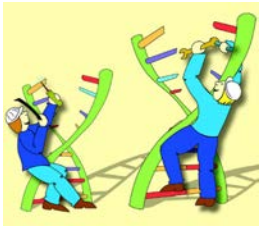
Further insight into the multifaceted roles of NER proteins and their implications in human pathologies

Donata Orioli

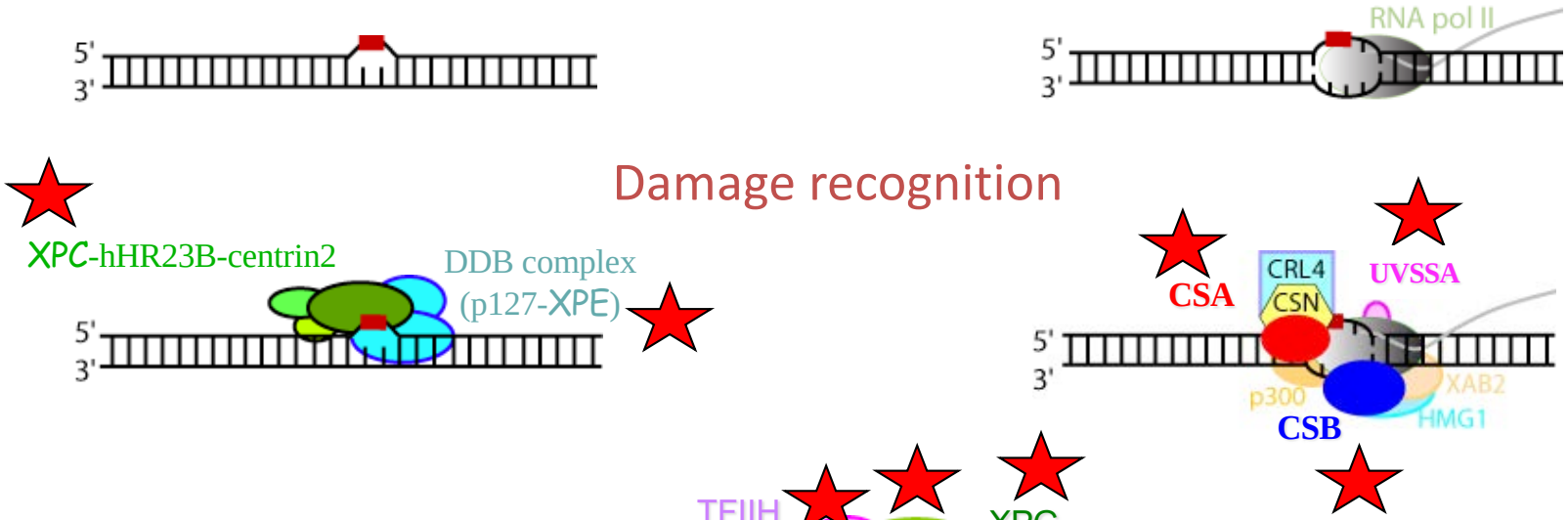
DNA Repair Interest Group, May 17, 2022



NER sub-pathways in human cells

Global genome repair

Transcription-coupled repair

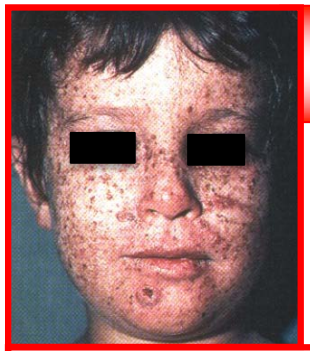


Open complex formation

Incision / Excision

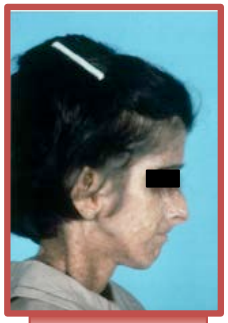
DNA repair synthesis
Ligation

NER-defective hereditary disorders



XERODERMA PIGMENTOSUM
- XP-

Cutaneous alterations and tumors in the sun-exposed areas of the skin



XP/CS

XPA, XPC, XPE, XPF

XPG ***XPB, XPD*** ***TTDA/p8***

COCKAYNE SYNDROME
-CS-

CSA, ***CSB***

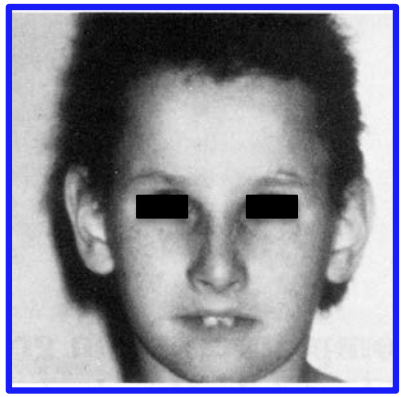
TRICHOTHIODYSTROPHY
TTD

Premature aging

Physical and mental retardation

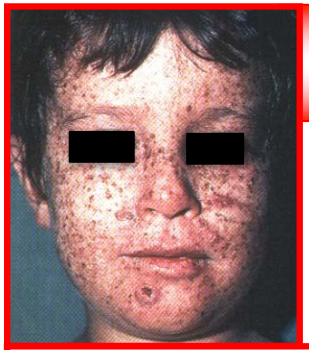
Hair abnormalities

Neurodegeneration



How can NER impairments give rise to such disease heterogeneity, with opposite skin cancer proneness and different appearance of progeroid features?

NER-defective hereditary disorders



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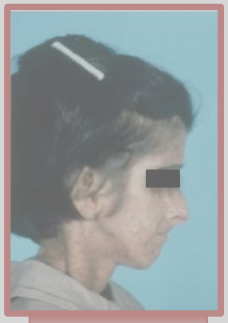

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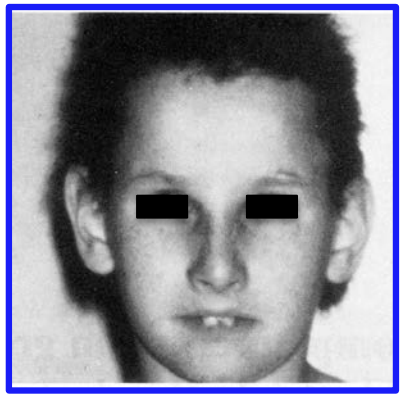
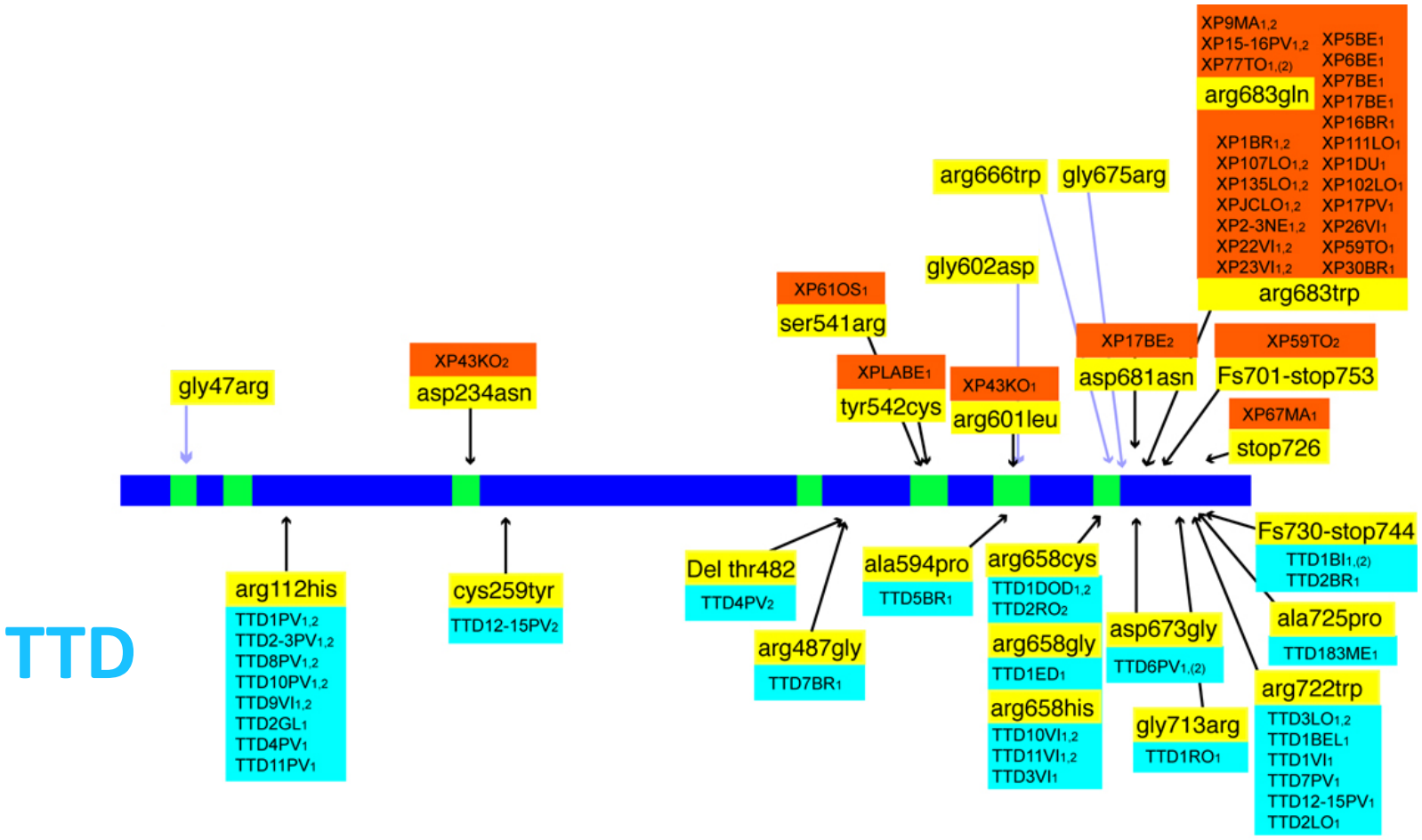
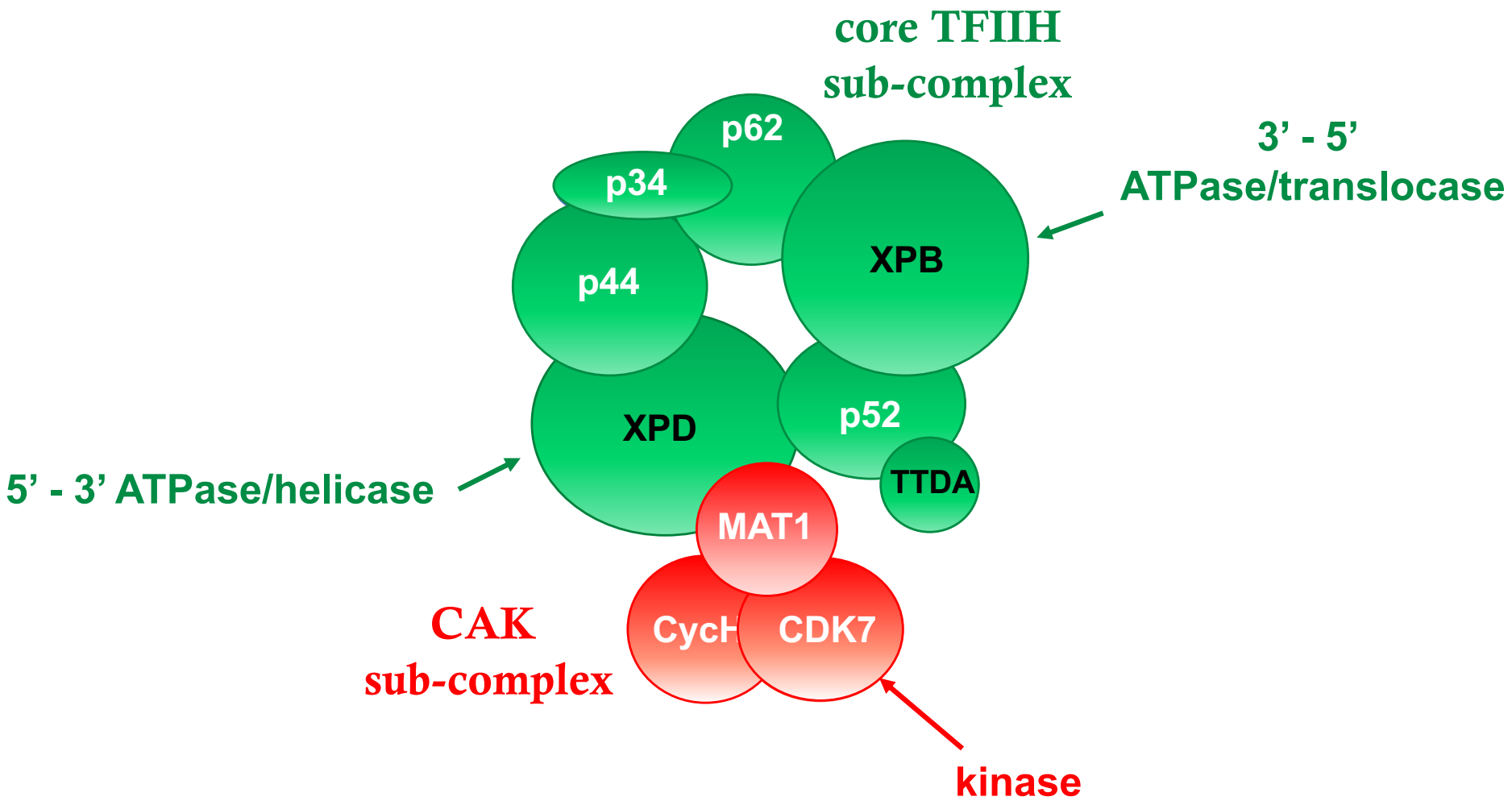


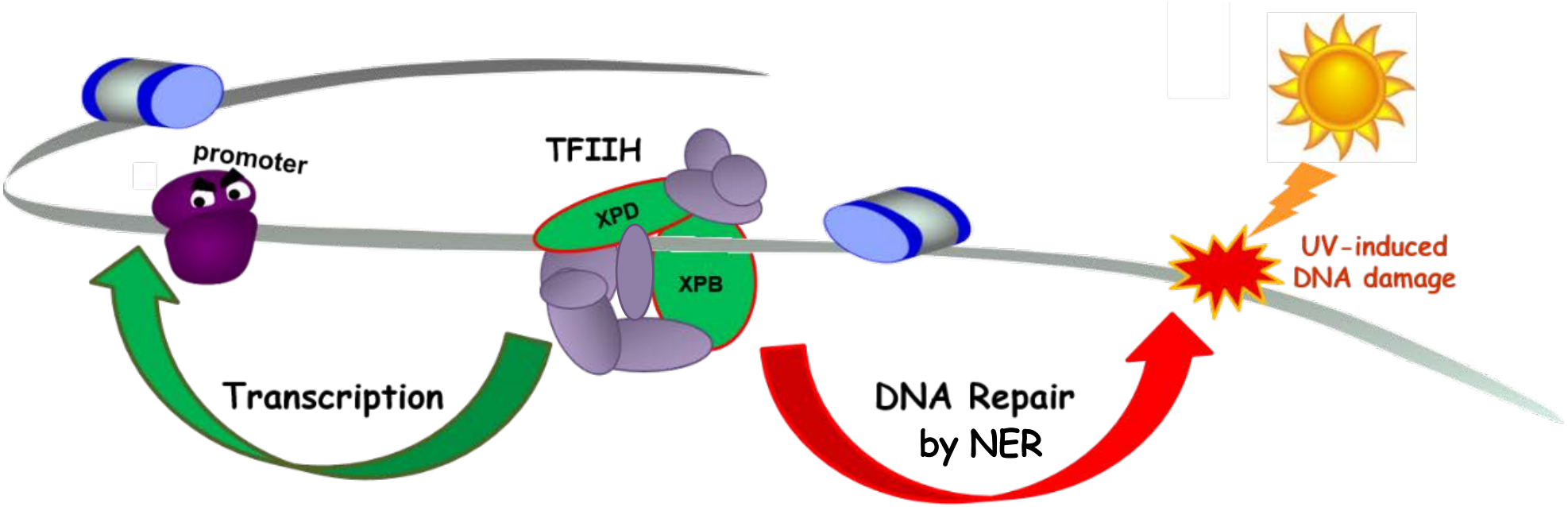
Mutation site determines the pathological phenotype

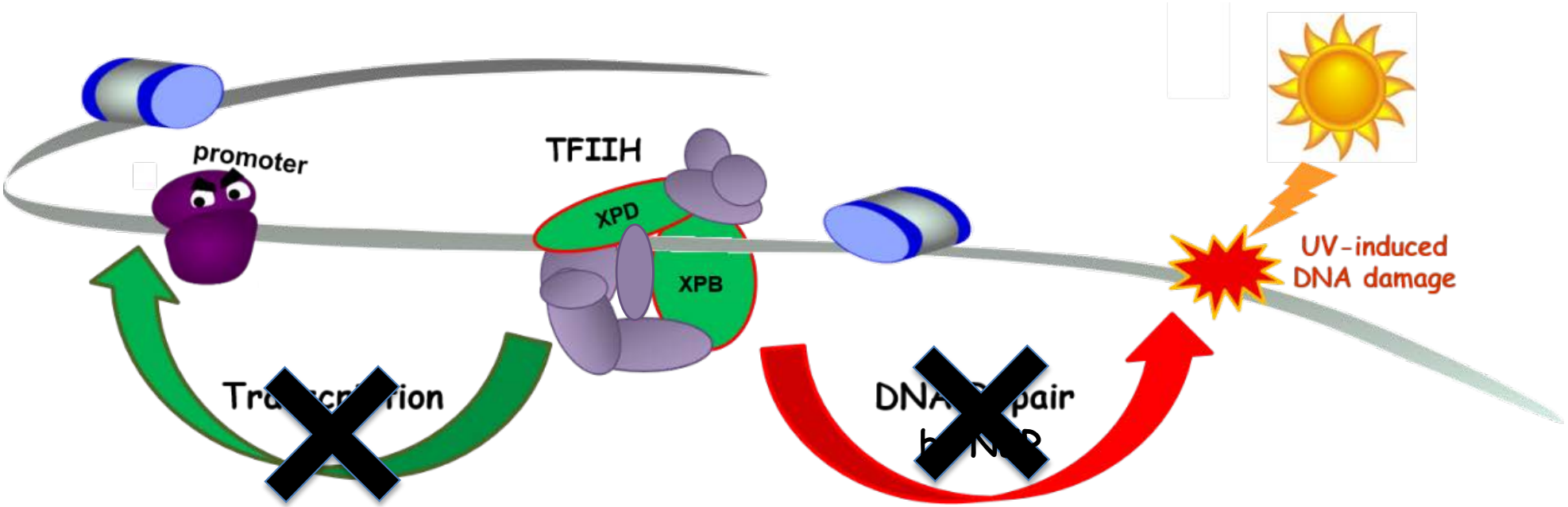


Alterations in the XPD protein in TTD and XP patients

The general transcription factor IIH (TFIIH)



