DNA damage, senescence and cancer

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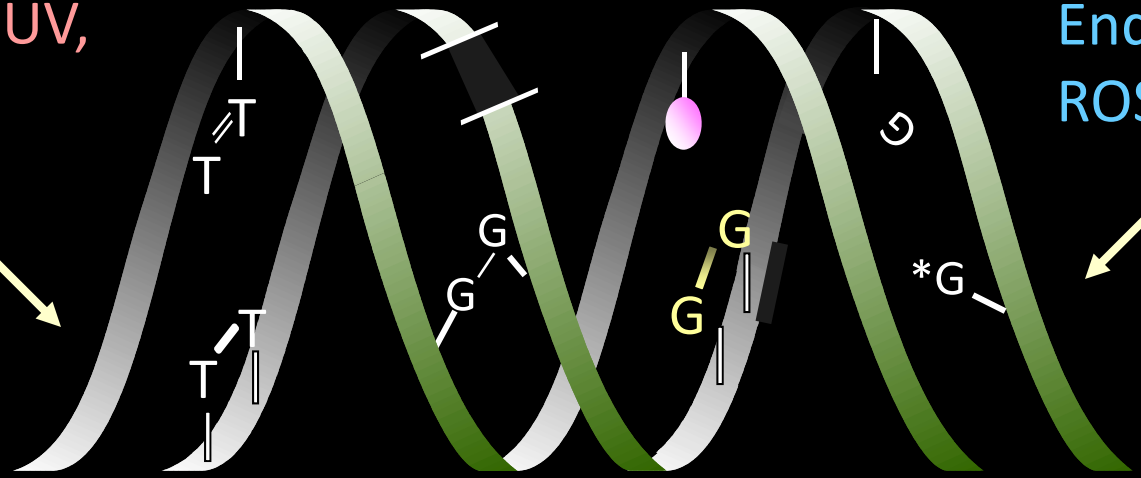
DNA damage

