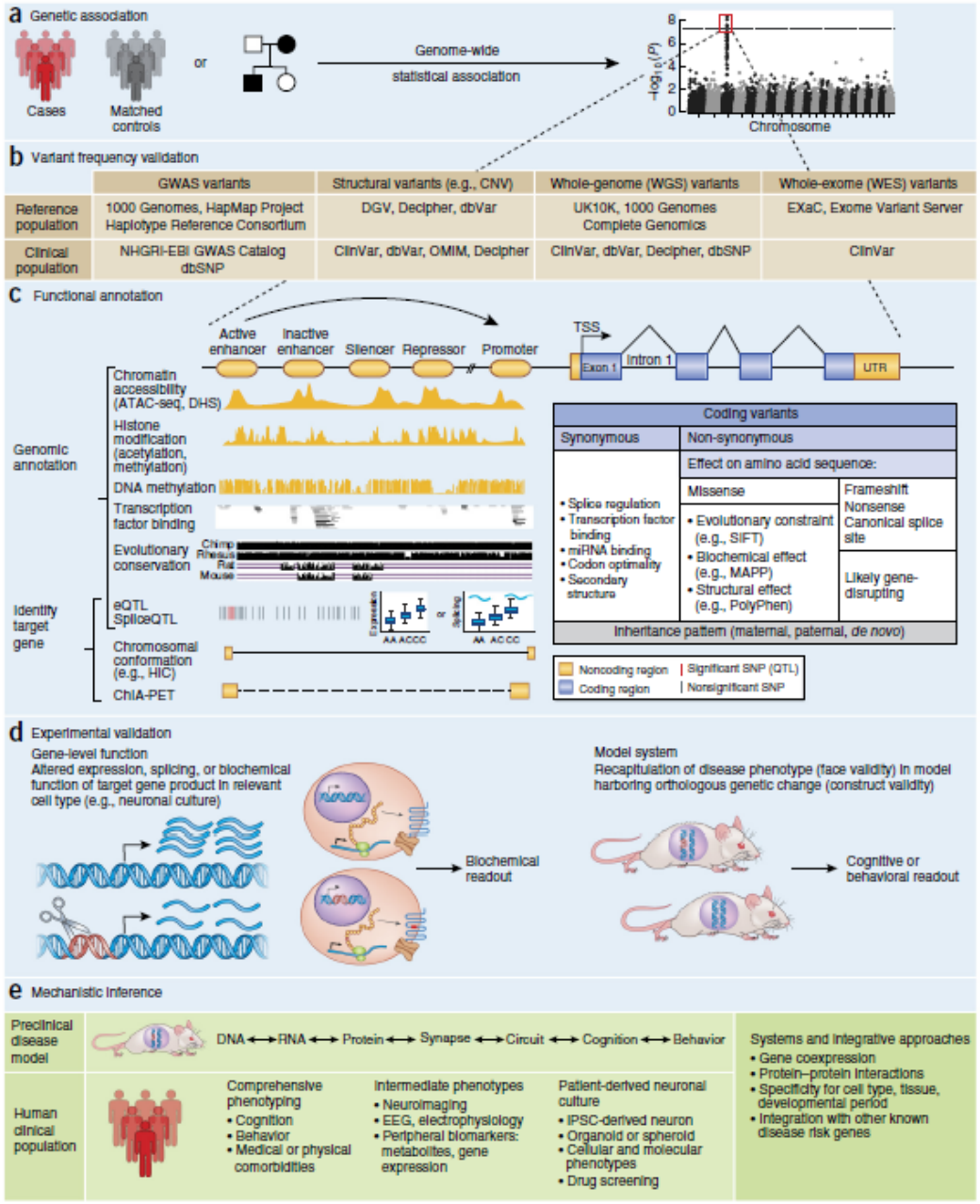
A way to modify a lncRNA, in this case lnc13, could be to use dCas13b, a catalytically dead Cas13 (dCas13) variant fused to the deaminase domain of human ADAR which naturally deaminates adenosines to inosines in dsRNA. The guide RNA specifies the target site by hybridizing to the bases contiguous to the target adenosine, forming a dsRNA structure for editing, and recruiting the dCas13b–ADAR fusion protein. As a result, a mismatched cytidine (C) in the crRNA opposite the target adenosine (A) promotes target adenosine deamination to inosine (A-to-I editing).