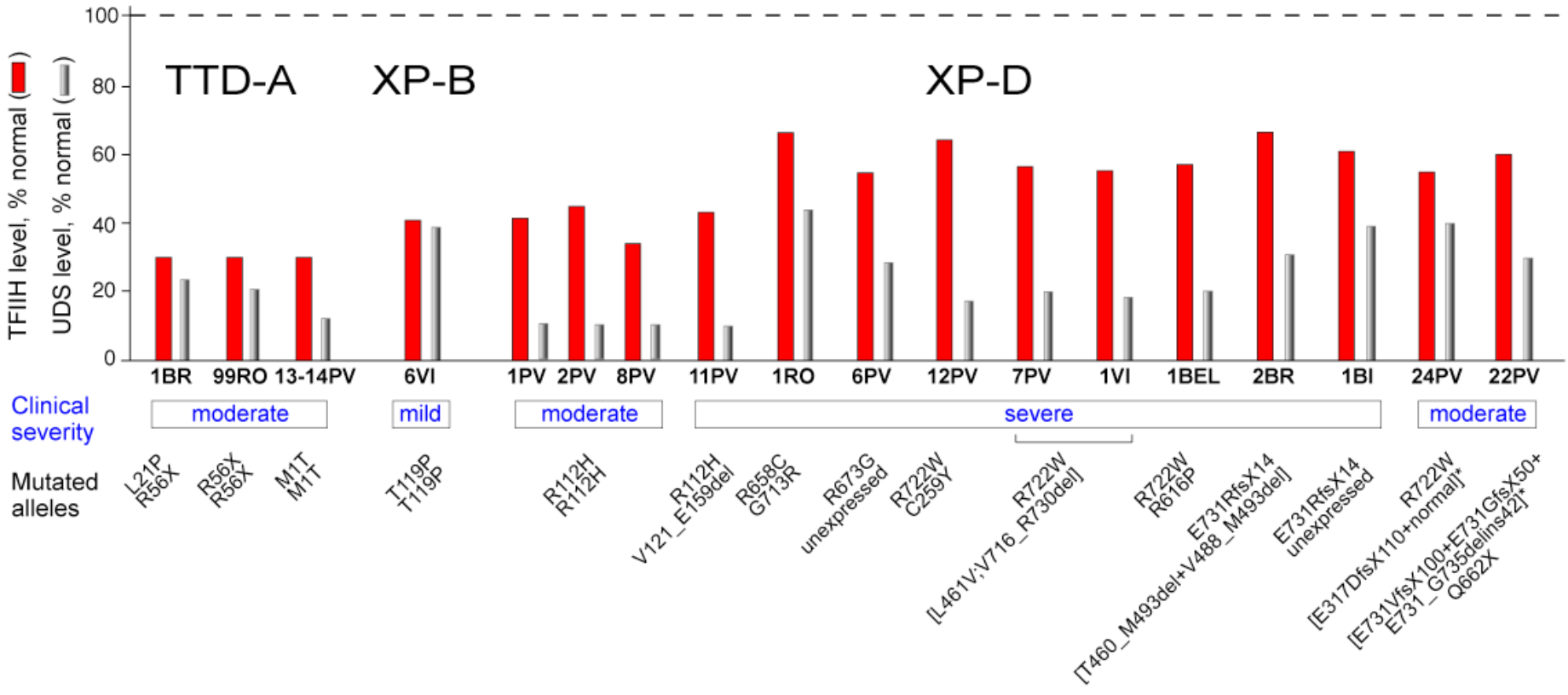

promoter
TFIIH
XPD
XPB
UV-induced
DNA damage
Transcription
DNA Repair
by NER



Does transcriptional failure affect **TTD** more than XP?

DNA damage accumulation affects **both XP and TTD**

All the mutations responsible for TTD cause a decrease by up to 70% in the cellular content of TFIIH

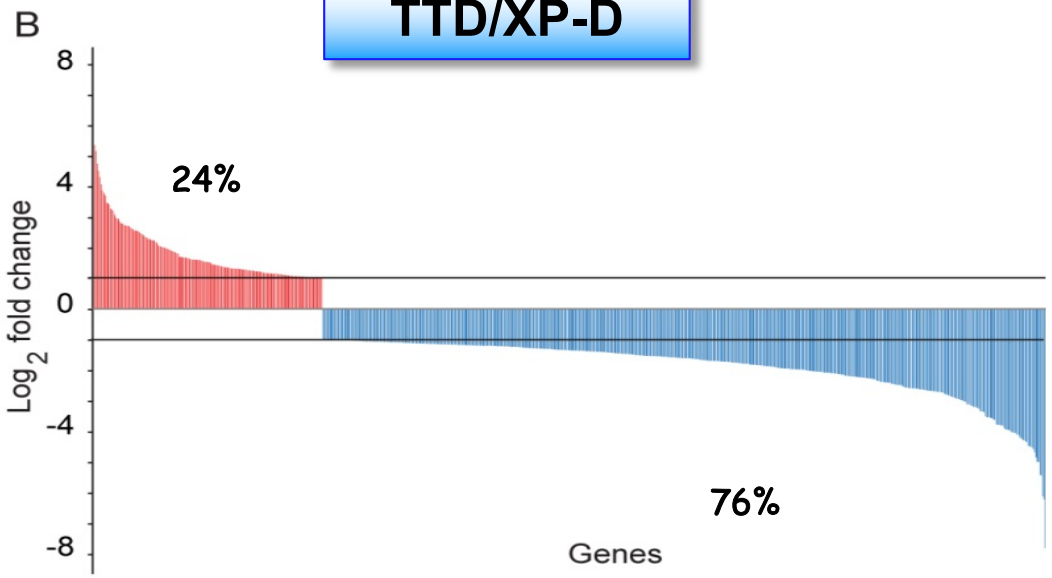


Vermeulen W *et al. Nat Genet, 2000*
Botta E *et al. Hum Mol Genet, 2002*

Gene expression profile analysis by RNA-seq

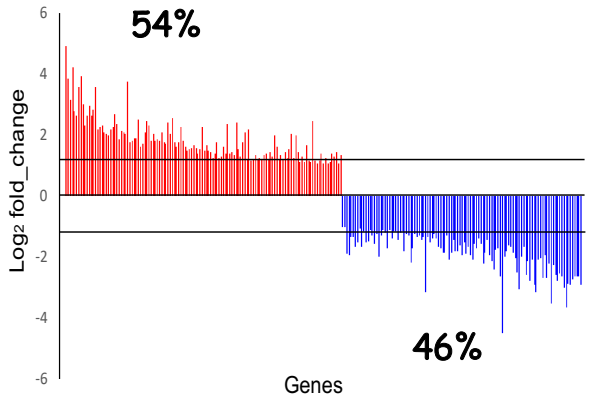
Differentially expressed genes are obtained by **comparing family members**

TTD/XP-D



718 coding genes differentially expressed

XP/XP-D



243 coding genes differentially expressed

Identification of altered signalling pathways distinctive of XP or TTD skin cells

TTD/XP-D, more than XP/XP-D, fibroblasts are affected by wide transcriptional impairments

Expression analysis in primary skin fibroblasts

Real Time RT-PCR in primary dermal fibroblasts from **TTD/XP-D** or **XP/XP-D** patients and corresponding **healthy parents**. Cells were cultured under basal condition **or following UV-induced DNA damage**

6 healthy controls

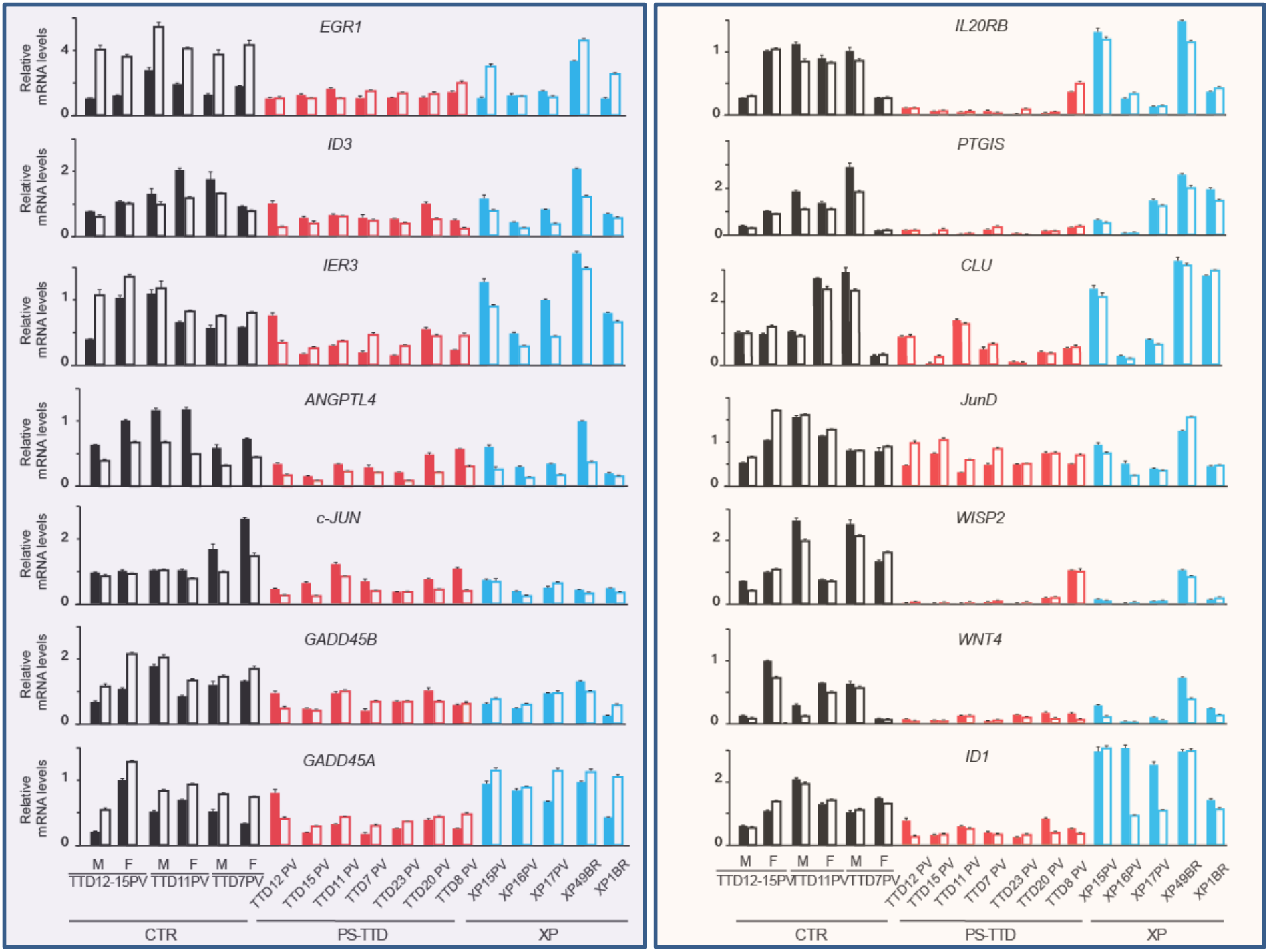
TTD and XP parents

7 TTD patients

representative of distinct clinical and molecular defects

5 XP patients

homozygous or combined heterozygous for the Arg683 substitutions

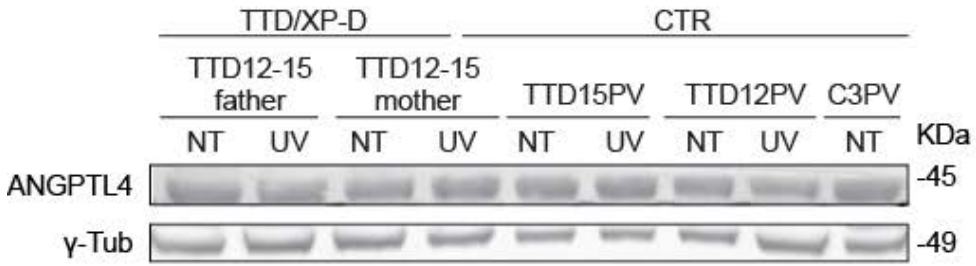


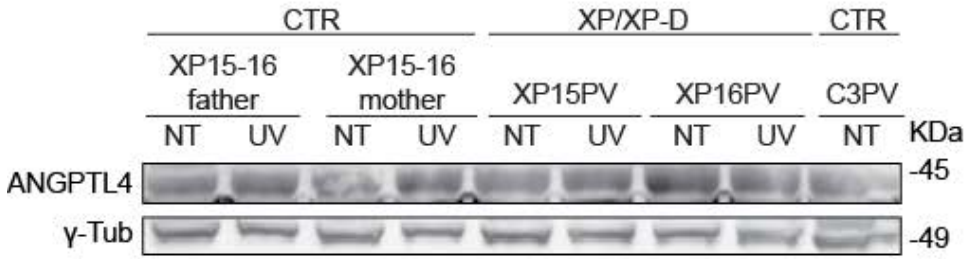
before UV irradiation

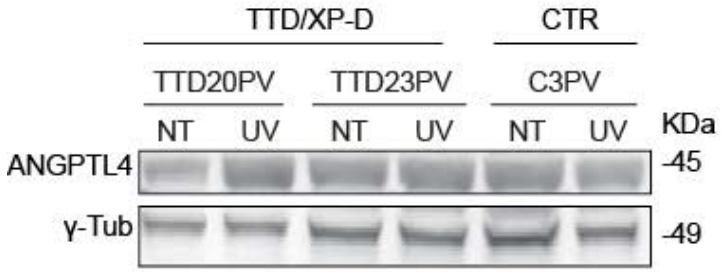
2 hrs upon UV irradiation

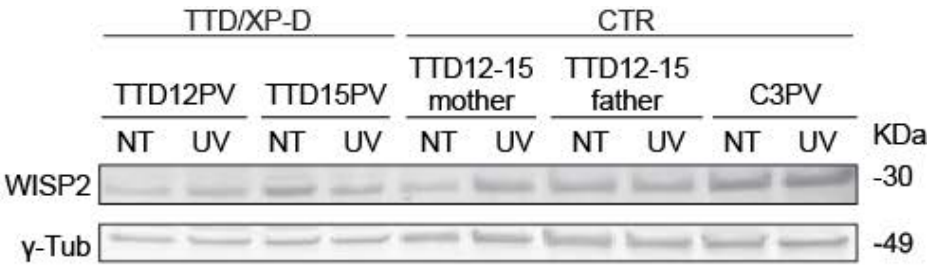
Lombardi A *et al. PNAS, 2021*

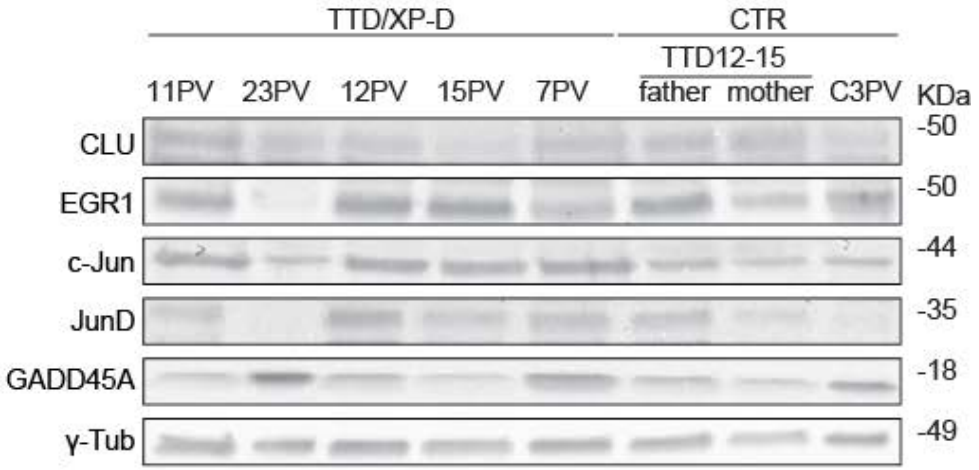
Most of TTD-specific transcription deregulations are masked by protein synthesis/stability

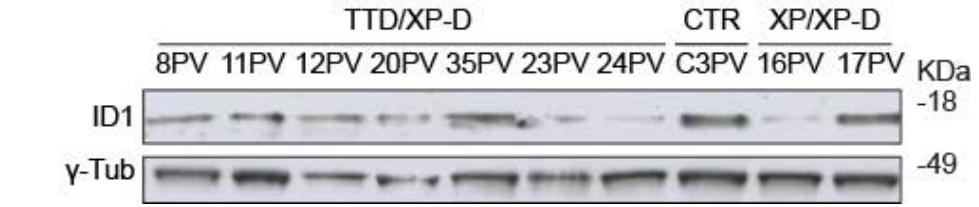






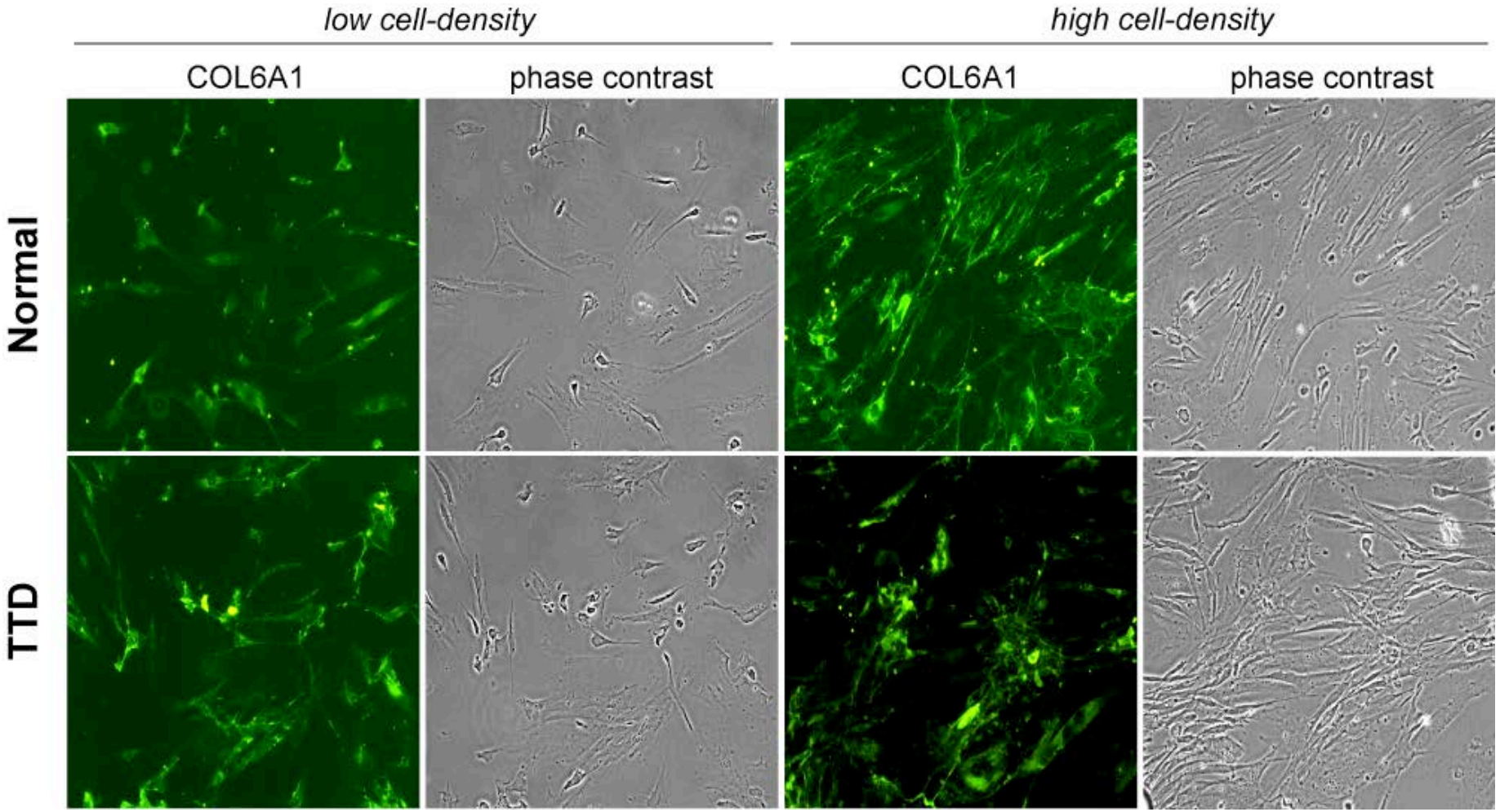






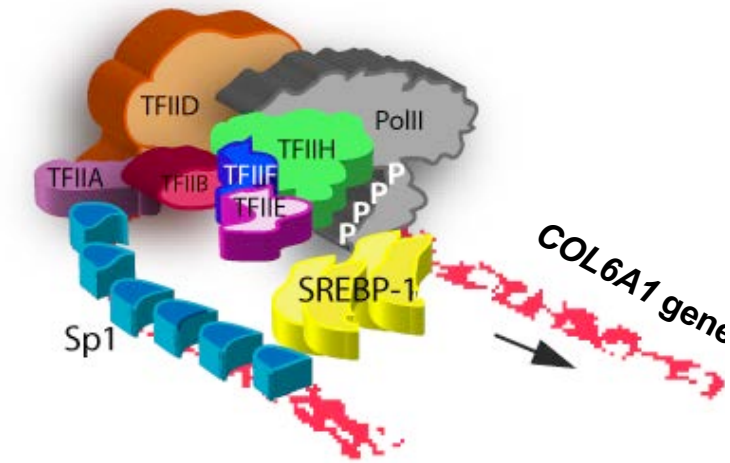
Lombardi A *et al. PNAS, 2021*

XPD mutations in trichothiodystrophy hamper collagen VI expression and reveal a role of TFIIH in transcription derepression