Exogenous: UV, radiation, chemicals

Endogenous: ROS, alkylation



misreplication

mutations

Cancer

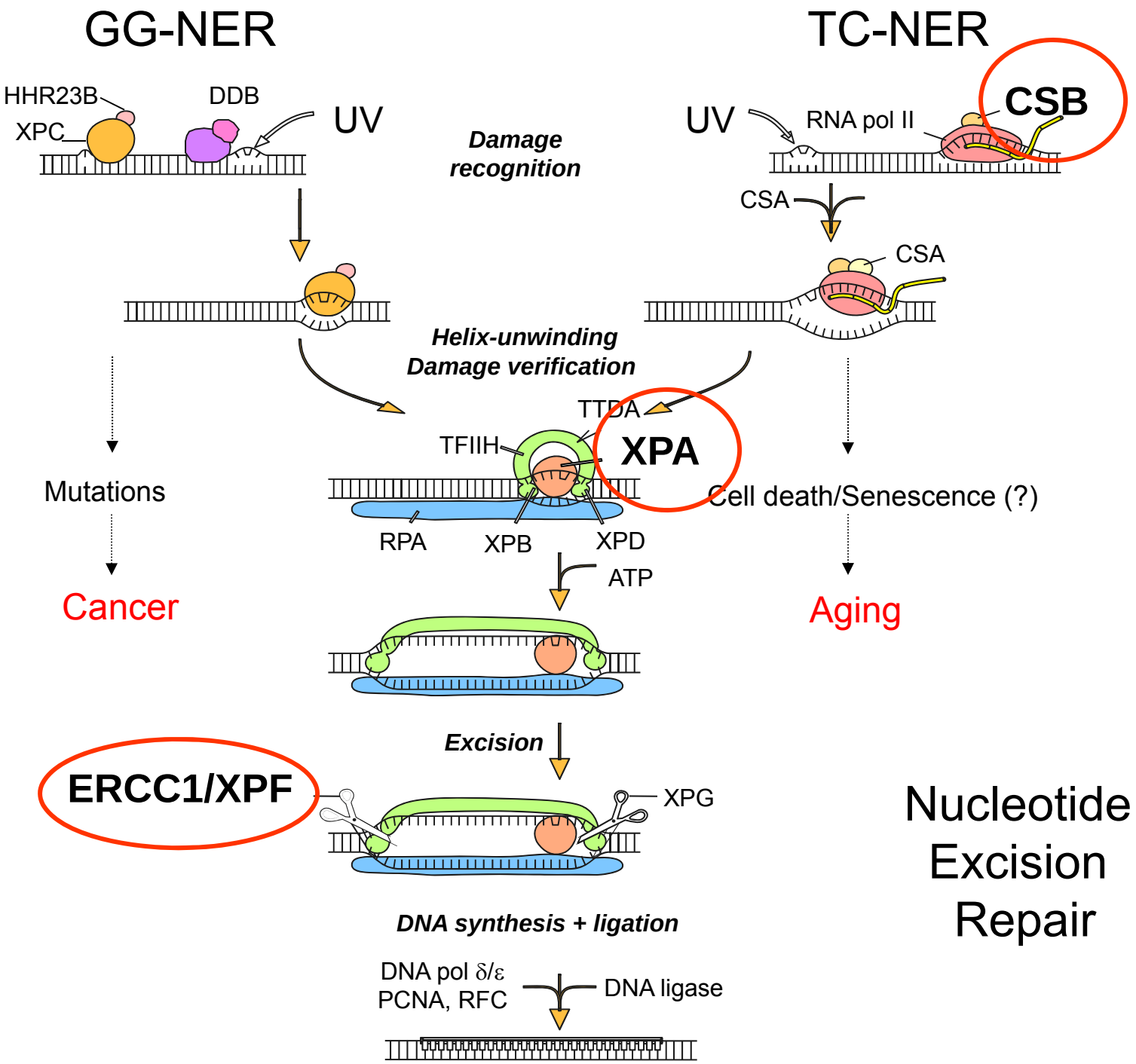
DNA repair systems

Developmental defects

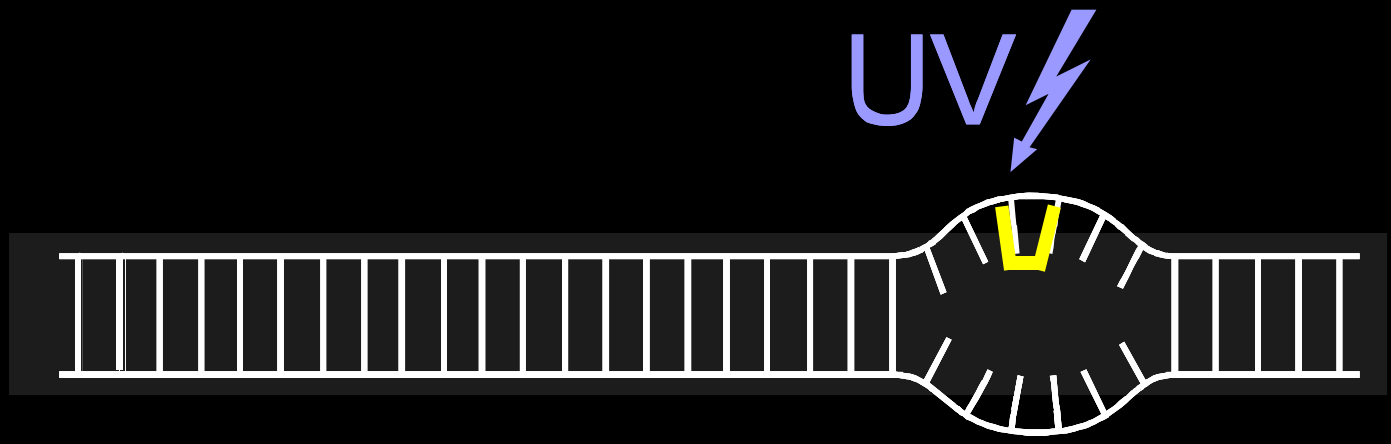
Blocked transcription

Cell cycle arrest

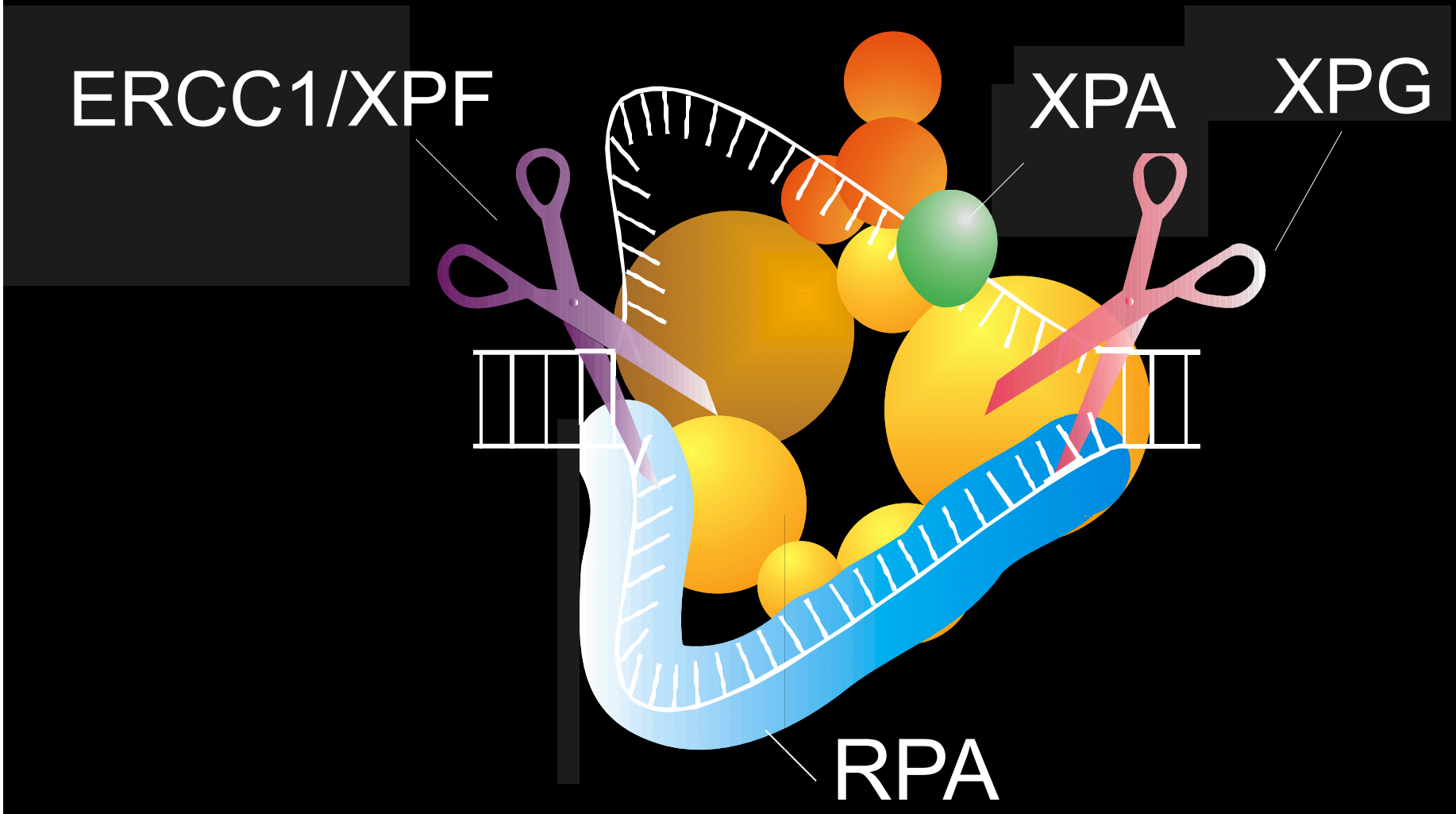
Senescence


GG-NER
TC-NER
HHR23B
DDB
XPC
UV
Damage recognition
UV
RNA pol II
CSB
CSA
CSA
Helix-unwinding
Damage verification
TTDA
TFIIH
XPA
Mutations
Cell death/Senescence (?)
RPA
XPB
XPD
ATP
Cancer
Aging
Excision
ERCC1/XPF
XPG
DNA synthesis + ligation
DNA pol δ/ε
PCNA, RFC
DNA ligase
Nucleotide Excision Repair

Global genome nucleotide excision repair (GG-NER)

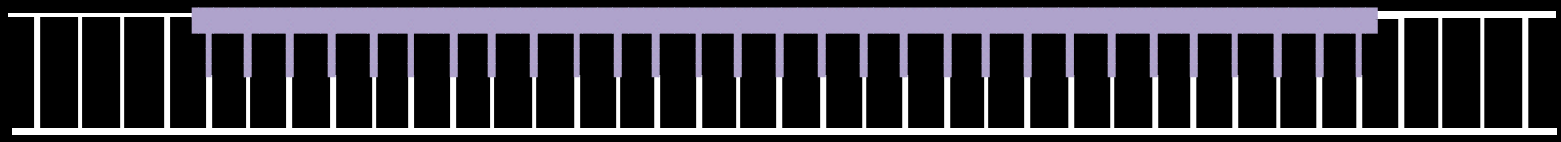


Global genome nucleotide excision repair (GG-NER)

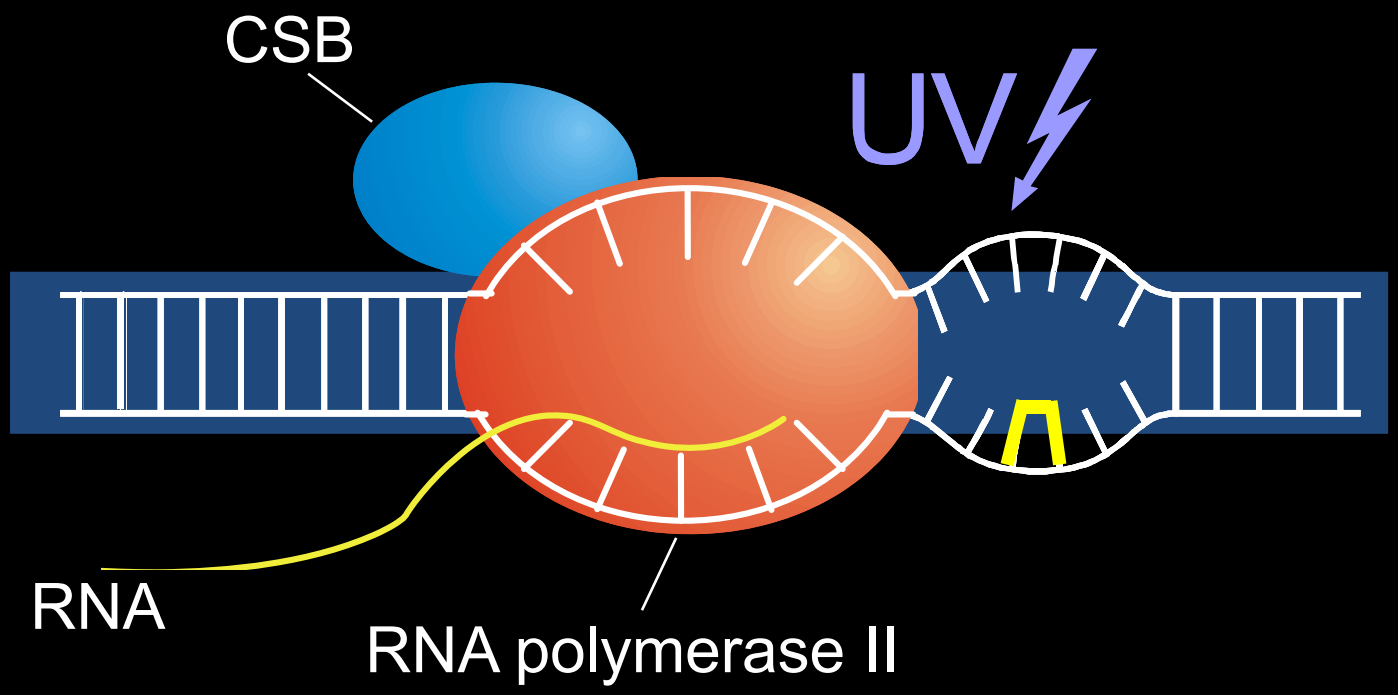


Global genome nucleotide excision repair (GG-NER)