Catalytically deficient Cas13 maintains the capacity to bind to the targeted RNA

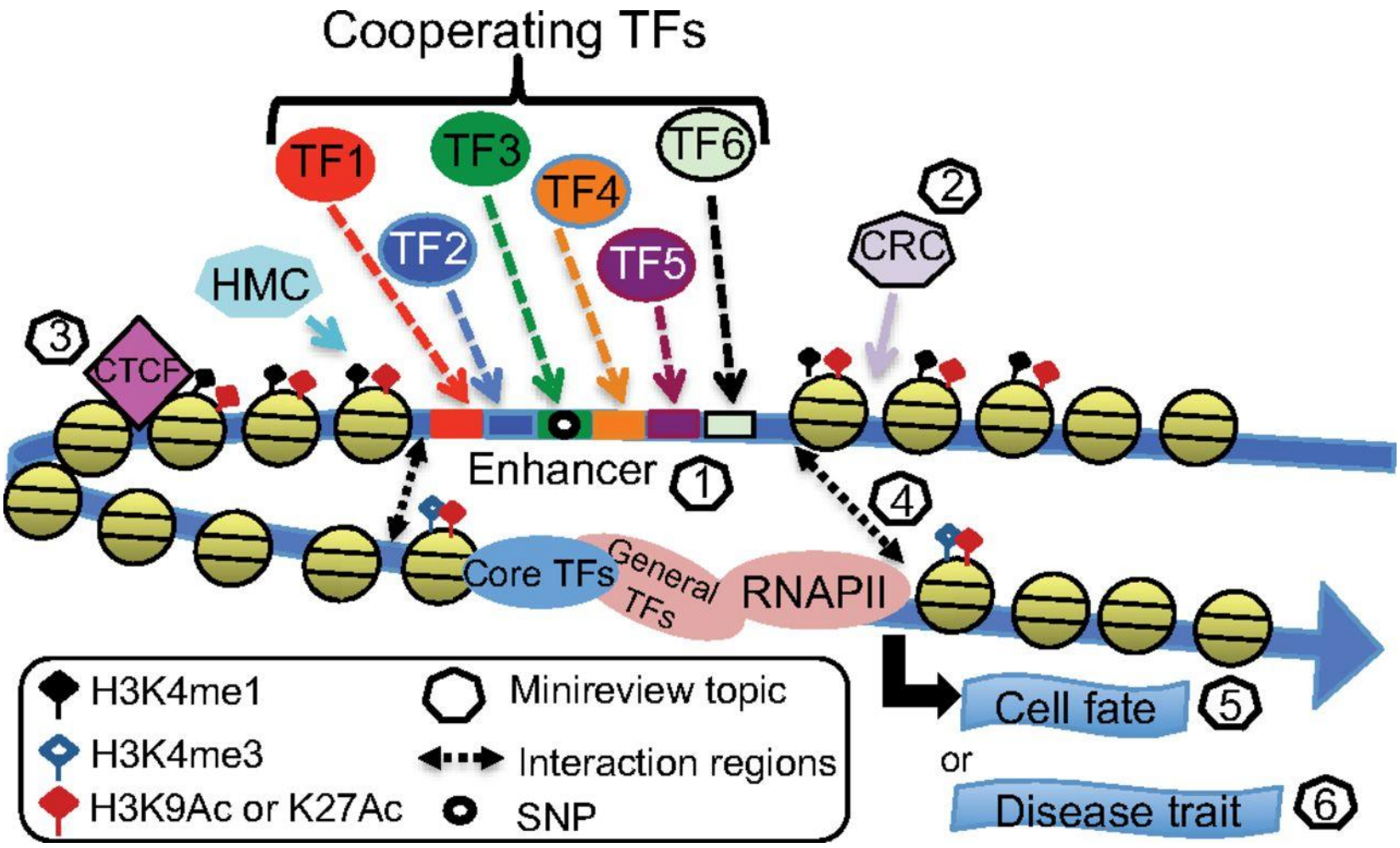
Adenosine deaminase acting on RNA (ADAR) can be fused to catalytically deficient Cas for RNA A→I base editing to correct disease-relevant mutations.



Framework for interpretation of individual disease-associated variants



Genome-wide characterizations of regulatory regions.



Peggy J. Farnham J. Biol. Chem. 2012;287:30885-30887

EXAM

Students are expected to demonstrate:

1. Knowledge of **basic** concepts
2. Understanding of **specific** concepts
3. Comprehension of experimental **methodology**
4. **Solving problem** that we have discuss during lesson

Evaluation:

EXAMS is based on lessons and is composed to 18 multiple choice questions and two open questions. Exam test time is 1 hour.