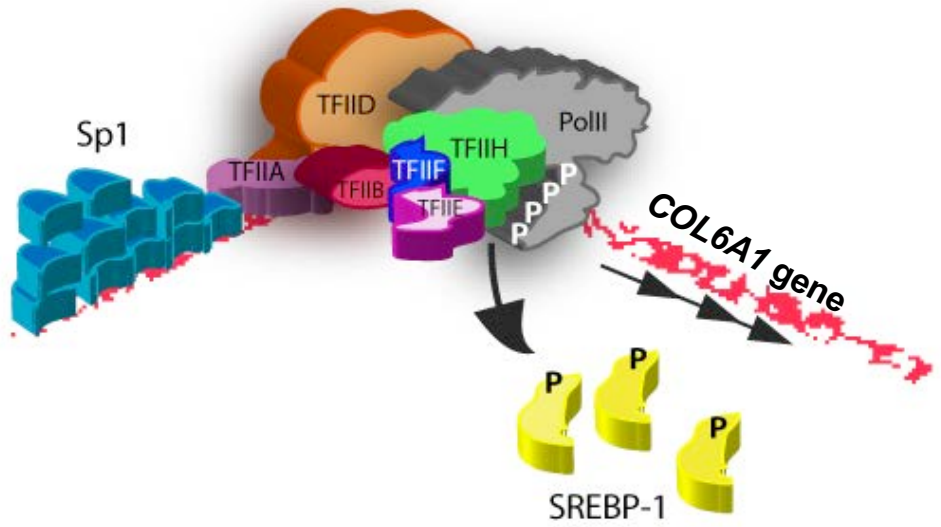


Orioli D *et al. Hum Mol Gen, 2013*

Normal dermal fibroblasts



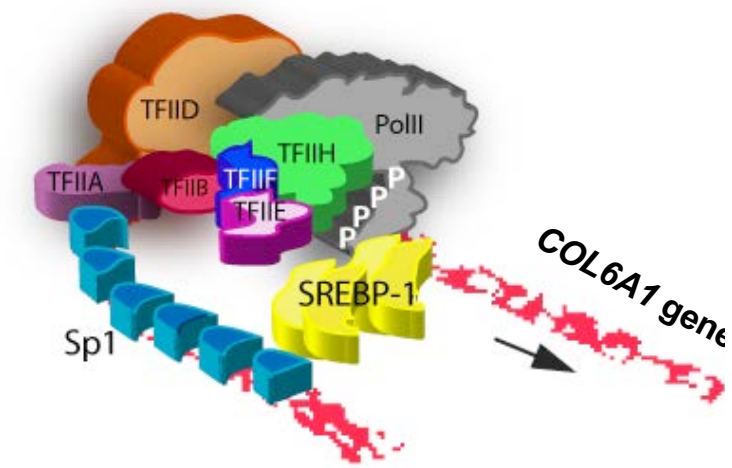
Low cell-density



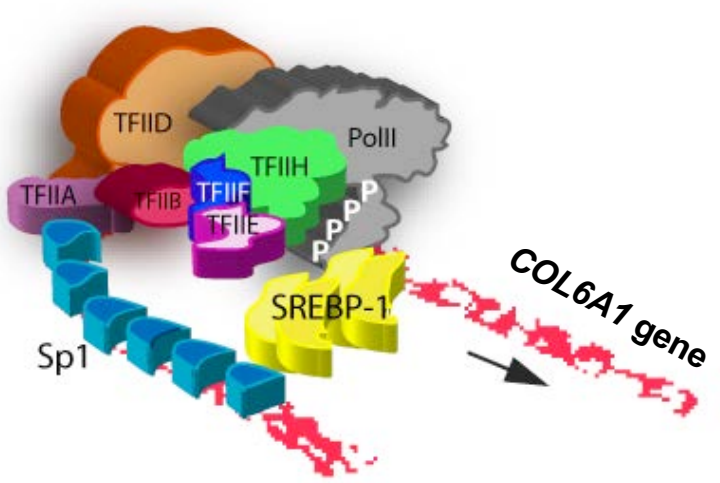
High cell-density

Orioli D *et al. Hum Mol Gen, 2013*

TTD dermal fibroblasts



Low cell-density

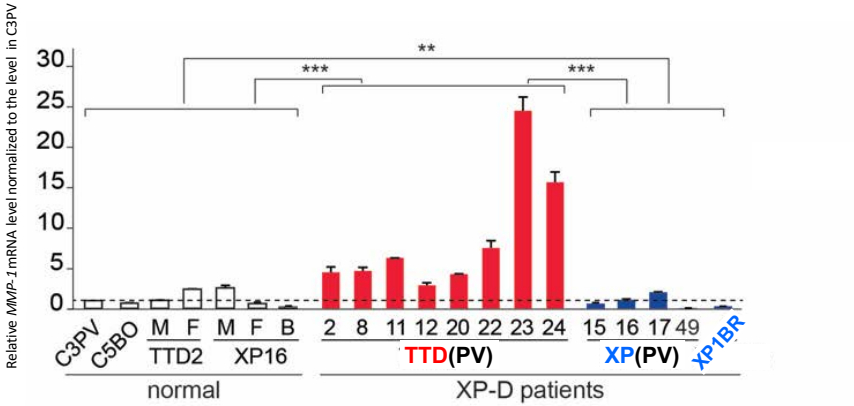


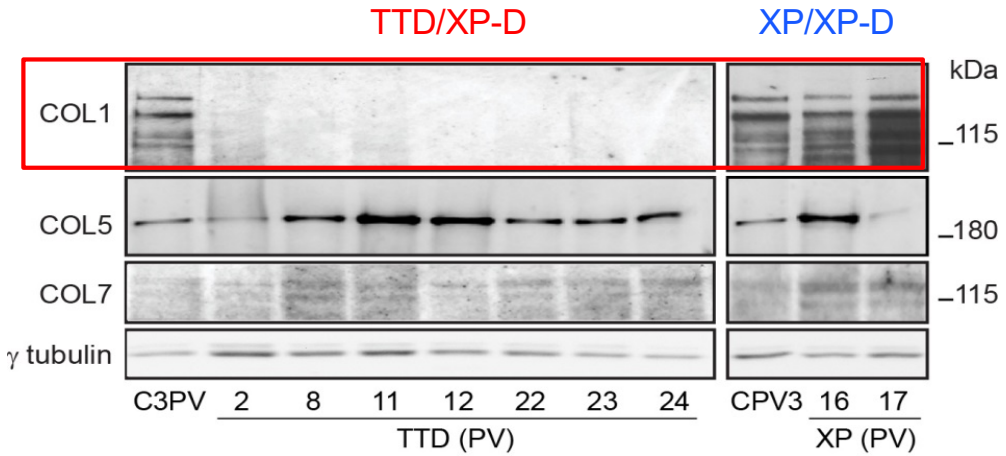
High cell-density

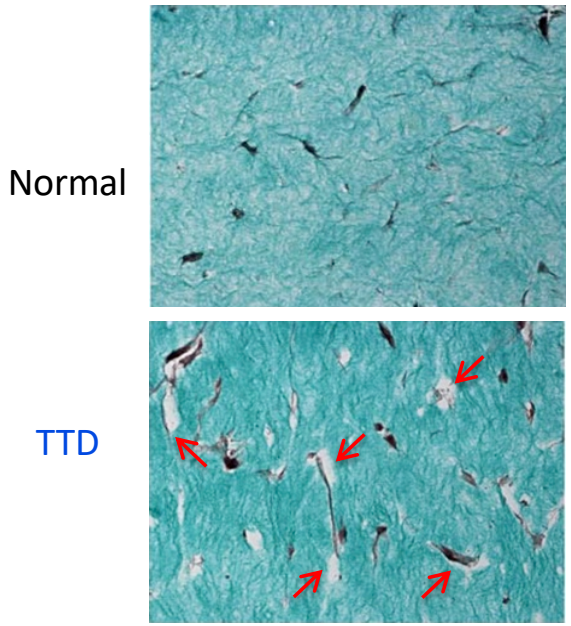
In TTD (but not in XP) fibroblasts, TFIIH fails to remove SREBP-1 from *COL6A1* promoter which maintains low the rate of transcription

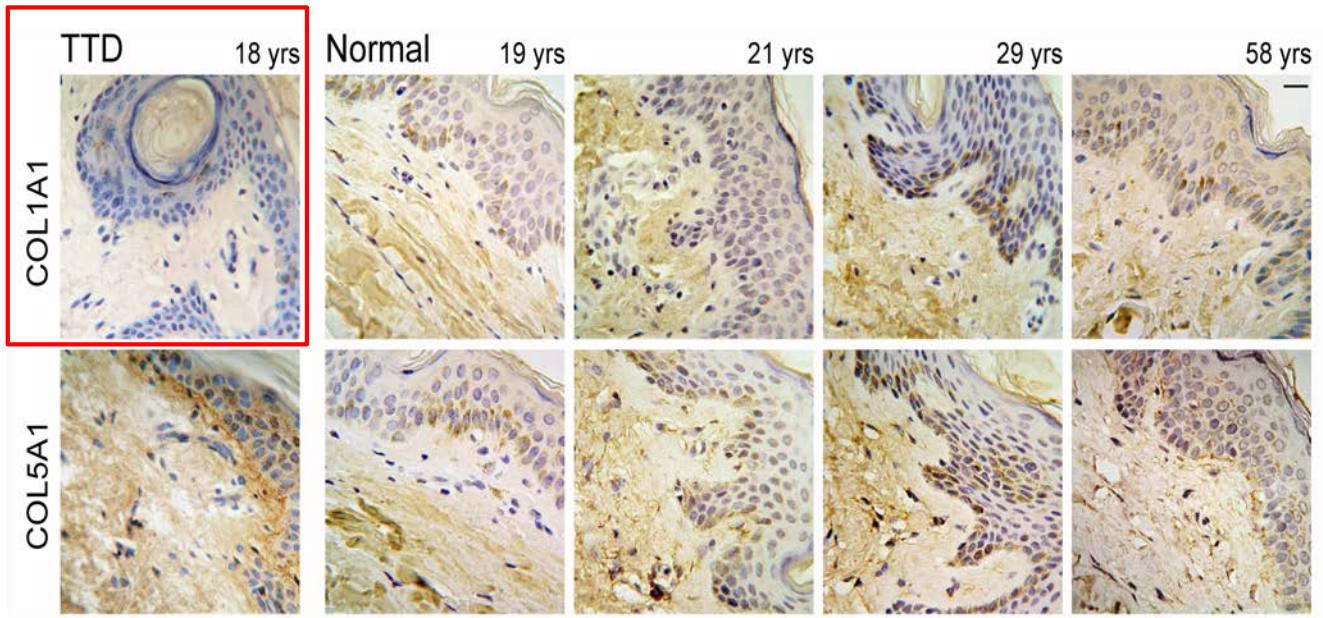
Orioli D *et al. Hum Mol Gen, 2013*

TFIIH-dependent *MMP-1* overexpression in trichothiodystrophy leads to extracellular matrix alterations in patient skin



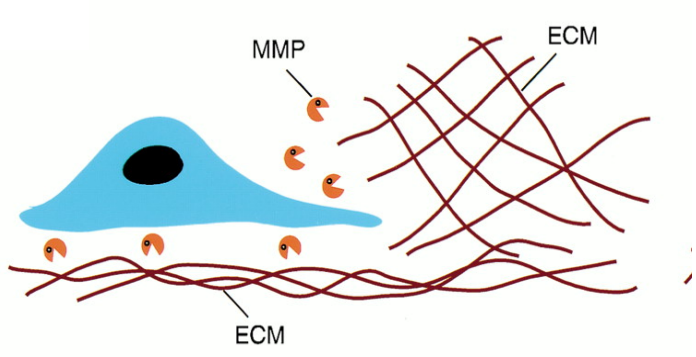


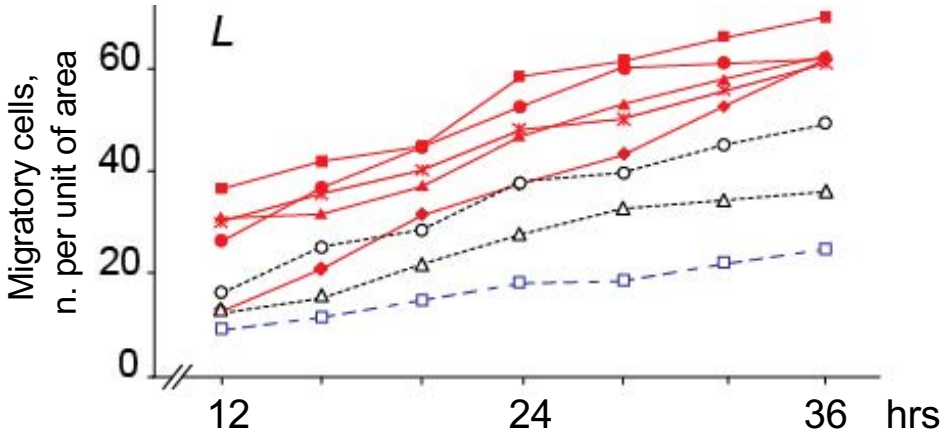


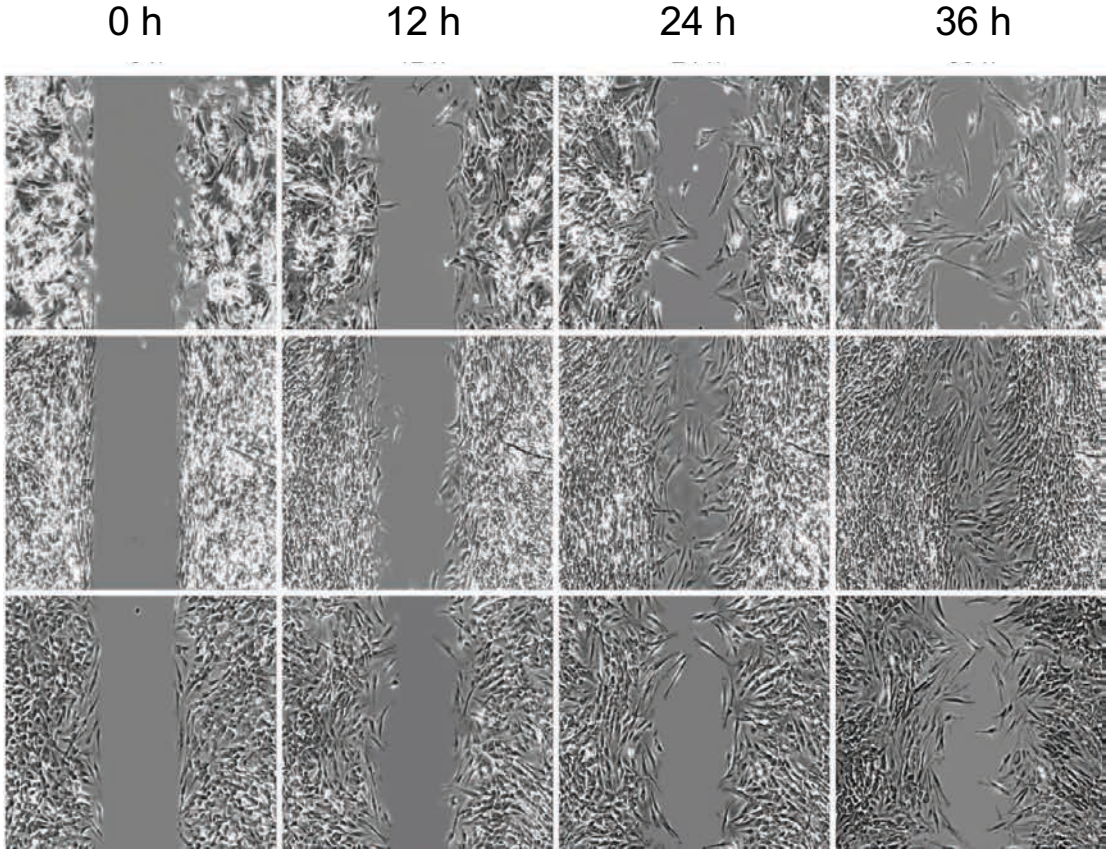


Arseni L *et al. PNAS, 2015*

Loose extracellular matrix (ECM) surrounds TTD fibroblasts







Normal

TTD24PV
TTD20PV
TTD23PV
TTD8PV
TTD12PV
XP17PV
C3PV
C377RM

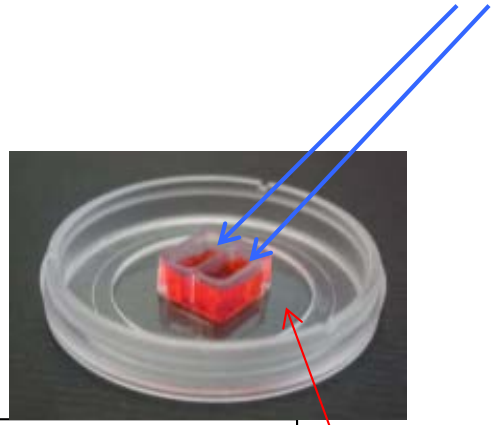
TTD/XP-D

XP/XP-D

Arseni L *et al. PNAS, 2015*

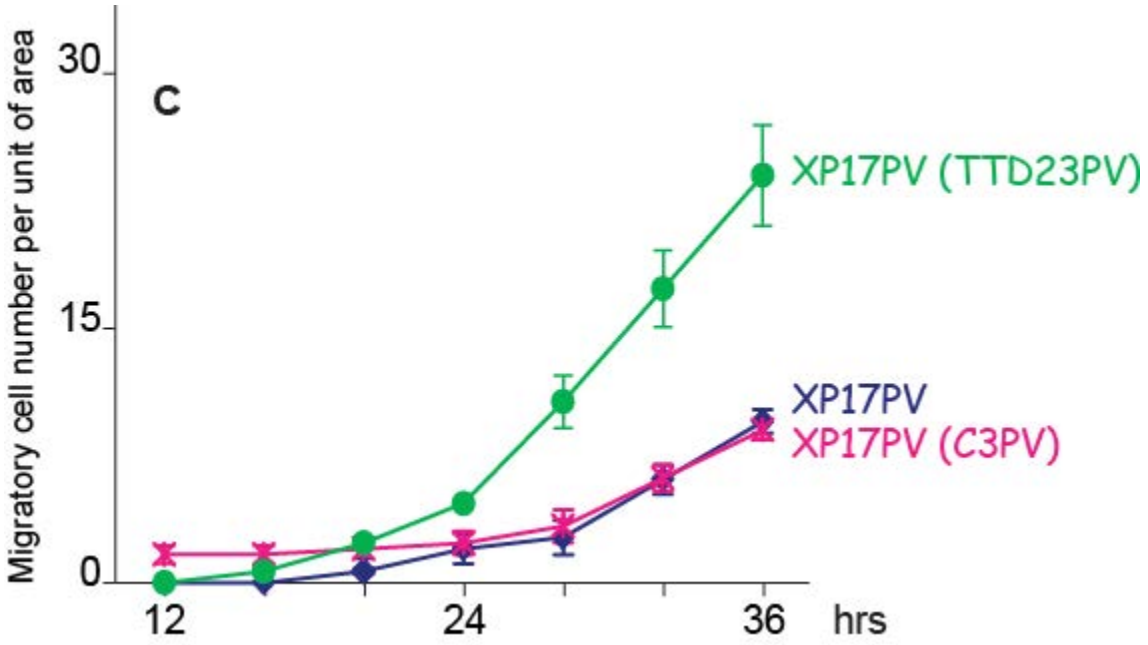
TTD cells secrete soluble factors capable of influencing cell migration