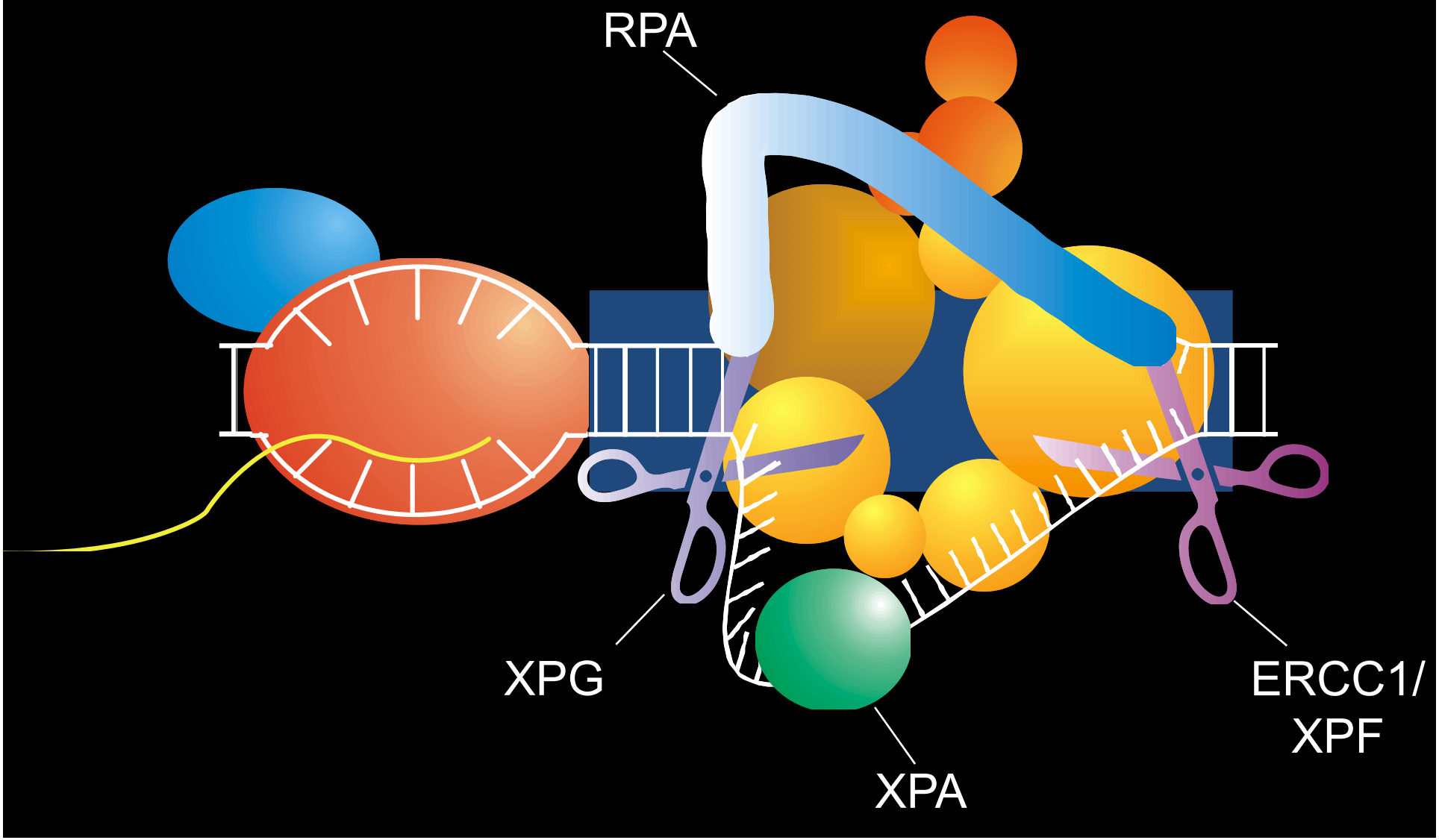
DNA polymerase δ/ε, RFC
PCNA and DNA ligase



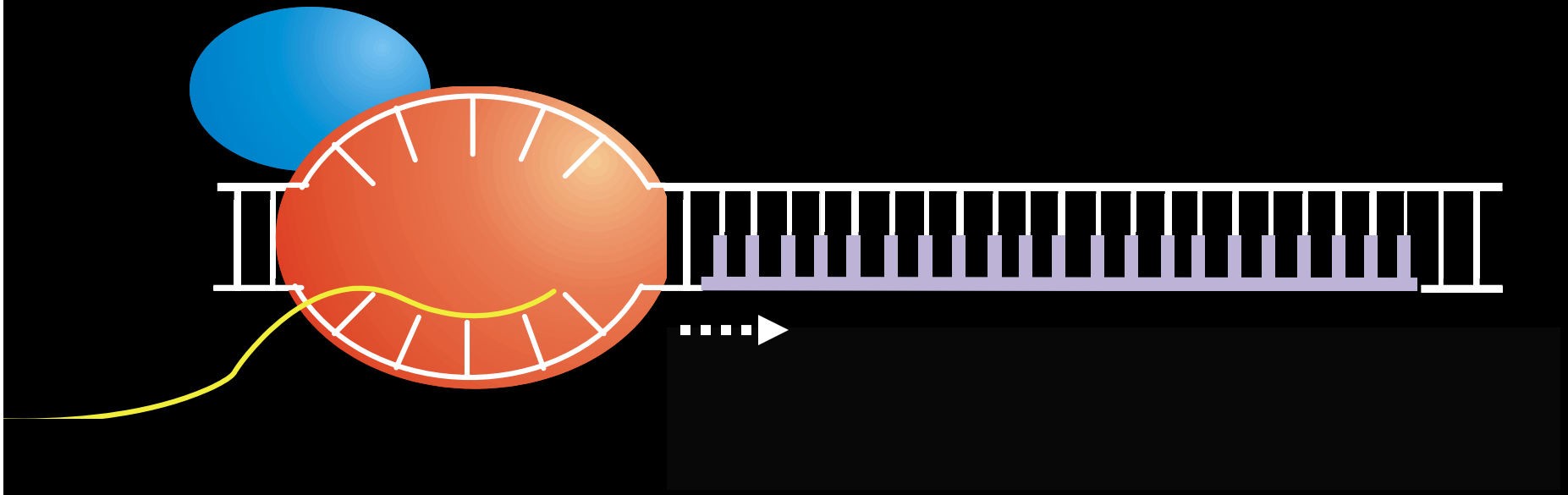
Transcription-coupled nucleotide excision repair (TC-NER)



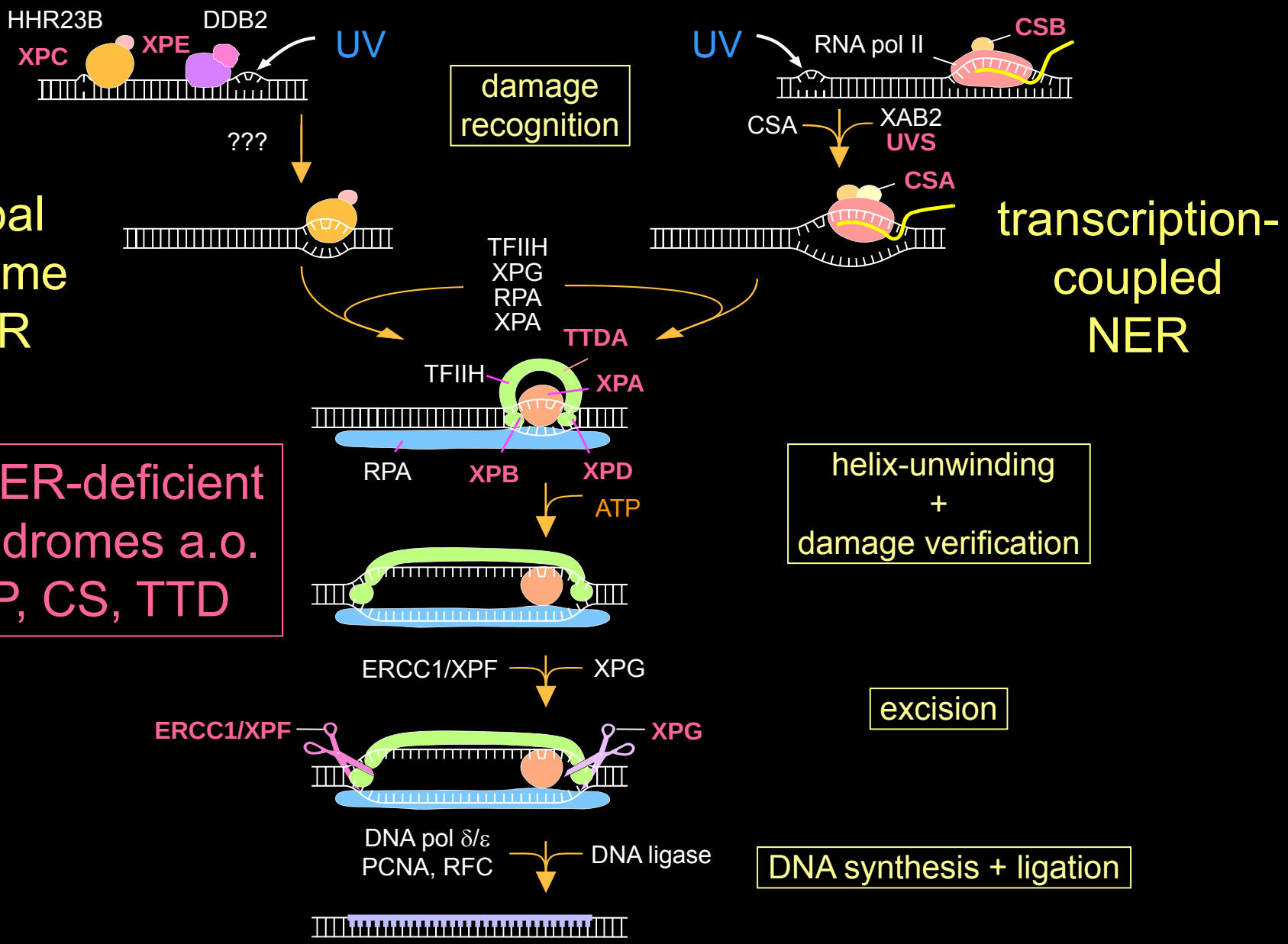
Transcription-coupled nucleotide excision repair (TC-NER)