XP fibroblasts

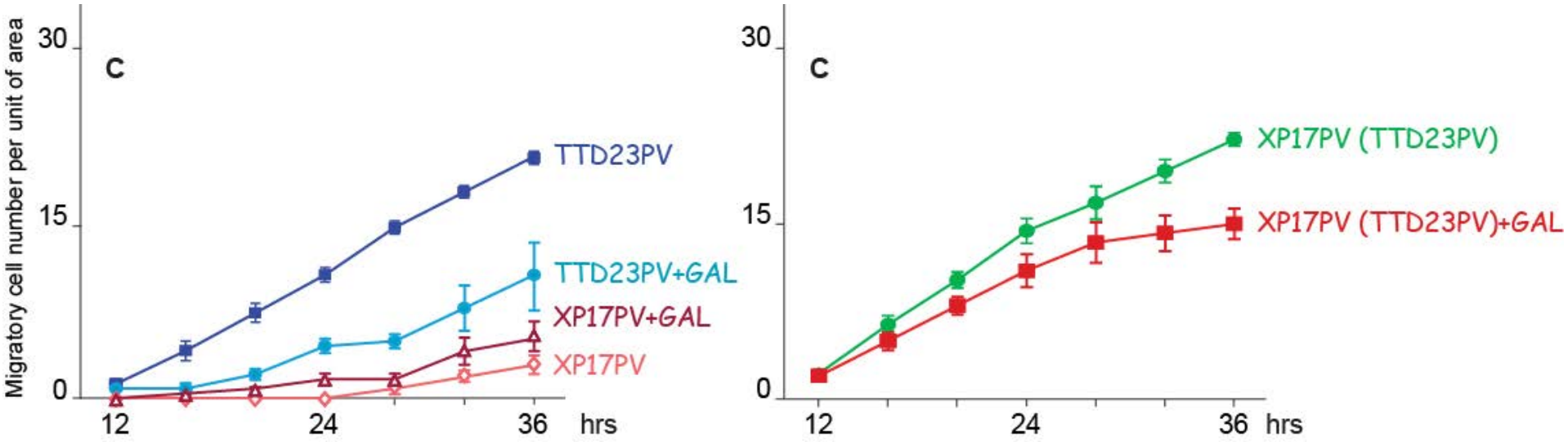


Wound healing assay in co-culturing conditions

Normal or
TTD fibroblasts



Synthetic MMP inhibitor (GAL) reduces the migration speed of TTD cells

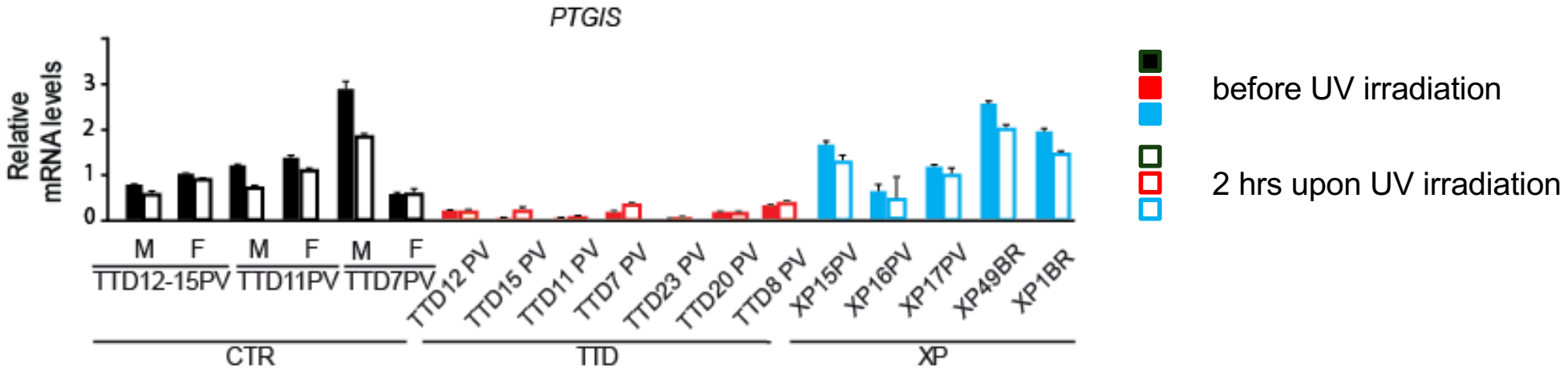


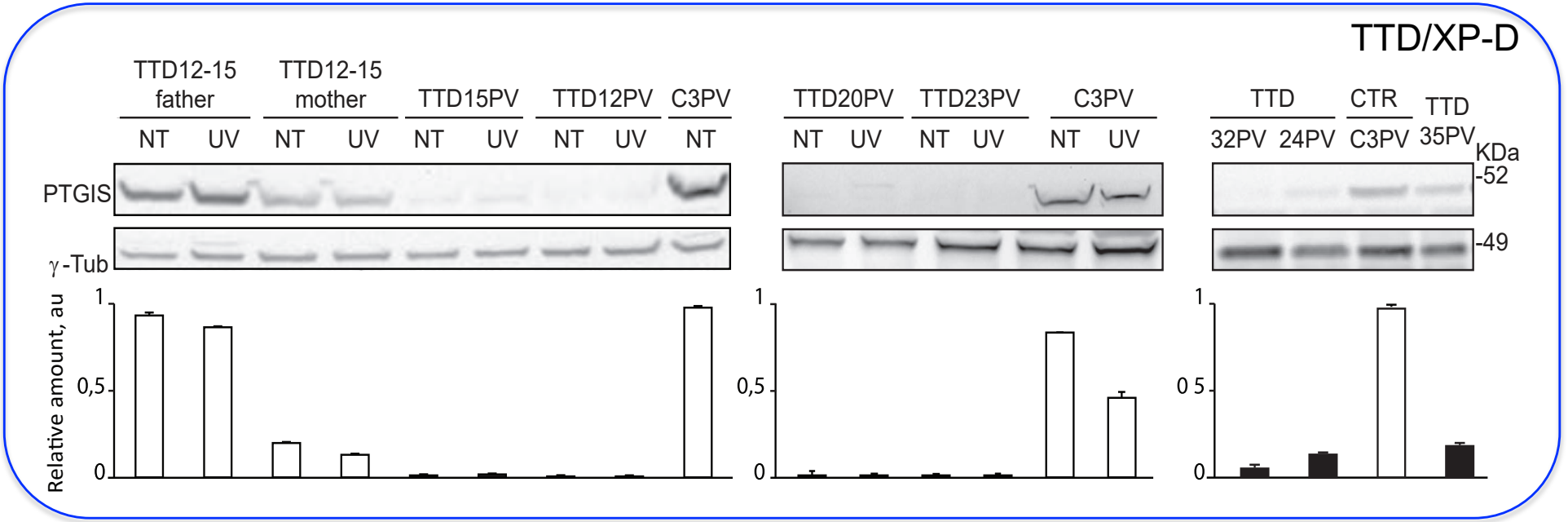
MMP-dependent degradation of ECM is the main event responsible for TTD migration defect

Could the *COL6A1* transcription deregulation account for the progressive muscle weakness, joint contractures and hypermobility of the distal joints observed in several TTD patients?

Could *MMP-1* over-expression explain the progressive TTD bone alterations?

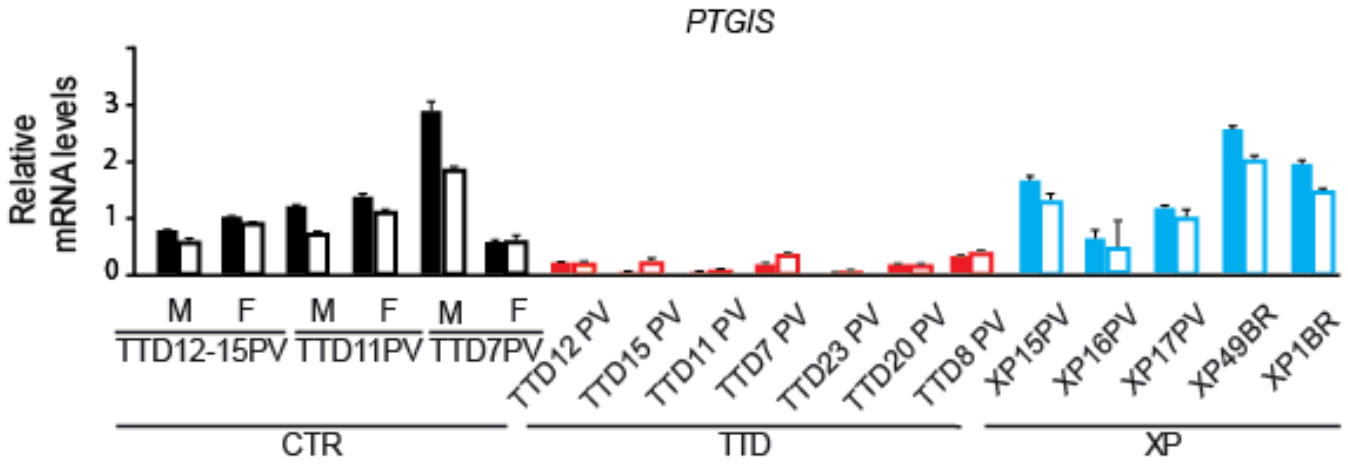
Reduced level of Prostaglandin I_2 Synthase is specific of TTD





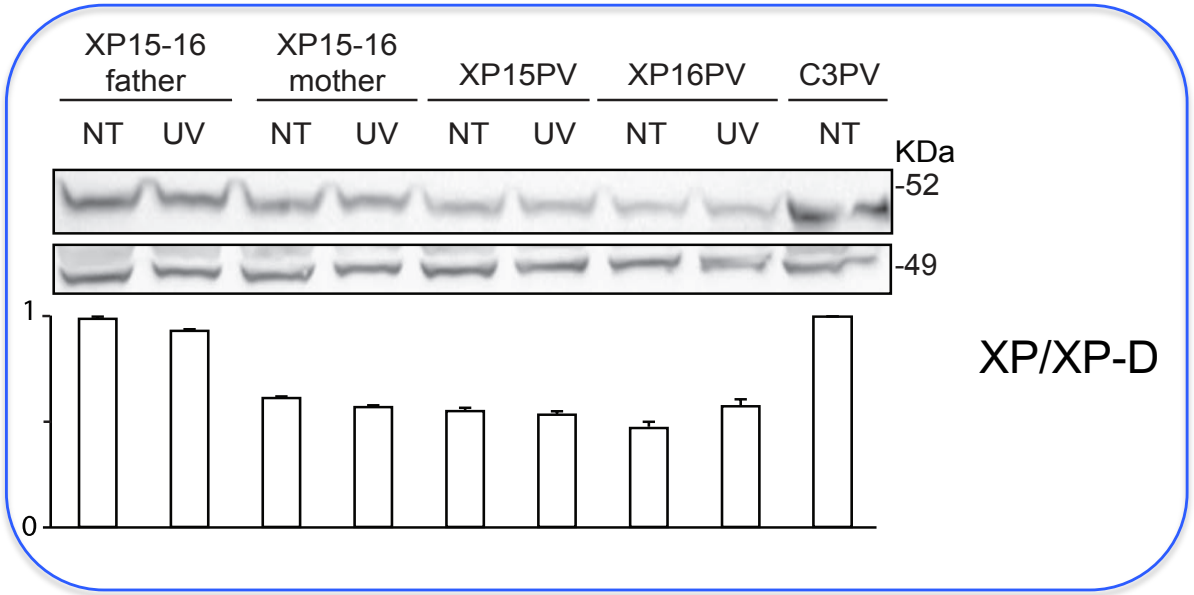
Lombardi A *et al. PNAS, 2021*

Reduced level of Prostaglandin I_2 Synthase is specific of TTD

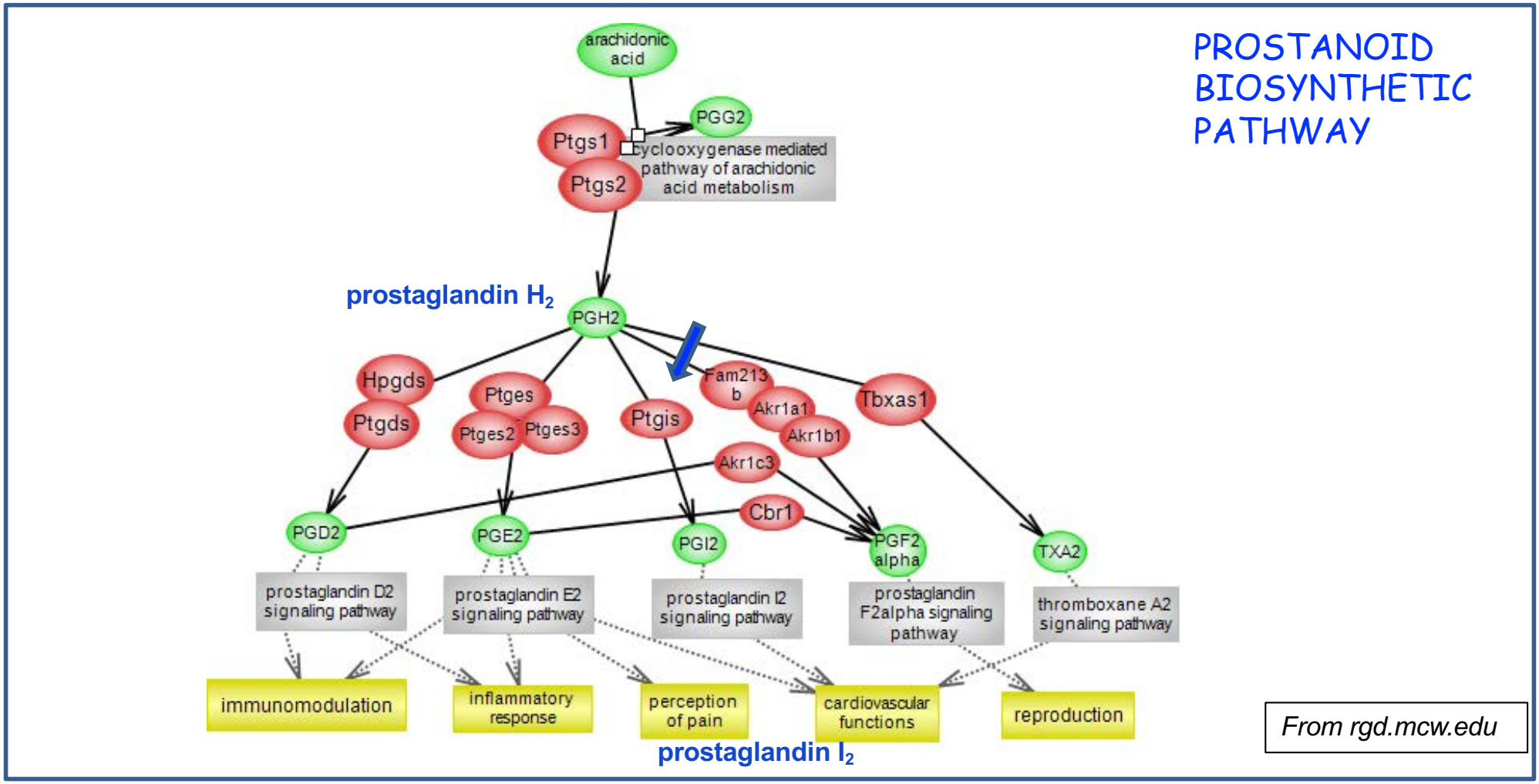


before UV irradiation

2 hrs upon UV irradiation



Lombardi A *et al. PNAS, 2021*



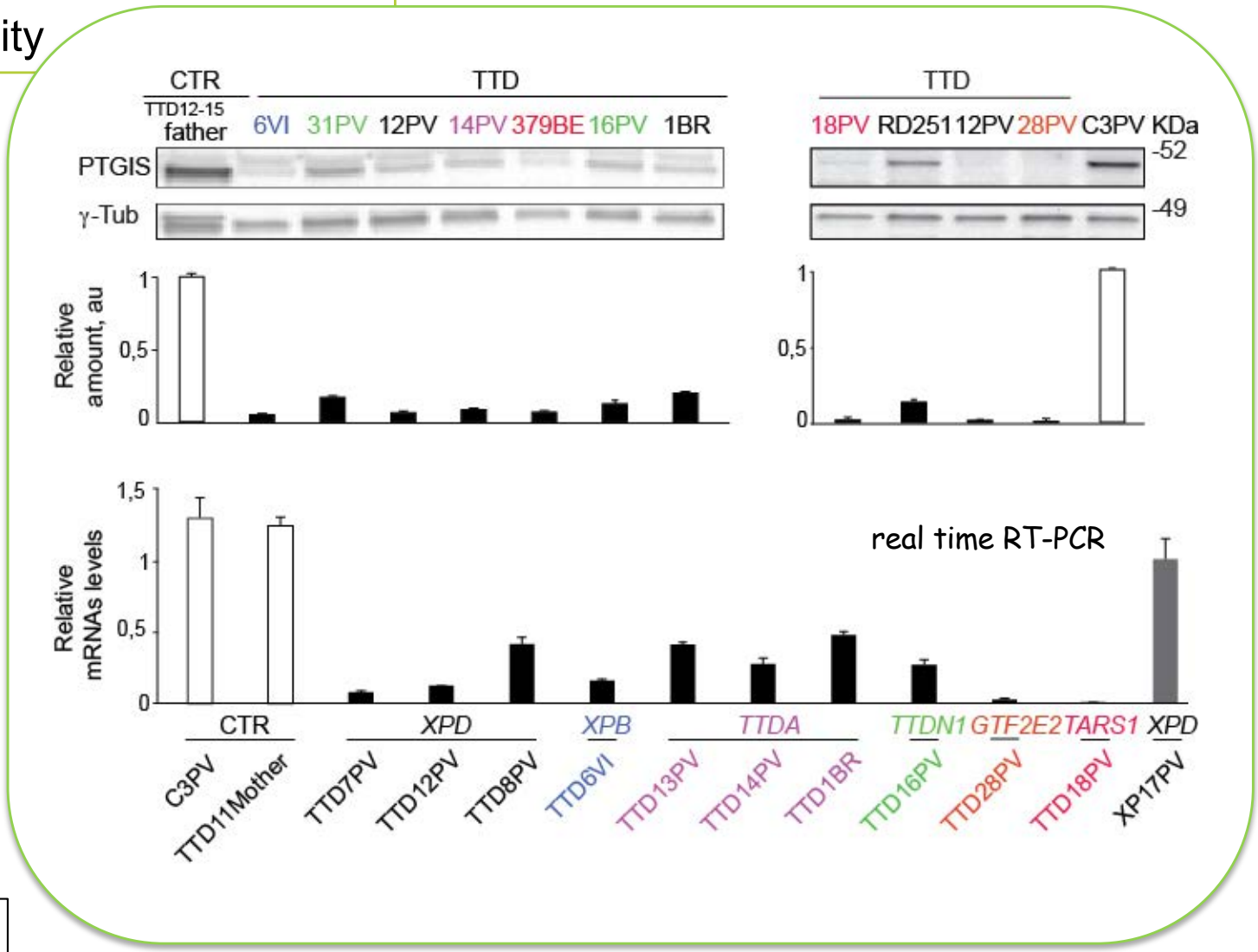
- PTGIS is implicated in the last step of prostaglandin I_2 (PGI_2) biosynthesis;
- PGI_2 is an active lipid with cardiovascular functions: it is a potent endogenous vasodilator and inhibitor of platelet aggregation;
- PGI_2 regulates the innate and adaptive immune system as well as adipocyte metabolism.
- PGI_2 over-production appears to sustain cancer progression.

Could some of TTD clinical features, including the weakening of the immune system, lack of subcutaneous fat and eventually the lack of skin cancer, result from reduced PGI_2 levels?

Non-photosensitive form of TTD (NPS-TTD)

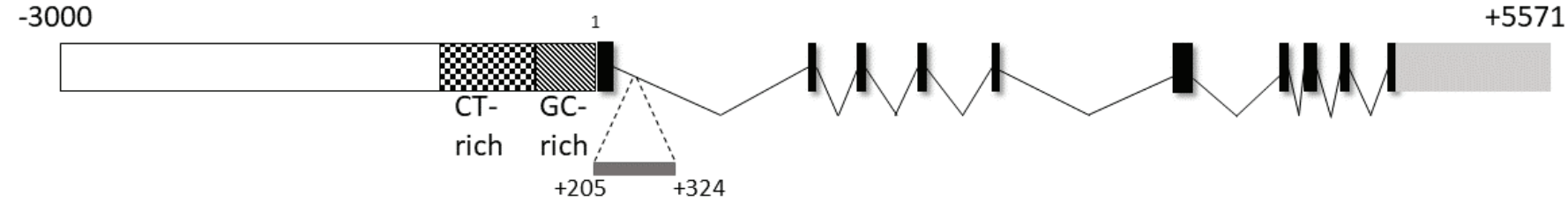
about 50% of the reported TTD cases

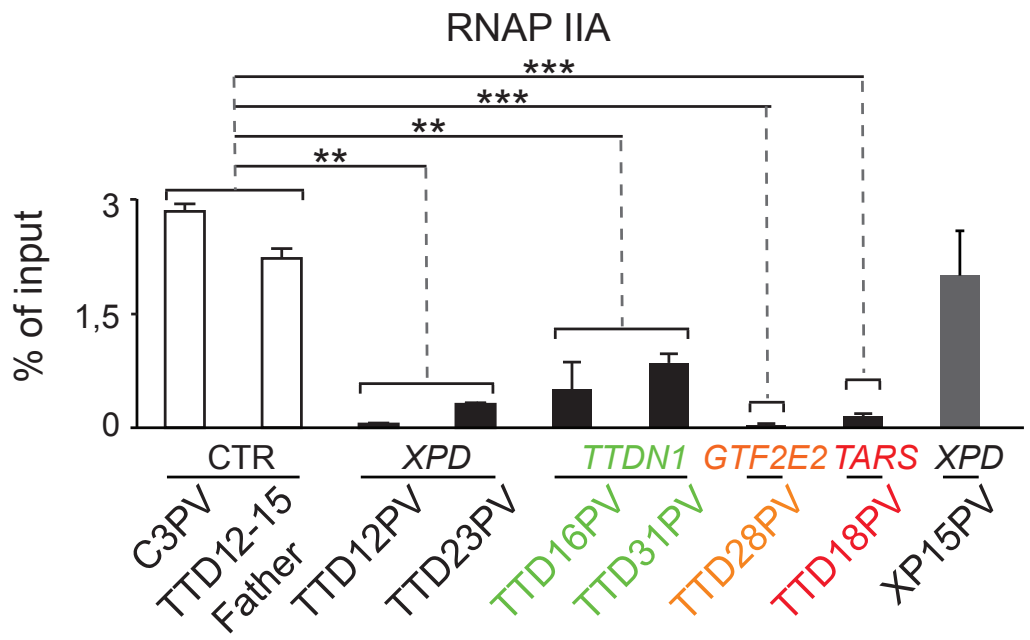
- ✓ Normal cellular response to UV
- ✓ Normal TFIIH
- ✓ Normal NER activity

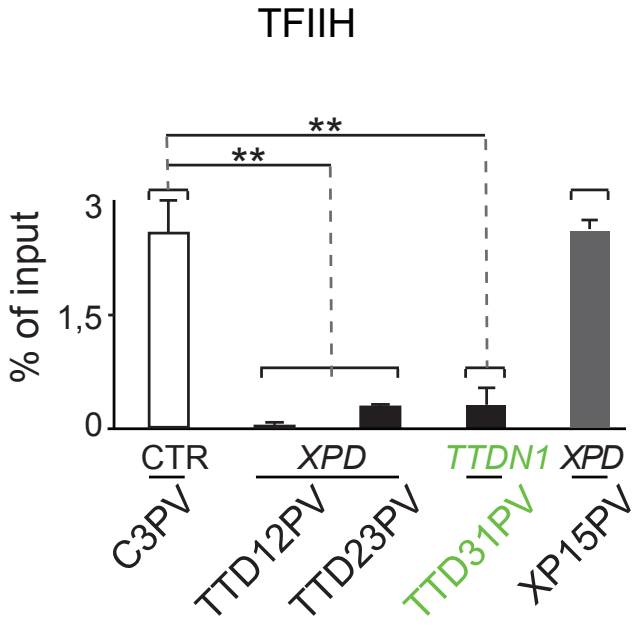


Lombardi *et al. PNAS, 2021*

No recruitment of RNA polymerase II transcriptional apparatus on *PTGIS* promoter in TTD cells







Lombardi A *et al. PNAS, 2021*

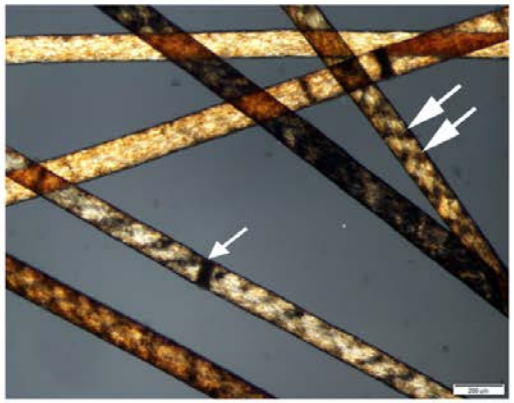
PTGIS reduction appears a gold standard marker for TTD, which may contribute to the clinical diagnosis

Non-photosensitive form of TTD (NPS-TTD)

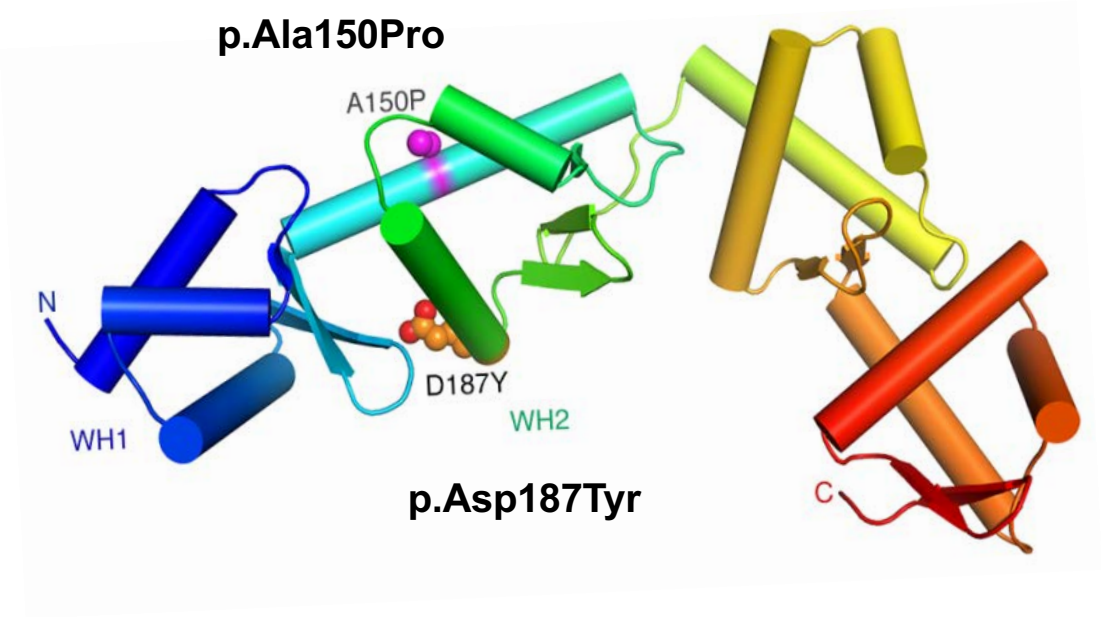
about 50% of the reported TTD cases

- ✓ Normal cellular response to UV
- ✓ Normal TFIIH
- ✓ Normal NER activity

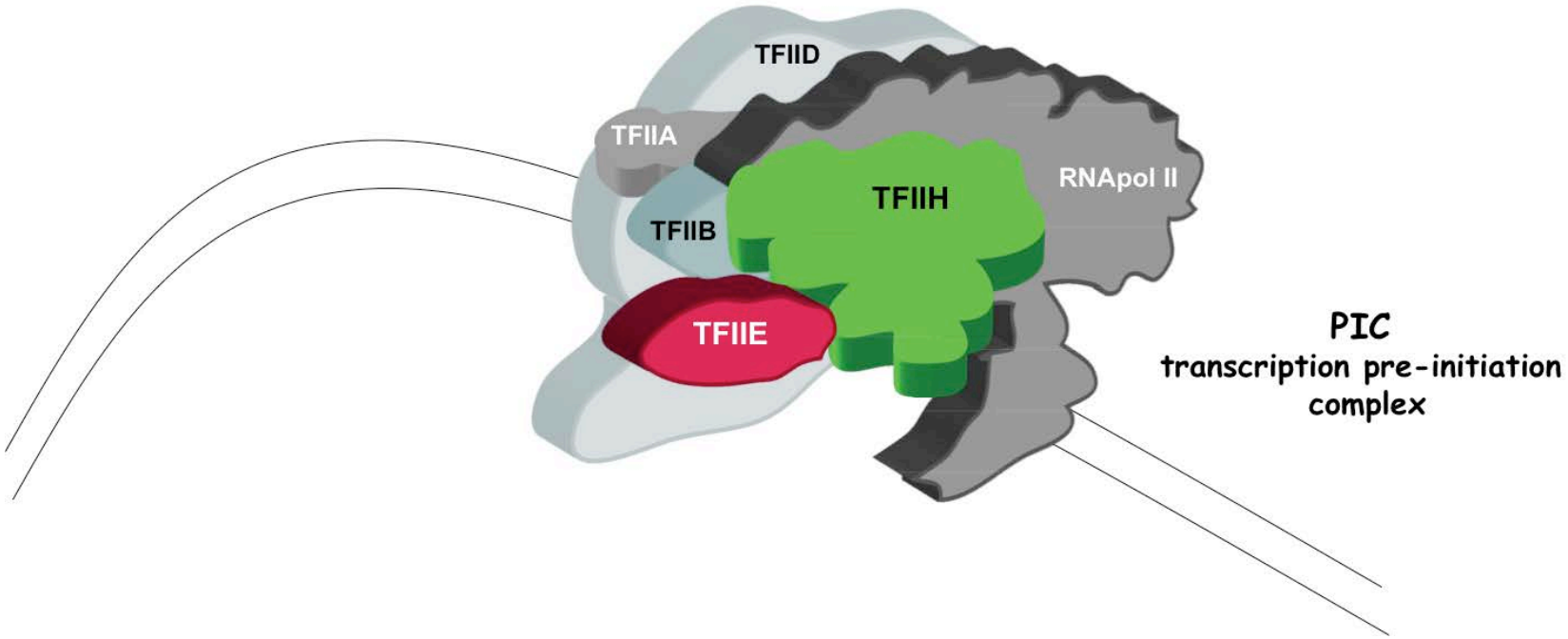
In collaboration with Prof. Ken Kraemer



Two TTD cases homozygotes for distinct missense mutations in ***GTF2E2*** encoding the 34 kDa TFIIEβ protein



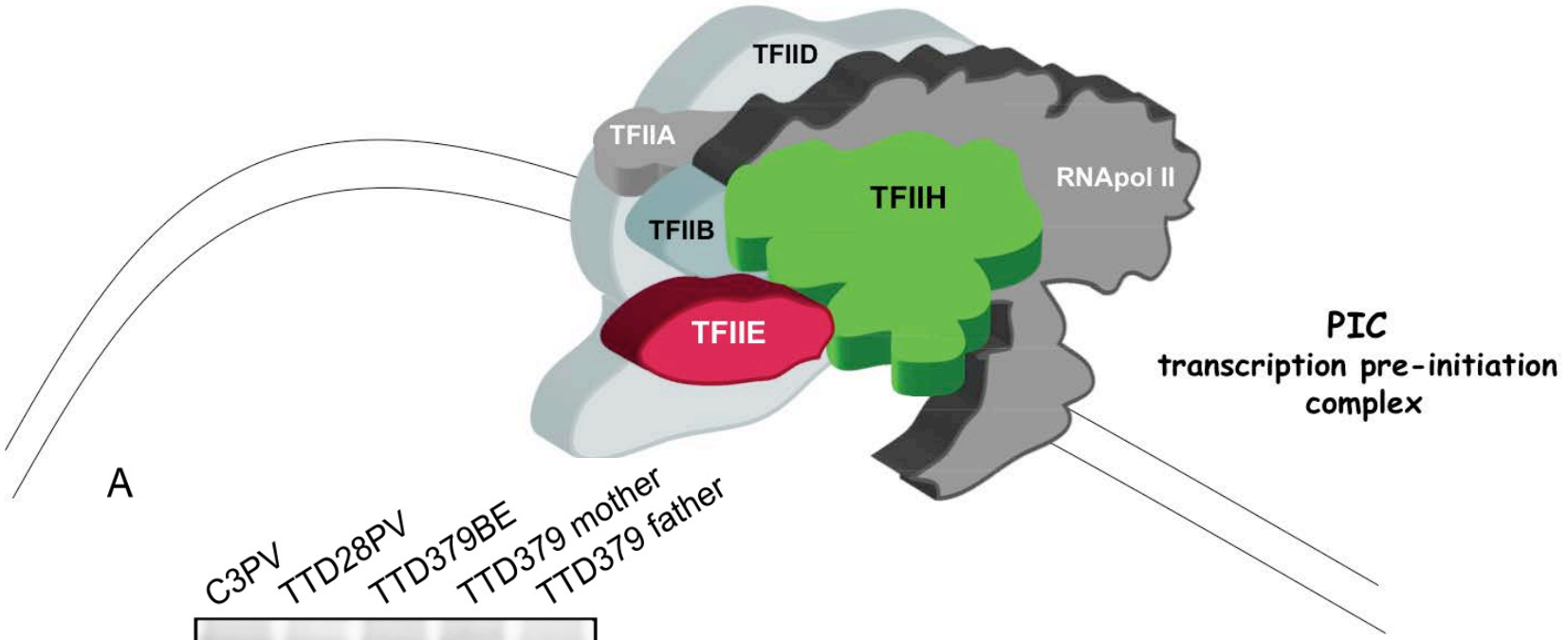
Kuschal C, Botta E et al. *Am J Hum Genet*, 2016

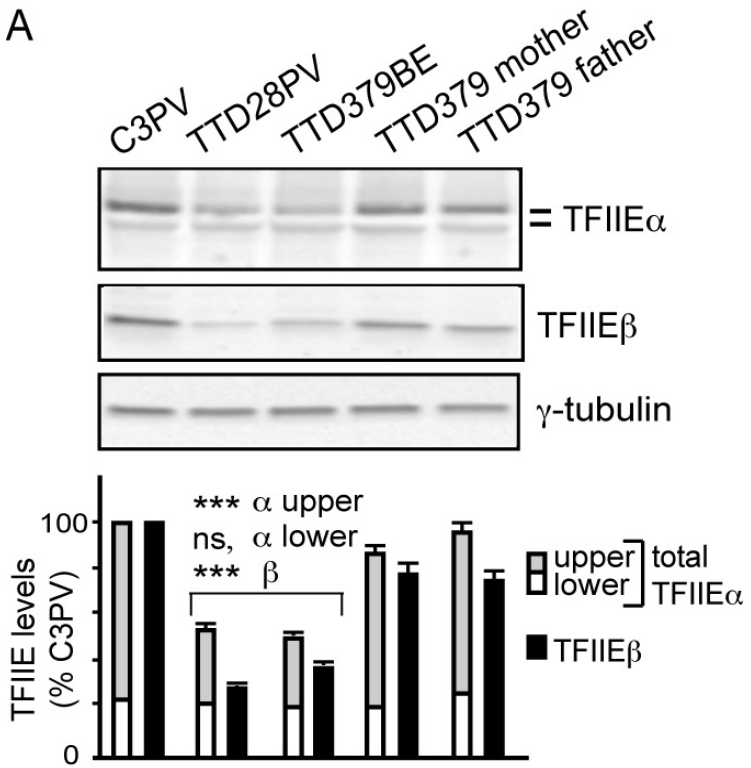


- TFIIE regulates the ATPase and kinase activity of TFIIH
- TFIIH phosphorylates TFIIEα

  [Ohkuma and Roeder RG, 1994. *Nature 368,* 160-3].