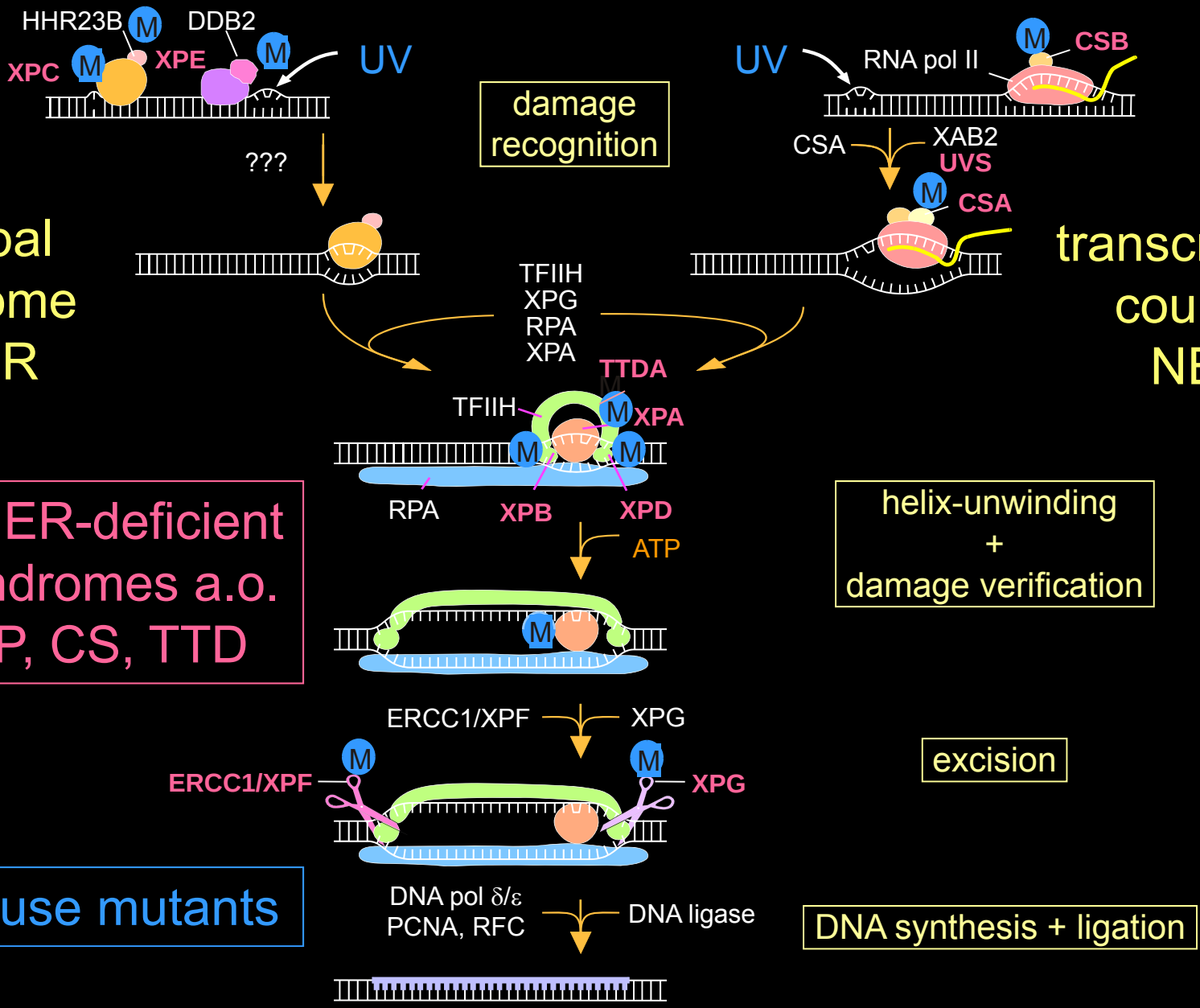
Transcription-coupled nucleotide excision repair (TC-NER)



Nucleotide Excision Repair (NER) - Patients



Nucleotide Excision Repair (NER) - Mouse mutants



Xeroderma Pigmentosum (XP)

- Photo (UV) sensitivity
- Pigmentation abnormalities
- Atrophic skin
- Skin cancer (>2000x↑)
- Accelerated neurological degeneration

7 genes involved:

XPA - XPG

Skin tumors in xeroderma pigmentosum

Management of XP cancer

- very rigorous sun-light protection

- regular dermatologic intervention

- preventive oral retinoids (~50%)

(Isotretinoid, Accutane®)

Neglect in patient XP20RO (XPC)

Cockayne Syndrome (CS)

- Photo (UV) sensitivity
- Growth failure
- Neurological abnormalities
- Retinal degeneration
- Cachexia
- Impaired sexual developm.

No skin cancer !

5 genes: *CSA,CSB*

combined with XP:

XPB, XPD, XPG

7 y

Defects in NER may trigger aging and tumor development

Global Genome Nucleotide Excision Repair

Xeroderma Pigmentosum (XP)
(predominantly a GG-NER defect)

- sun (UV) sensitivity
- pigmentation abnormalities
- dry, atrophic skin
- ***skin cancer (>2000x ↑)***
- accelerated neurological degeneration

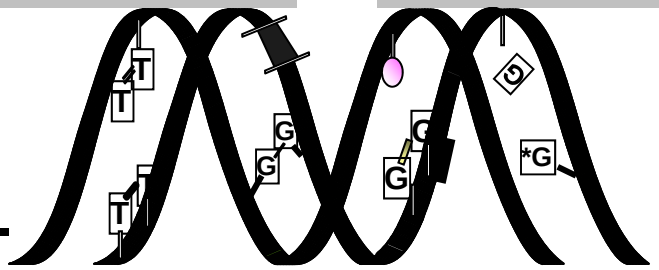
- 7 genes involved:
  XPA - XPG

Transcription coupled Nucleotide Excision Repair

Cockayne Syndrome (CS)
(TC-NER defect)

- sun (UV) sensitivity
- growth failure
- neurological abnormalities (dysmyelination)
- retinal degeneration
- ***accelerated aging***

2 genes: *CSA,CSB*
no skin cancer !



misreplication

GG-NER

mutations

Cancer

blocked transcription & replication

TCR, DSB, ICLr

cell cycle arrest & apoptosis

Progeria

Can mouse models mimic the patients?

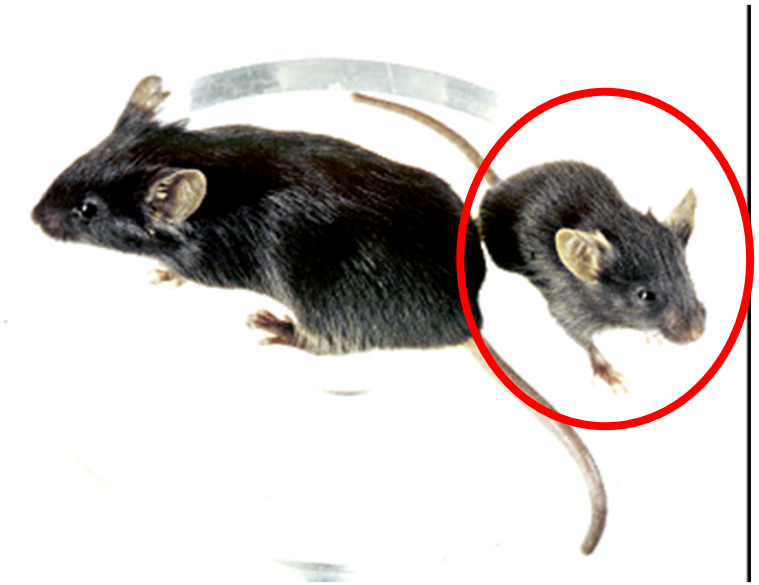
Summary phenotype of *Csb$^{m/m}$/Xpa$^{-/-}$* mutant mice

- Normal embryonic development
- Born at submendelian frequency (likely due to birth stress)
- Runted, growth retarded, cachexia
- Purkinje cell loss cerebellum
- Ataxia
- Kyphosis
- Osteoporosis?
- Enhanced retinal cell loss
- Premature death (around weaning)
- Absence of apoptosis or proliferative defects in the liver