GTF2E2 mutations destabilize the general transcription factor complex TFIIE in individuals with DNA Repair-proficient TTD





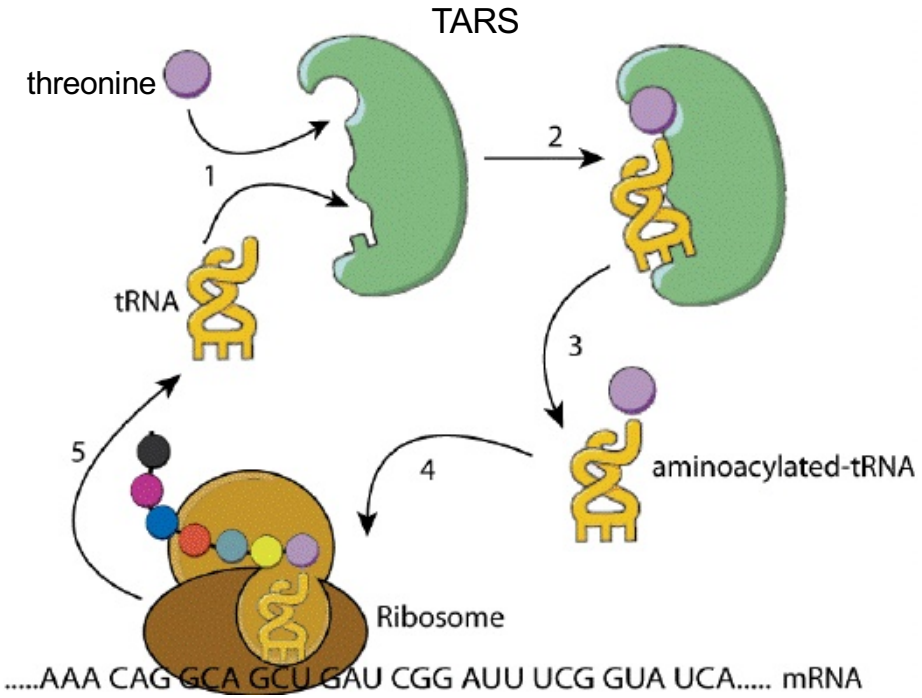
GTF2E2-defective primary fibroblasts:

- Normal UV damage repair ability
- Normal levels of TFIIH complex
- Reduced levels of TFIIE complex

Kuschal C, Botta E et al. *Am J Hum Genet*, 2016

In collaboration with Prof. Wim Vermeulen

TTD is a ‘’gene-expression’’ disorder



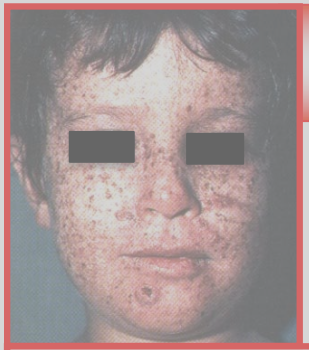
Protein instability associated with AARS1 and MARS1 mutations causes Trichothiodystrophy-related features

Theil A, Botta E *et al. Am J Hum Gen*, 2019

Bi-allelic TARS mutations are associated with brittle hair phenotype

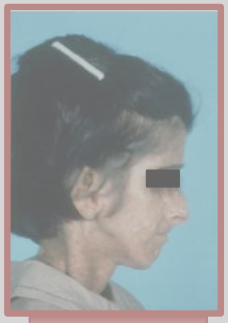
Botta E, Theil A *et al.* Hum Mol Genet, 2021

NER-defective hereditary disorders



XERODERMA PIGMENTOSUM
- XP-

Cutaneous alterations and tumors in the sun-exposed areas of the skin



XP/CS

XPA, XPC, XPE, XPF

XPG ***XPB, XPD*** ***TTDA/p8***

COCKAYNE SYNDROME
-CS-

CSA, ***CSB***

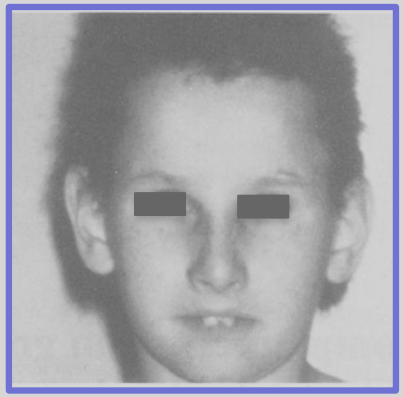
Premature aging

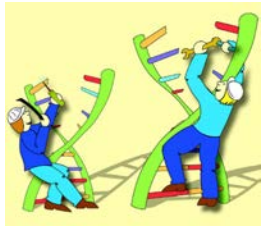
Physical and mental retardation

Neurodegeneration

TRICHOTHIODYSTROPHY
PS-TTD

Hair abnormalities

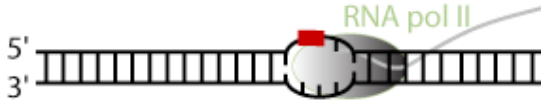


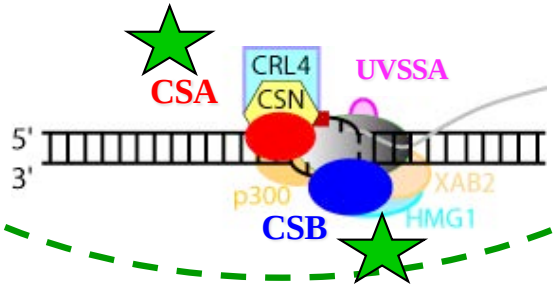


NER sub-pathways in human cells

Global genome repair

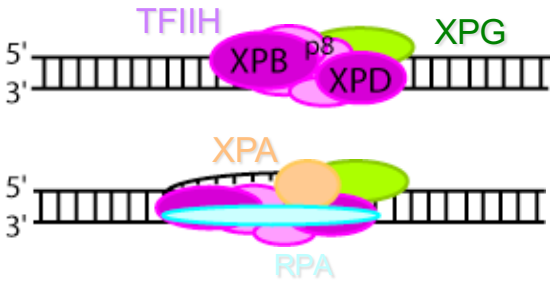
Transcription-coupled repair





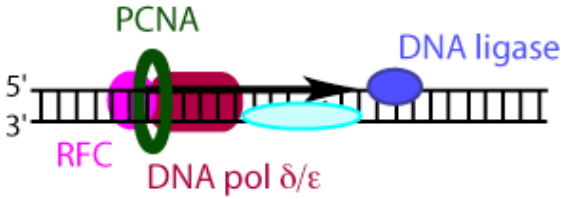
Damage recognition

Open complex formation

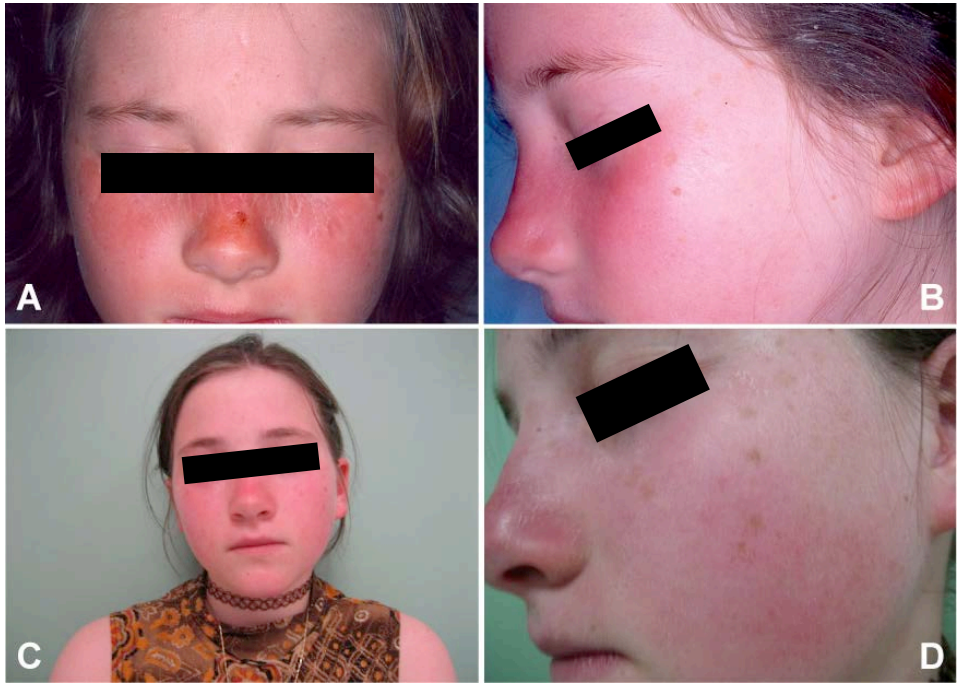


Incision / Excision

DNA repair synthesis
Ligation



A UV-sensitive syndrome patient with a specific CSA mutation reveals separable roles for CSA in response to UV and oxidative DNA damage



In collaboration with Prof. Alain Sarasin

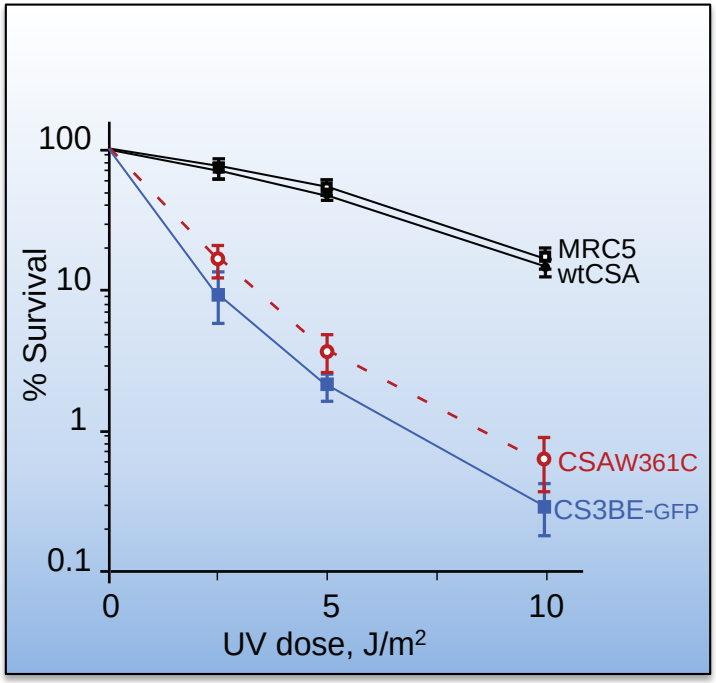
Clinical features:

- Extreme sun-sensitivity with erythema and freckling;
- No cutaneous tumors;
- Physical and mental development within normal ranges.

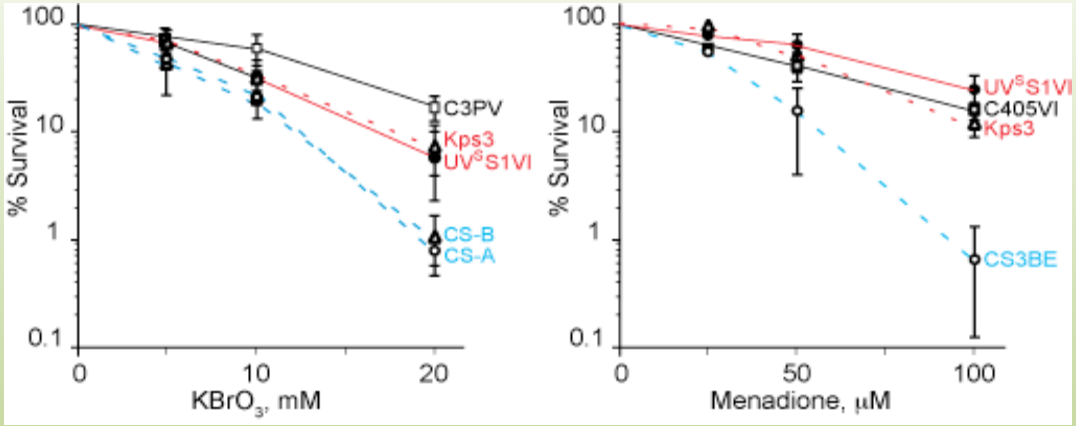
Molecular defect: the patient is homozygous for a novel missense mutation (p.Trp361Cys) in the *ERCC8/CSA* gene.

Nardo T et al. PNAS, 2009

Cellular features: patient cells showed an altered response to UV similar to that typically observed in CS-A and CS-B fibroblasts (TC-NER defects).



Differently from all the other CS-A cells showed a normal sensitivity to oxidative stress



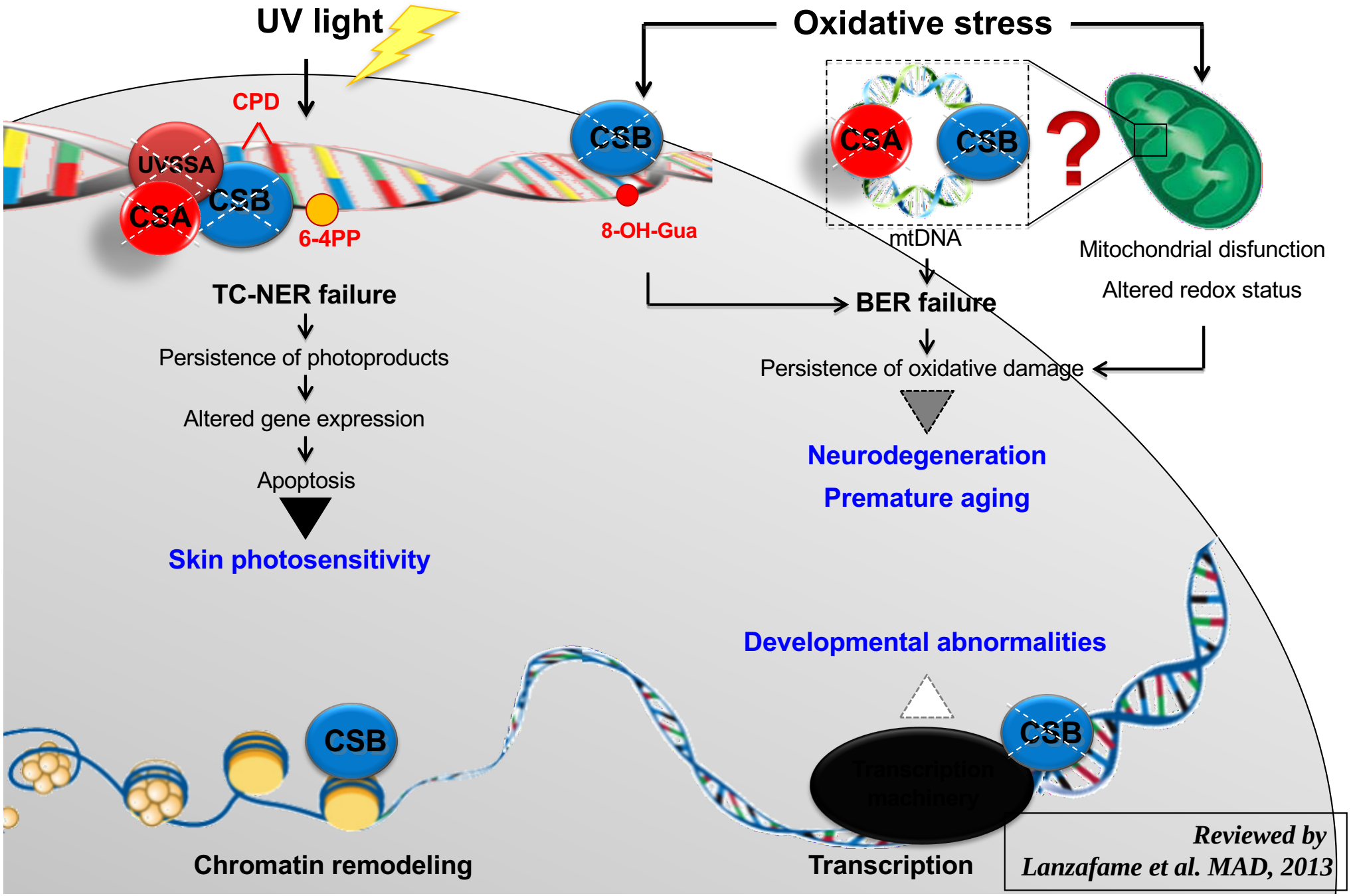
The Trp361Cys mutation impairs the CSA activity in TC-NER dependent removal of UV-induced DNA damage but does not affect the role of CSA in the oxidative stress response.

Nardo T et al. PNAS, 2009

✓ Defective TC-NER mainly results in cutaneous alterations

✓ The neurological and physical deterioration with premature ageing features that affect CS patients may reflect additional roles of the CSA (or CSB) protein outside TC-NER

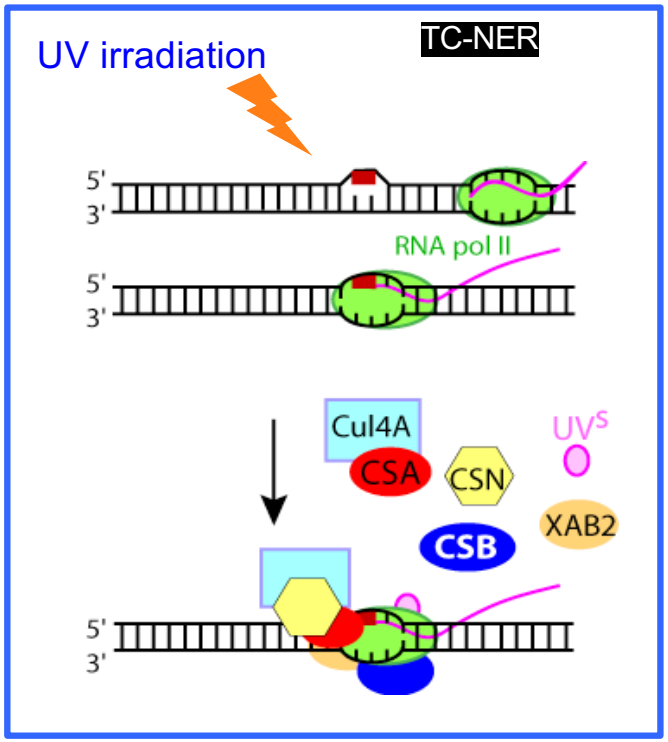
Multifaceted role of the CS proteins

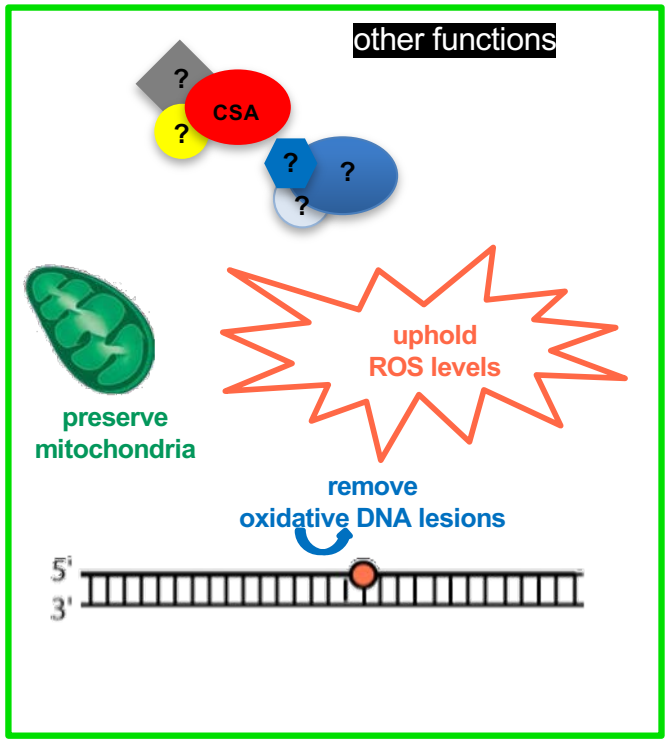


Reviewed by
Lanzafame et al. MAD, 2013

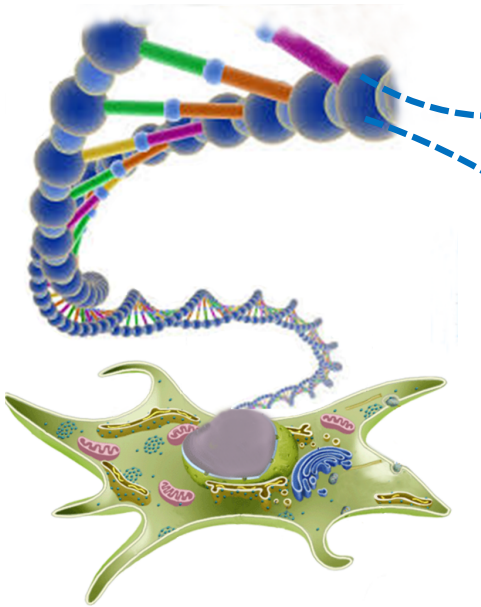
working hypothesis

May CSA interact with different activities/complexes to perform its various functionalities?