Day 21

PLoS Biology 2007

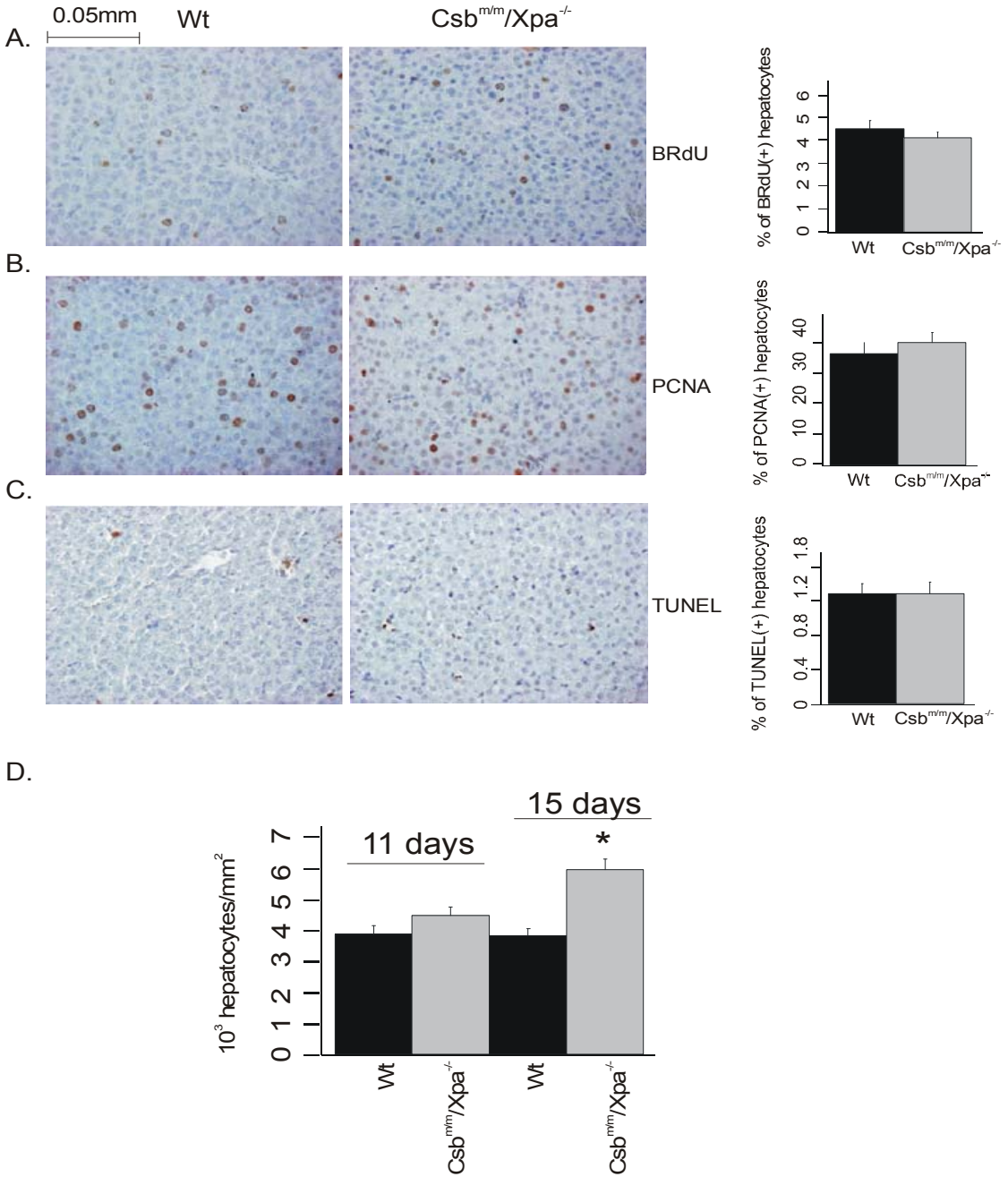
Summary phenotype of Ercc1$^{-/-}$ mice



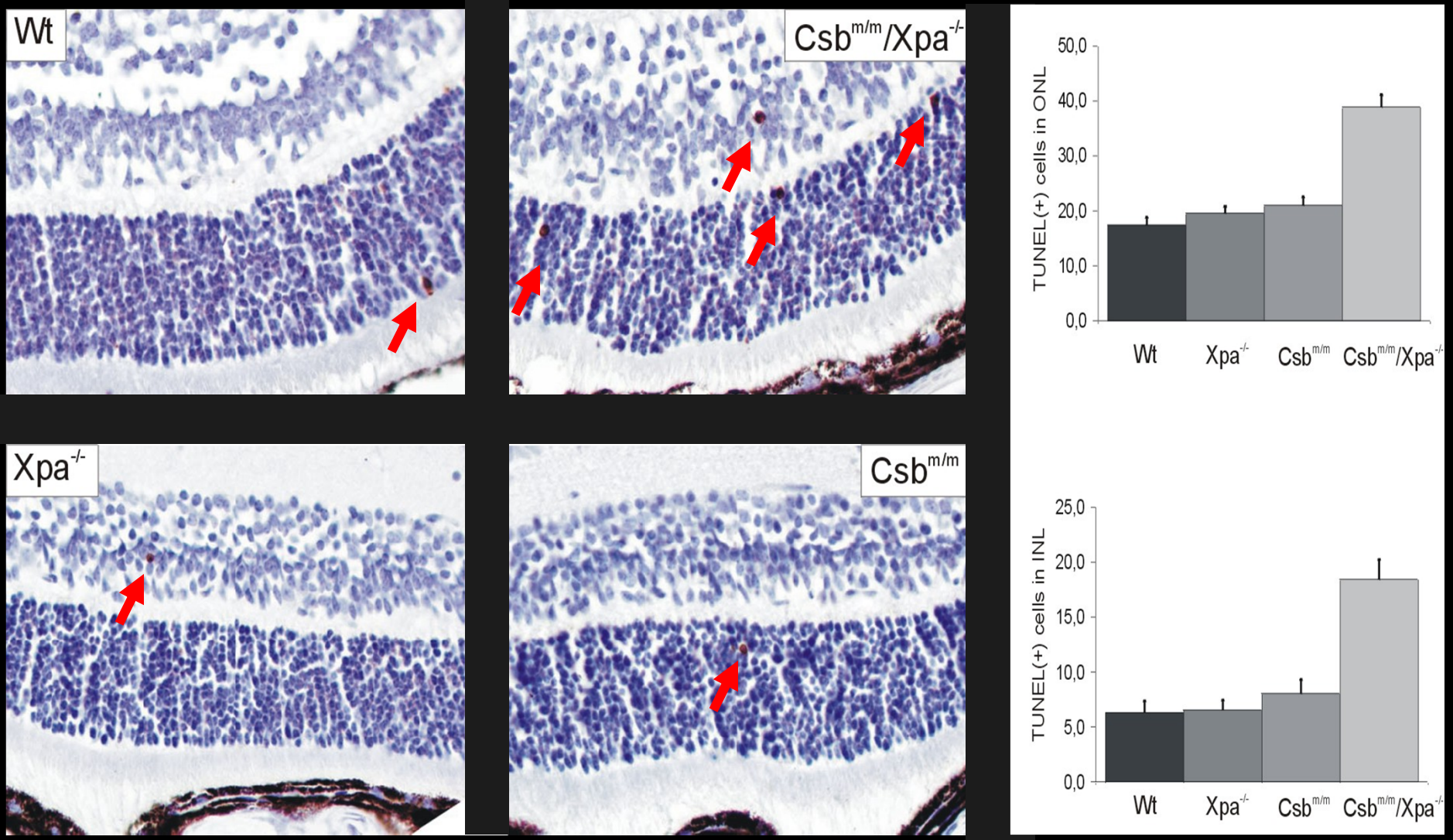
Growth delay
Kyphosis
Progressive ataxia
Infertility
Cachexia
Sarcopenia
Polyploidization in liver/kidney
Premature death at ~3 weeks

Nature 2007

Csb-Xpa mice: hepatocytes are significantly smaller



Enhanced retinal degeneration in *Csb$^{m/m}$/Xpa$^{-/-}$* mice



Photoreceptor loss by apoptosis (assayed by TUNEL)