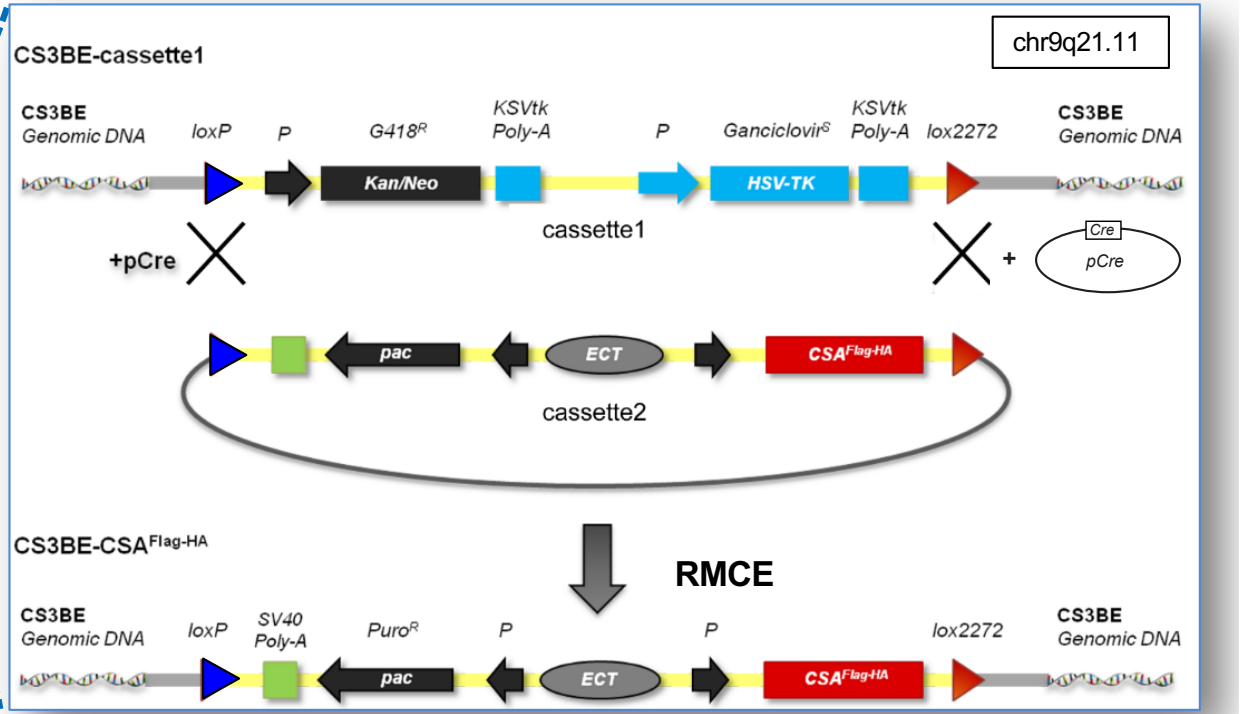




Generation of isogenic cell lines expressing “normal” levels of wtCSA$^{Flag\text{-}HA}$



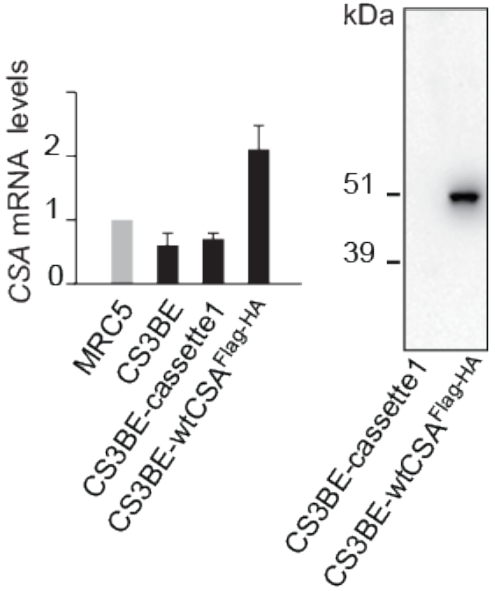
CS3BE/CS-A cells

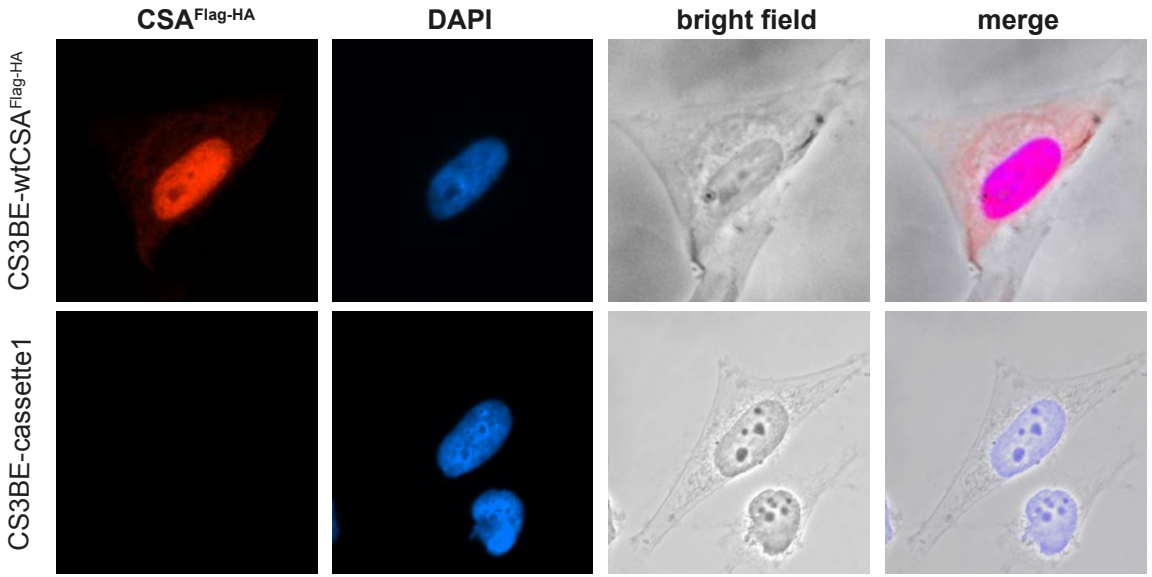


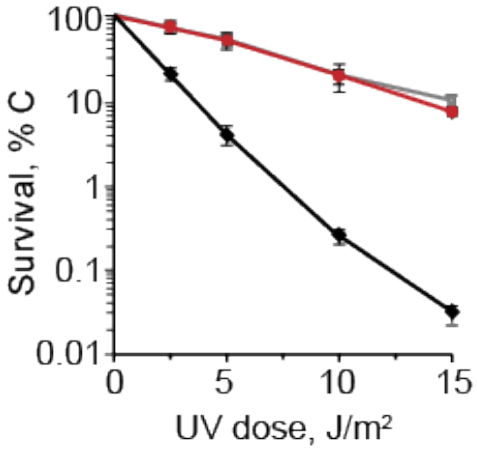
wtCSA$^{Flag\text{-}HA}$

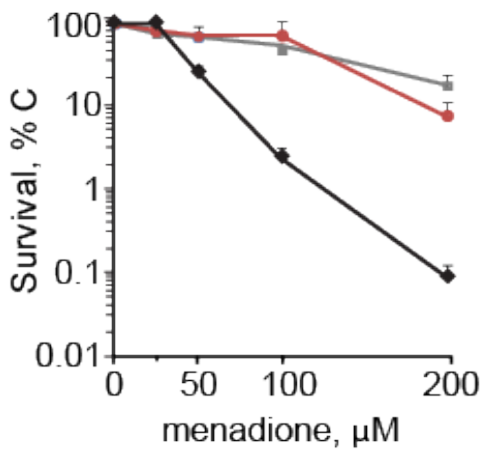
✓single-copy integration of a DNA cassette expressing wtCSA$^{Flag\text{-}HA}$ into chr9q21.11

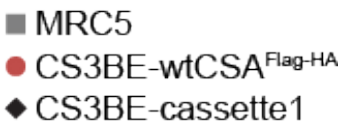
Lanzafame M, Branca G *et al. NAR*, 2021











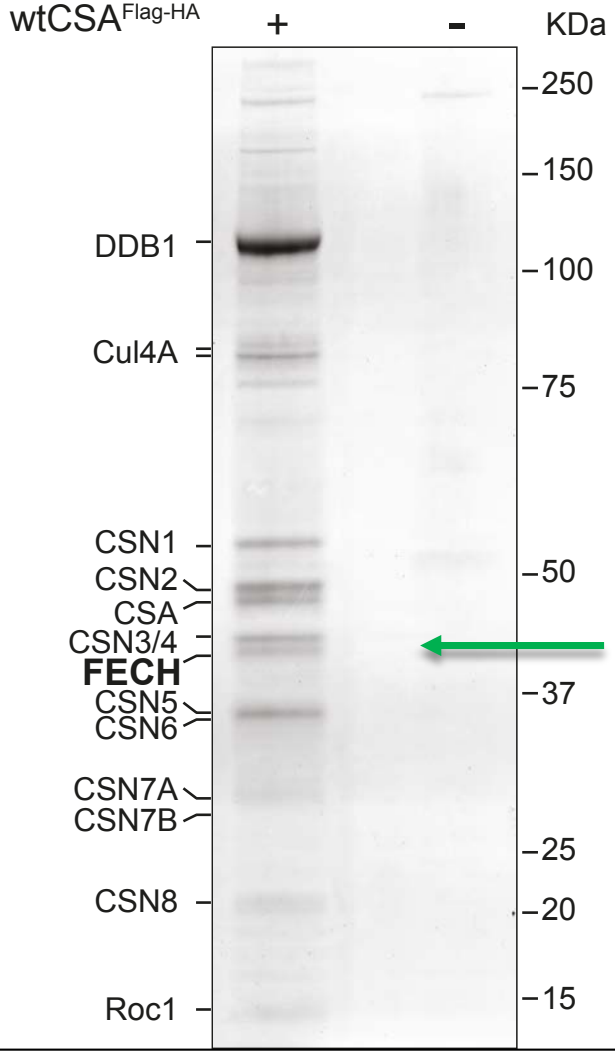
Lanzafame M, Branca G *et al. NAR,* 2021

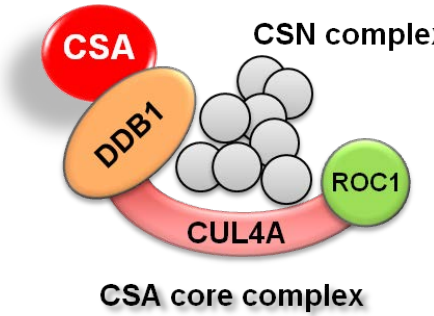
Identification of novel CSA-interactors

Tandem Affinity Purification (TAP)

Mass Spectrometry (MS)

All proteins of the CSN and CSA-core complex have been identified





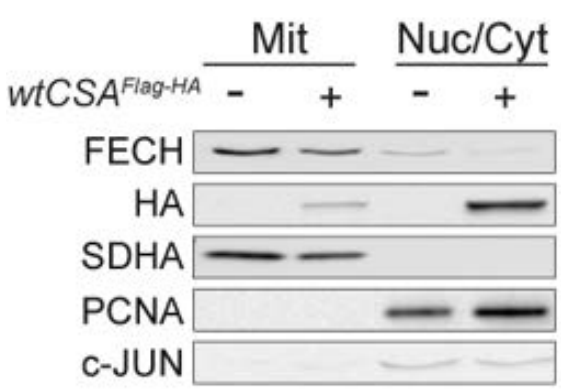
Ferrochelatase (FECH)

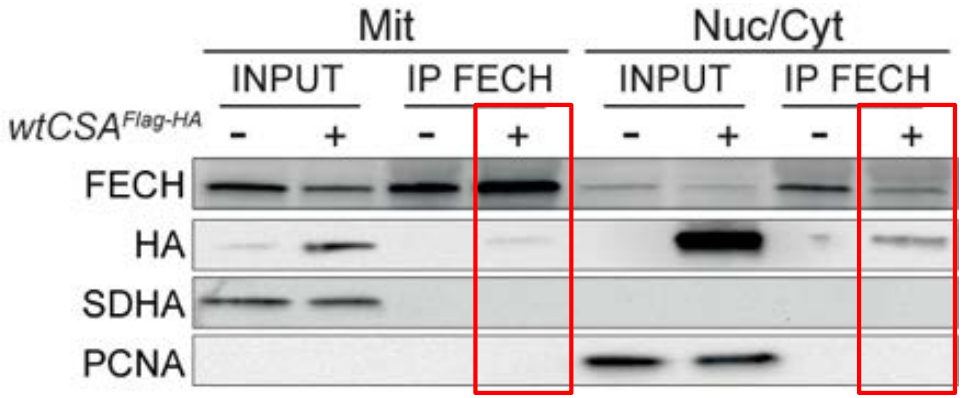
Ferrochelatase (FECH) is a novel interactor of CSA

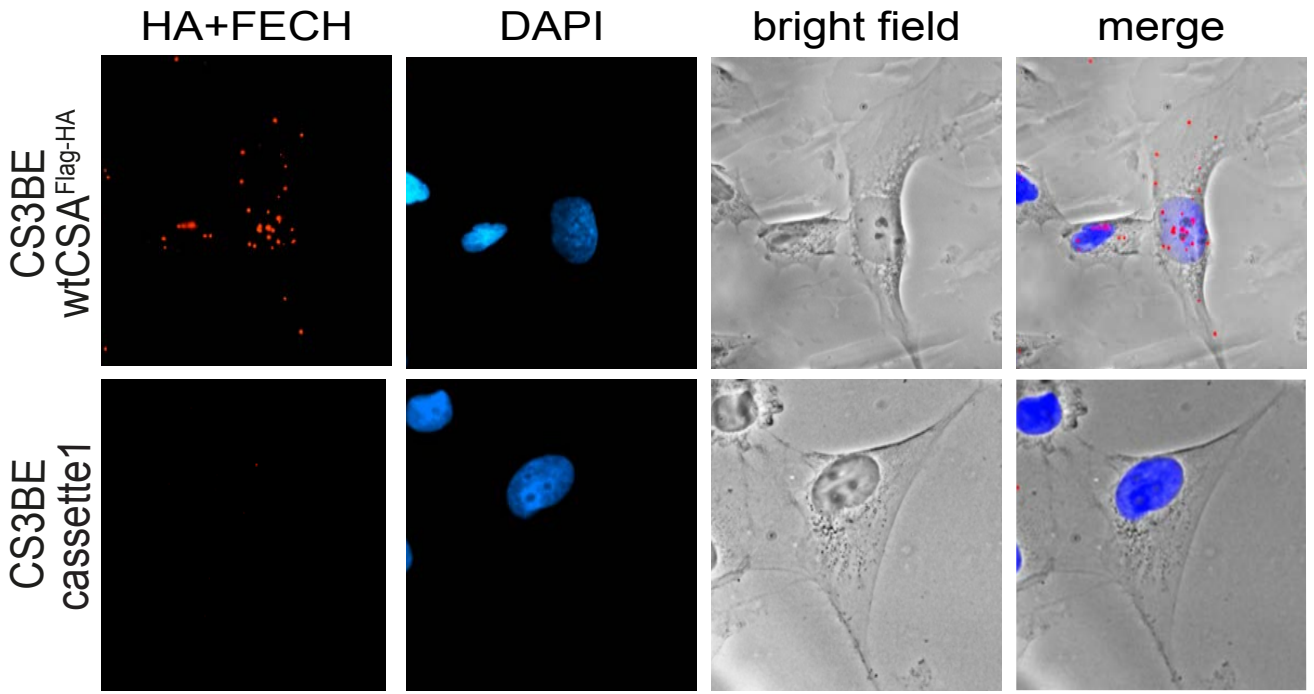
- **FECH is a mitochodrial protein.** It is the terminal enzyme in the heme biosynthesis pathway where it catalyzes the insertion of the ferrous form of iron (Fe^{2+}) into protoporphyrin IX.

- ***FECH* mutations cause erythropoietic protoporphyria (EPP),** characterised by severe and painful photo-toxic reactions in the skin and the liver, due to the accumulation of protoporphyrin in the blood.