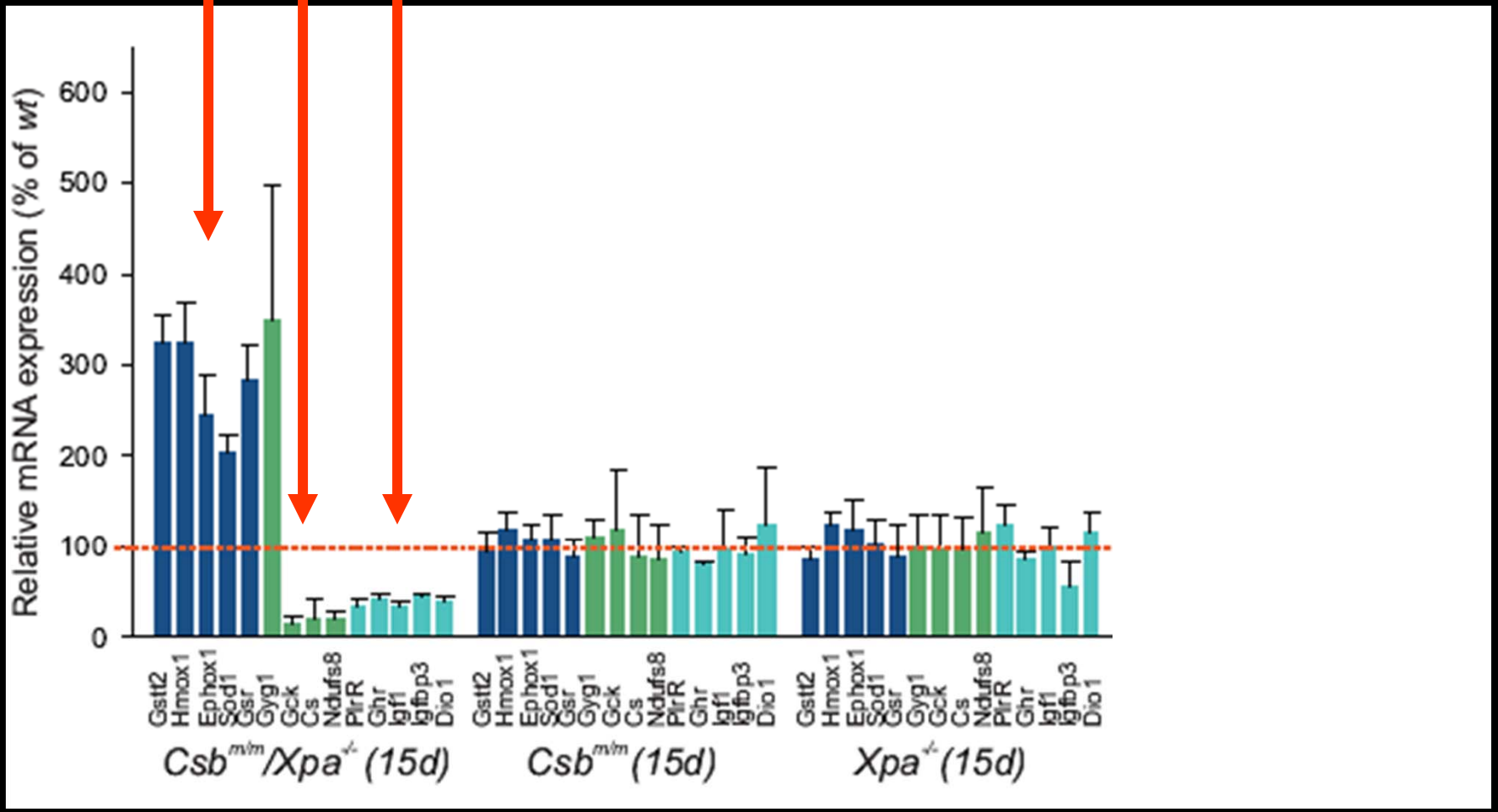
Mol Cell Biol 2007

Transcriptome analysis/Q-RT-PCR confirmation reveals

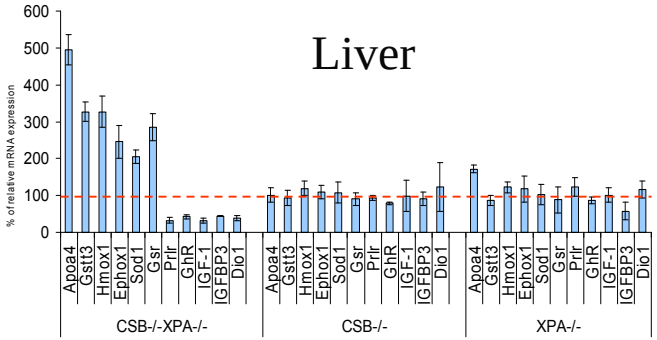
up-regulation anti-oxidant defense

down-regulation catabolic metabolism (glycolysis, Krebs, ox.phos.)

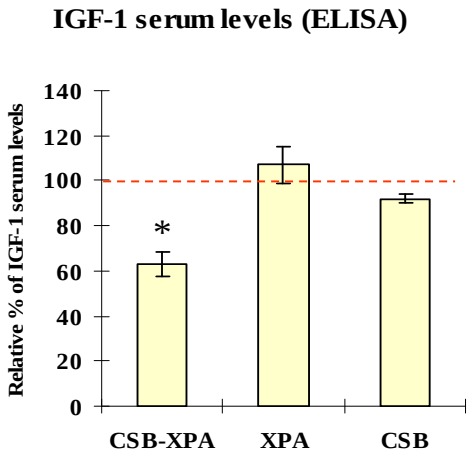
down-regulation GH/IGF1 growth axis

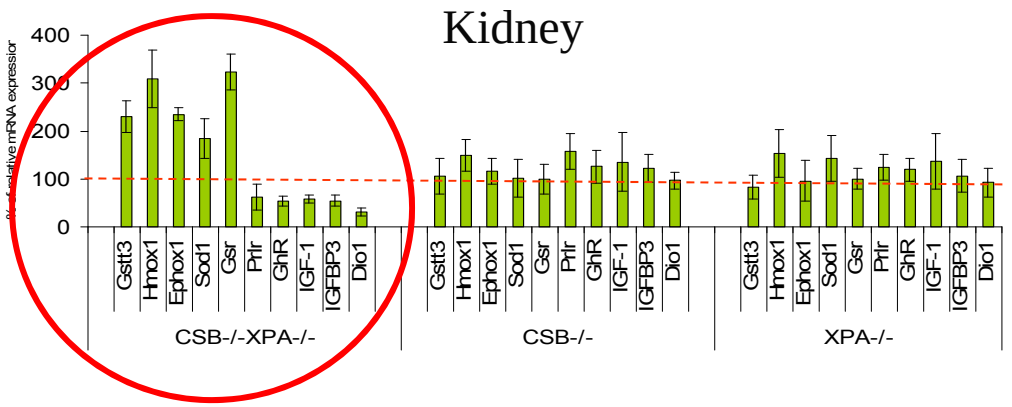


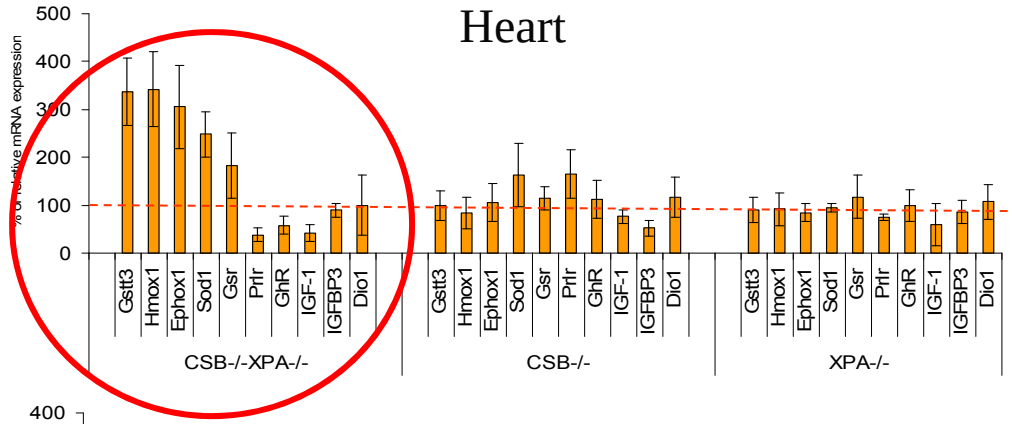
This response is systemic:

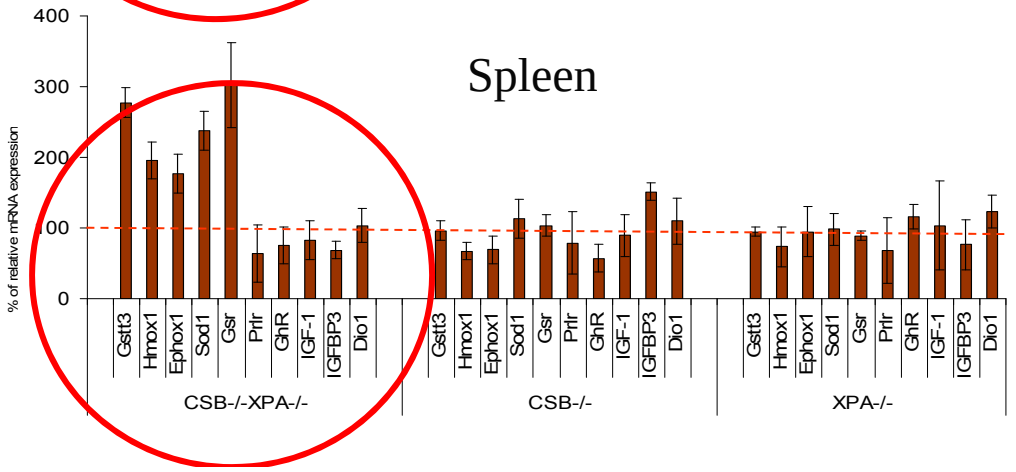


hypoglycemia



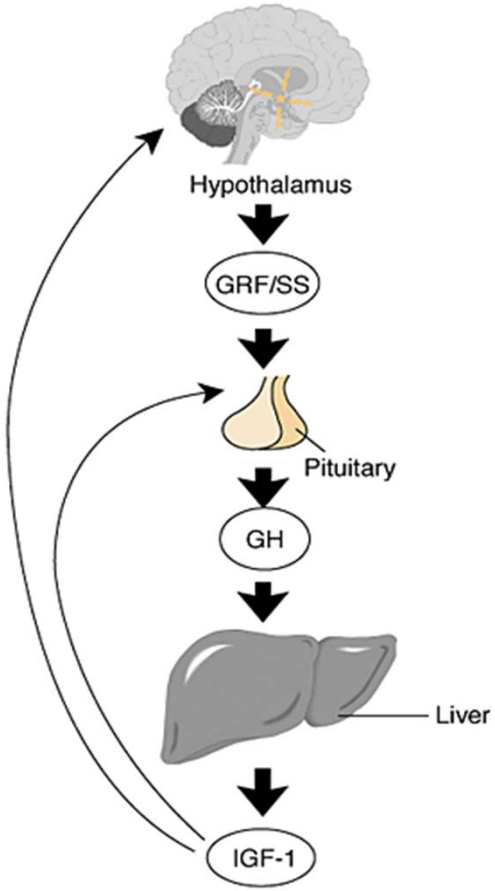




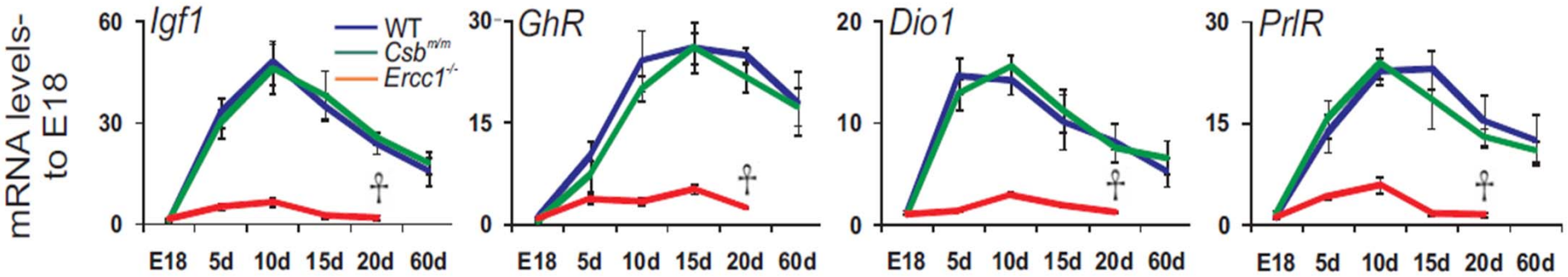


↓IGF-1
↓GH-R
↓PRL-R
↓DIO1

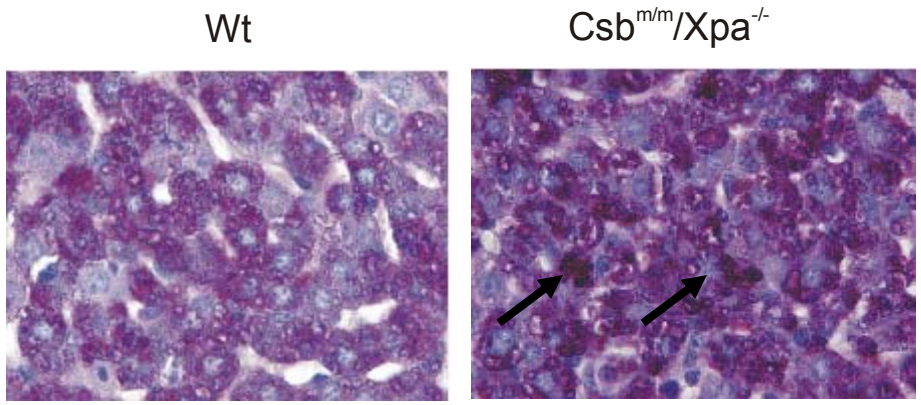
E18 → P9



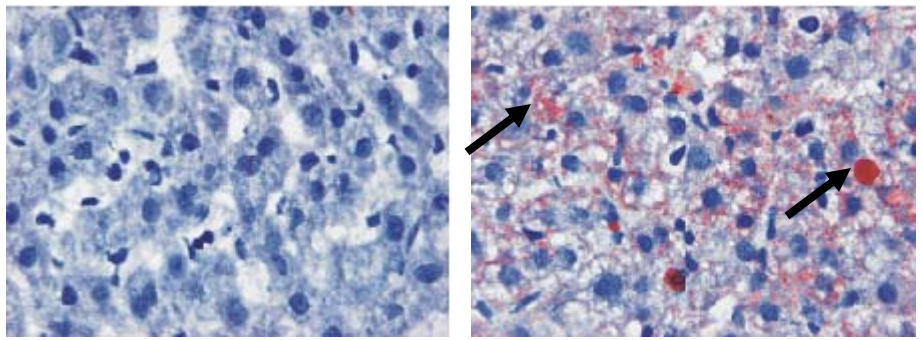
Suppression of GH/IGF1 axis leads to growth defect



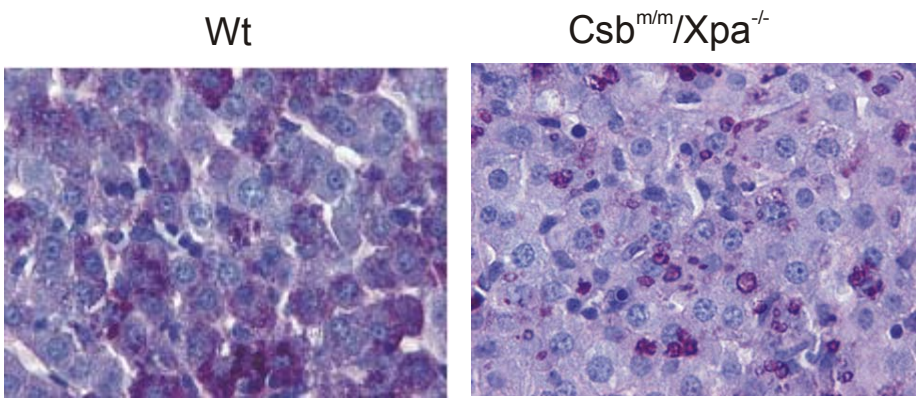
$Csb^{m/m}/Xpa^{-/-}$ appear to store glycogen and fat



Glycogen



Triglycerides



Glycogen (upon fasting)

Gene expression profiles of NER progeroid mutants show remarkable similarity to those of naturally aged mice

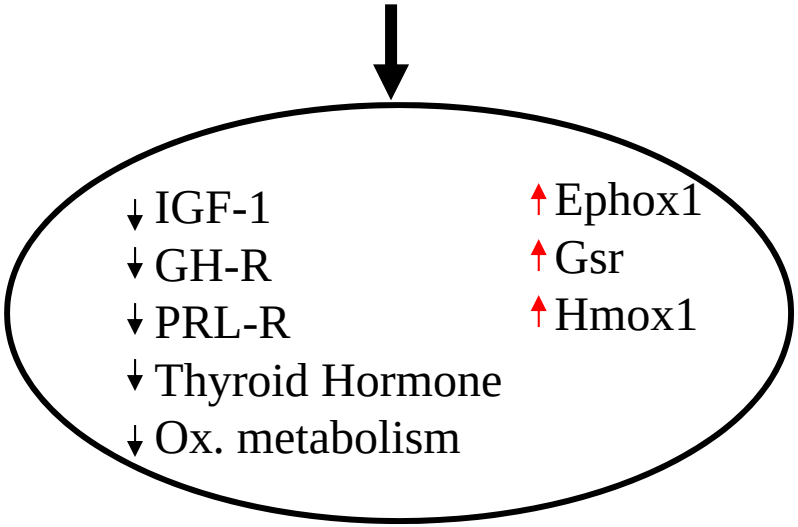
NER progeroid mice respond normally by mimicking the response of old mice:

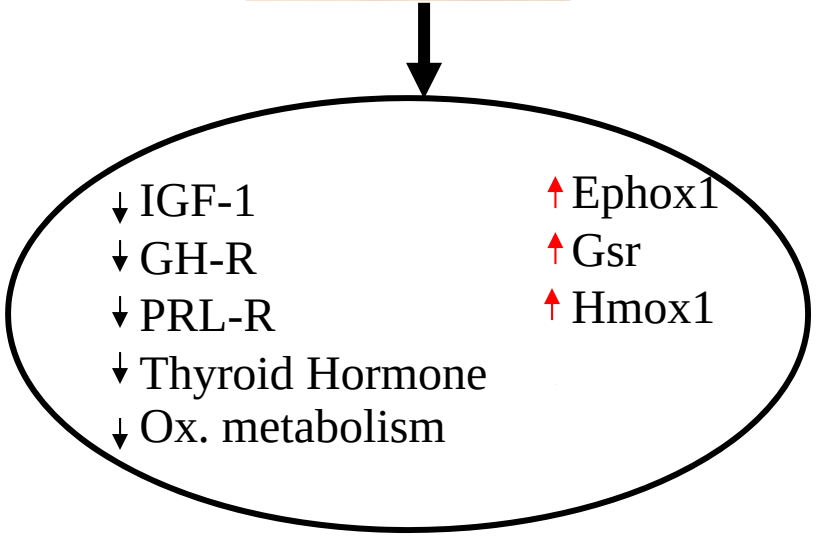
15-day old

XPA$^{-/-}$/CSB$^{m/m}$

Endogenous DNA damage

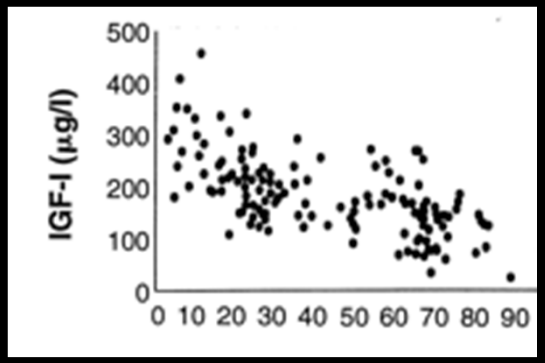
2.5-year old





Comparison with human ageing

1. Both GH and IGF-1 decline with advancing age:



Lamberts et al 1997

somatopause:

- Reduced muscle mass
- Increased visceral fat mass
- Attenuated bone mineral density
- Cardiovascular changes
- Reduced elasticity of the skin
- Cognitive performance

(van Dam et al 2000)

GH/IGF1 mouse models live very long…

1. Gh-r KO mice - profound decrease of hepatic IGF-1 (GH-resistant)
   - reduced somatic growth within 2 to 4 weeks after birth
   - decreased body size
   - live longer
   - increased antioxidant defense mechanisms (Bartke et al 2003)

2. Hypopituitary Ames/dwarf mice (deficient in GH, PRL and TSH)
   - exhibit 40-65% extension of their lifespan
   - reduced body size
   - increased anti-oxid. defense mechanisms (Brown-Borg et al. 1996).