CSA-FECH interaction mainly occurs in the nuclear compartment







Proximity Ligation Assay

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Identification of novel CSA-interactors

Tandem Affinity Purification (TAP)

Mass Spectrometry (MS)

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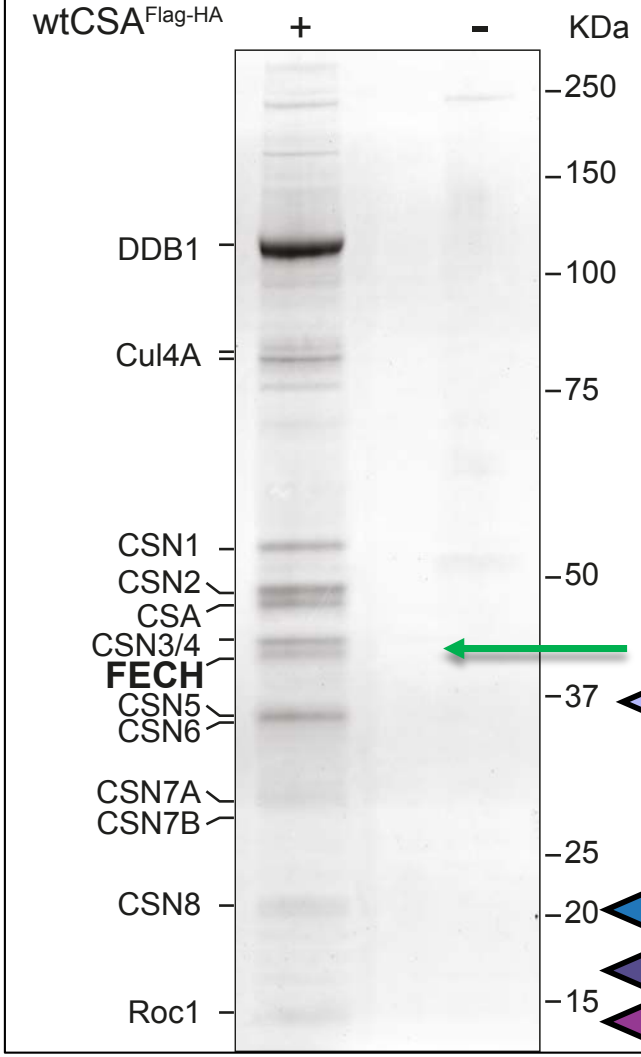
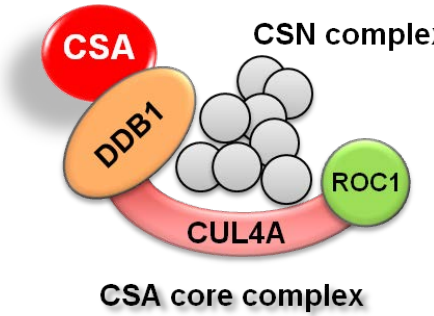




FECH

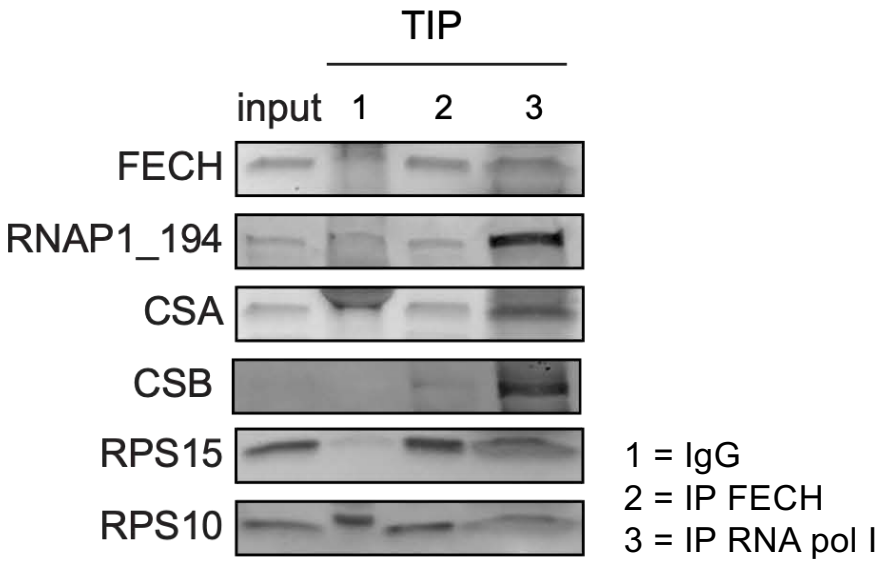
RPSA

RPS10

RPS15

RPS28

CS-FECH-RPs is a novel chromatin-bound protein complex associated to RNA polymerase I



Two-Step Co-Immunoprecipitation (TIP)

FECH-RNApol1-CSA-CSB-RPS10-RPS15 are part of a unique protein complex, named CS-FECH-RP

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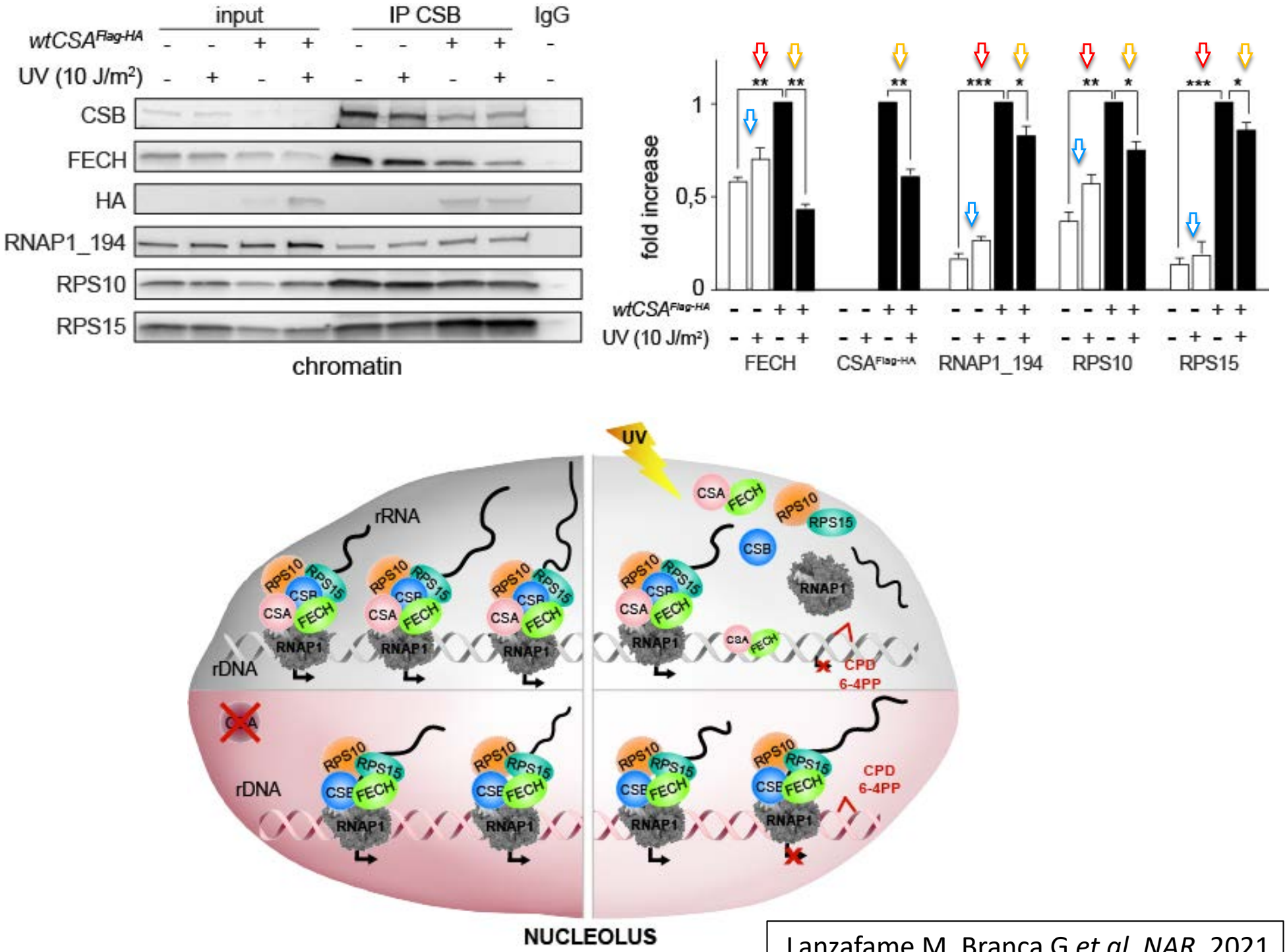

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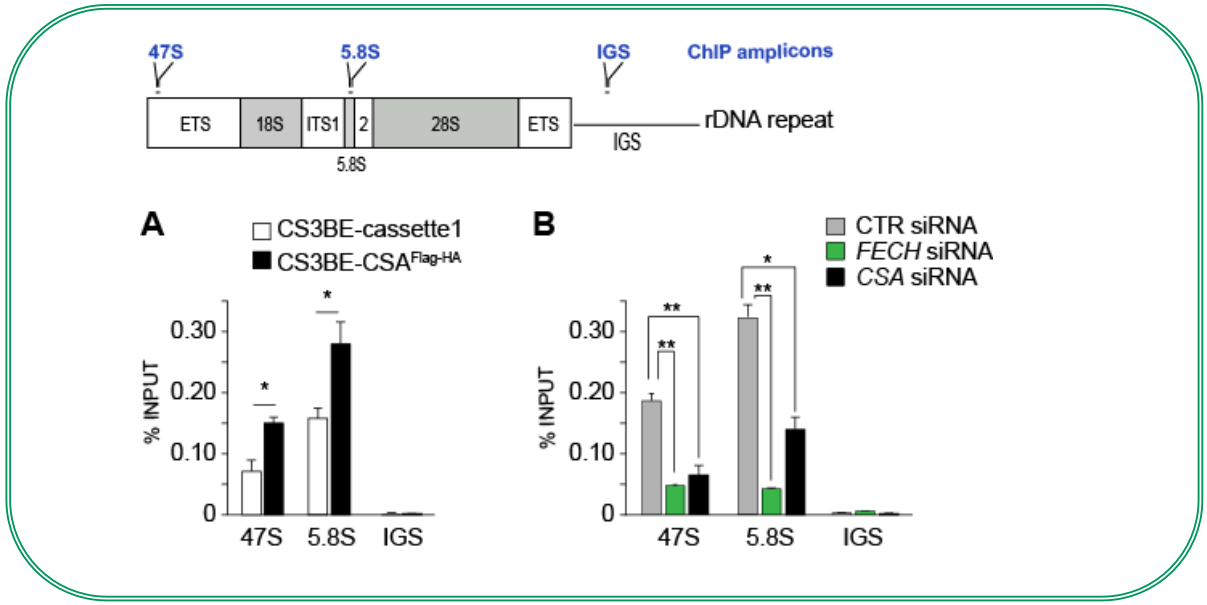
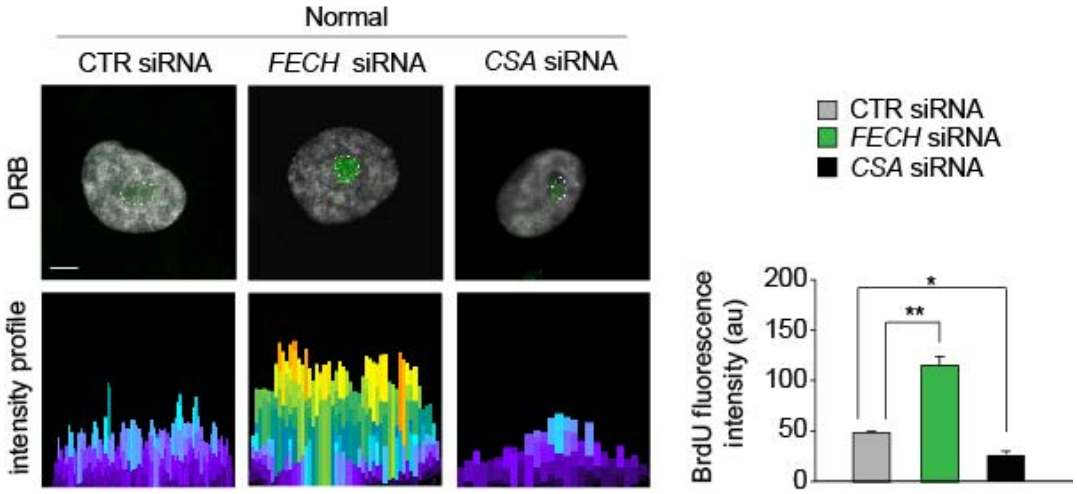


Chromatin-Immunoprecipitation (ChIP)



In situ Run-on

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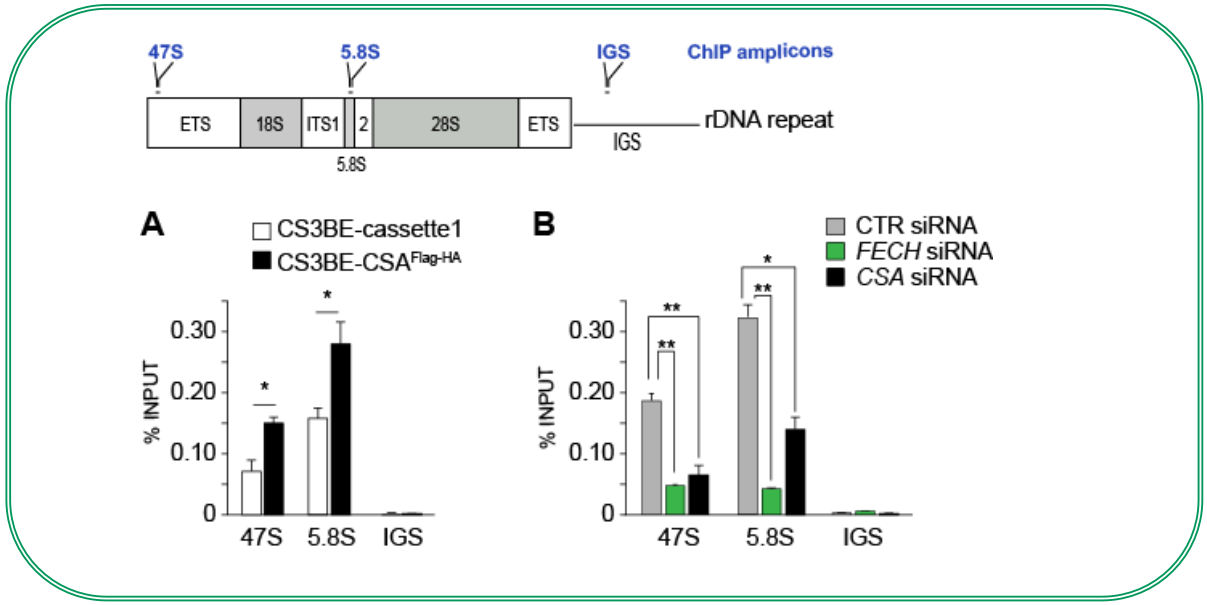
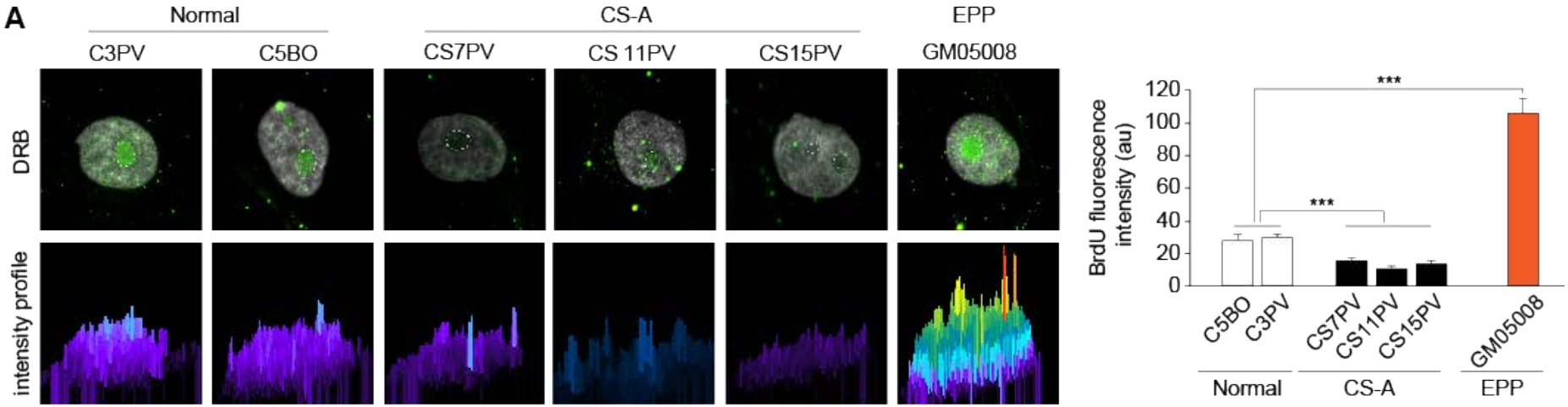


Chromatin-Immunoprecipitation (ChIP)



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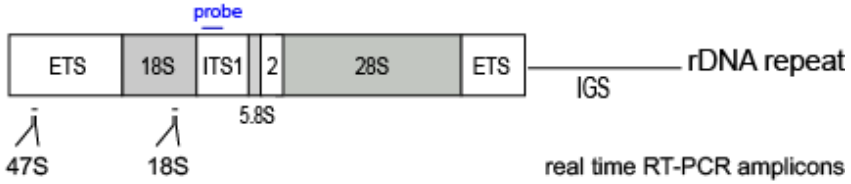
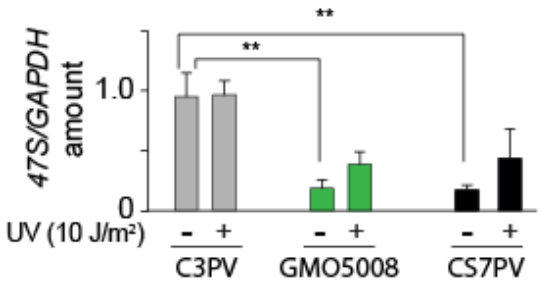
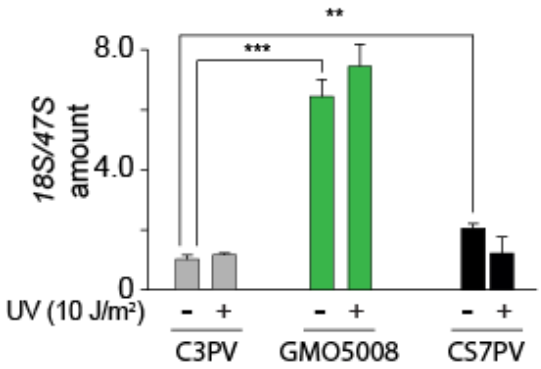
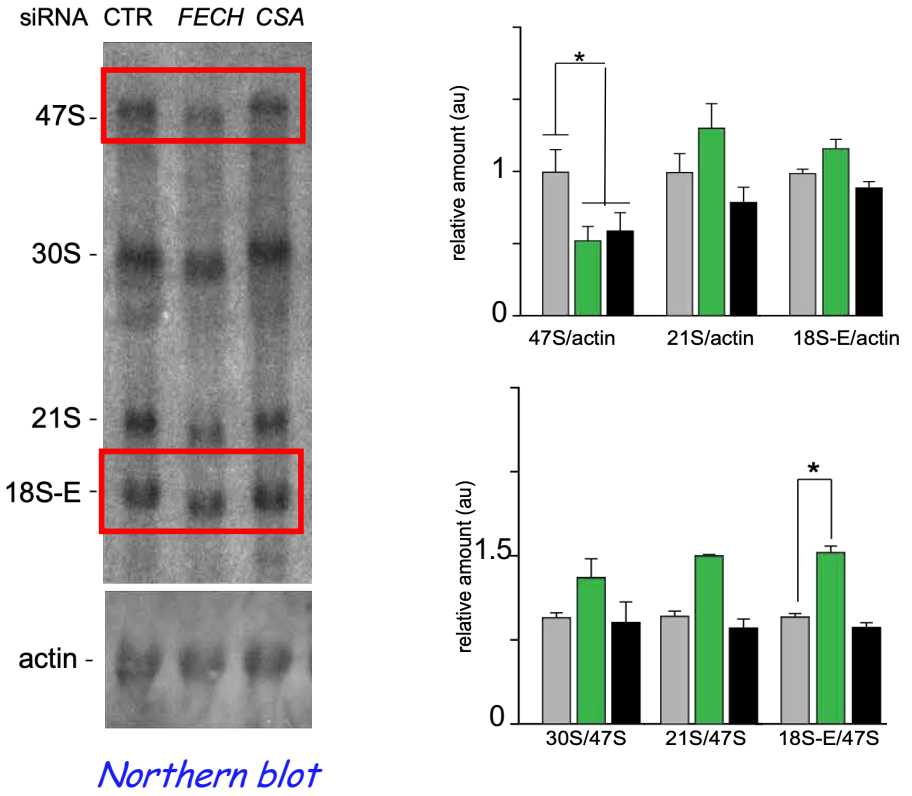
FECH and CSA play a role in nucleolar transcription and ribosomal biogenesis







Real time RT-PCR



Northern blot

Lanzafame M, Branca G *et al. NAR,* 2021

✓ The concerted action of CSA and FECH finely tune rRNA transcription and its response to UV irradiation

✓ CS and EPP patient cells reveal different ribosome biogenesis alterations

Could the altered function of CS proteins within the nucleoli account for the progeroid features of CS?

Miria Stefanini

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Manuela Lanzafame, CNR researcher	Anita Lombardi
Giulia Branca, post-doc	Debora Ferri
Erica Gandolfi, PhD student	Martina Uggè
Tiziana Nardo, CNR technician	
Roberta Ricotti, CNR technician	

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E. Dellambra e M. Teson, IDI Roma
L. Bini, University of Siena
V. Castelvetro, University of Pisa

CLINICIANS AND PATIENTS

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Italian Association XP (AIXP)

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- *Regione Lombardia*

AIRC

Associazione Italiana Xeroderma Pigmentoso