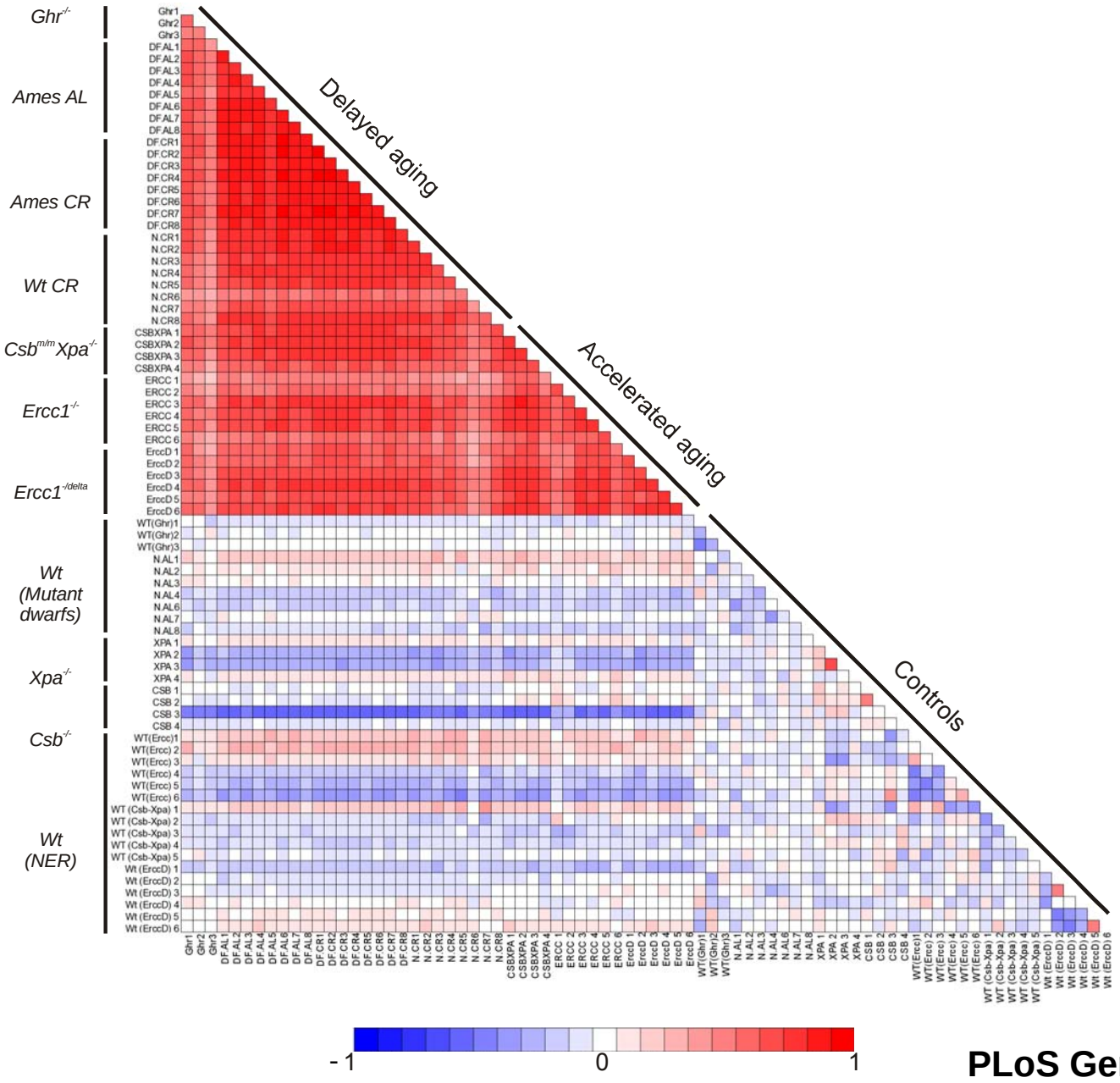
3. Ghrh mutant mice and heterozygous IGF-1R KO mice
   - live longer
   - reduced body size (Flurkey et al 2001, Holzenberger et al 2003 (resp.)

4. GH transgenic mice (carrying a bovine GH gene)
   - renal lesions
   - hepatic alterations
   - drastic reduction in lifespan (Carter et al 2002)

5. CR extends lifespan and down regulates both GH and IGF-1

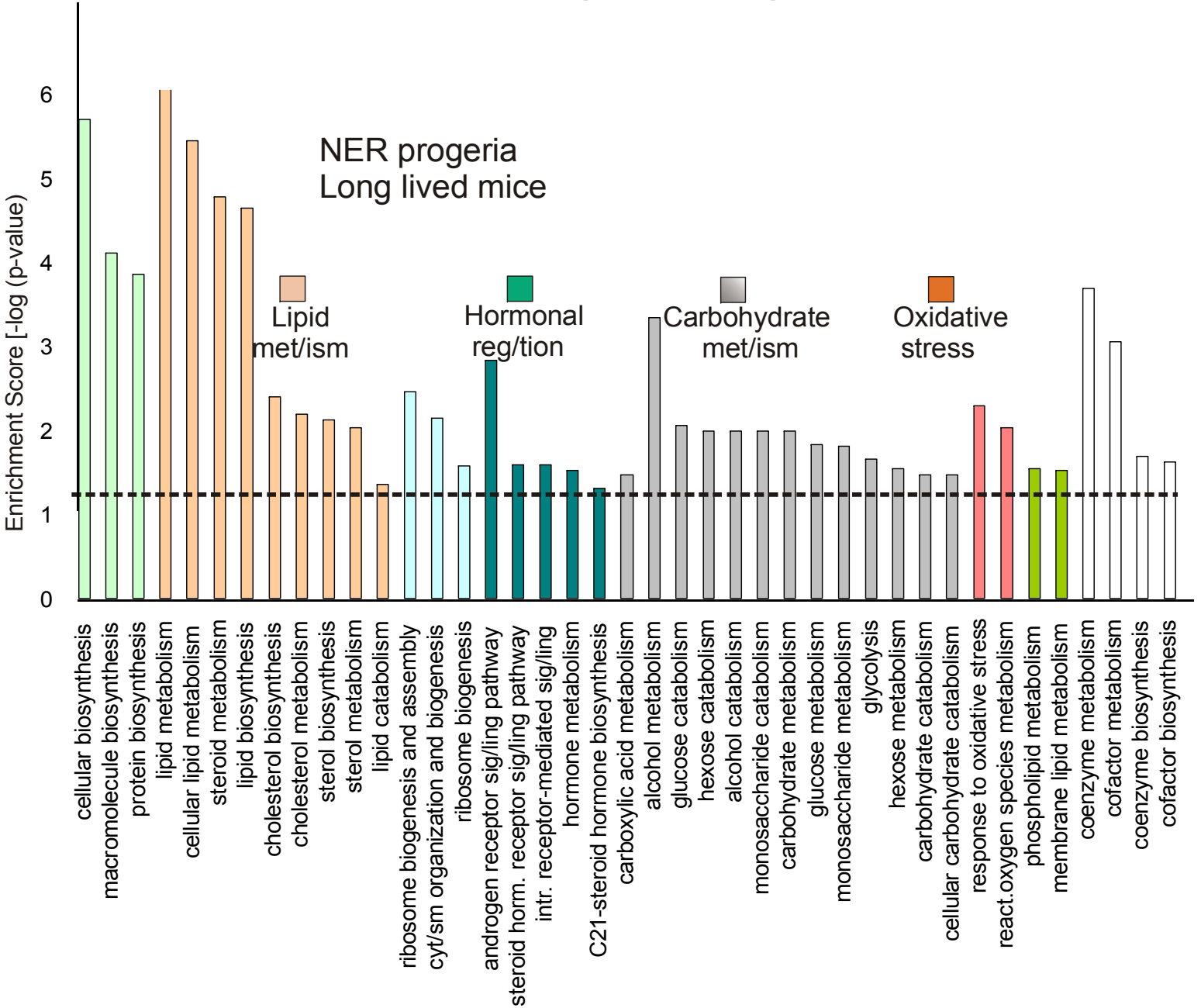
Correlation heat map: “mouse to mouse” correlation

NER progeroid mice are highly correlated to long-lived mutants

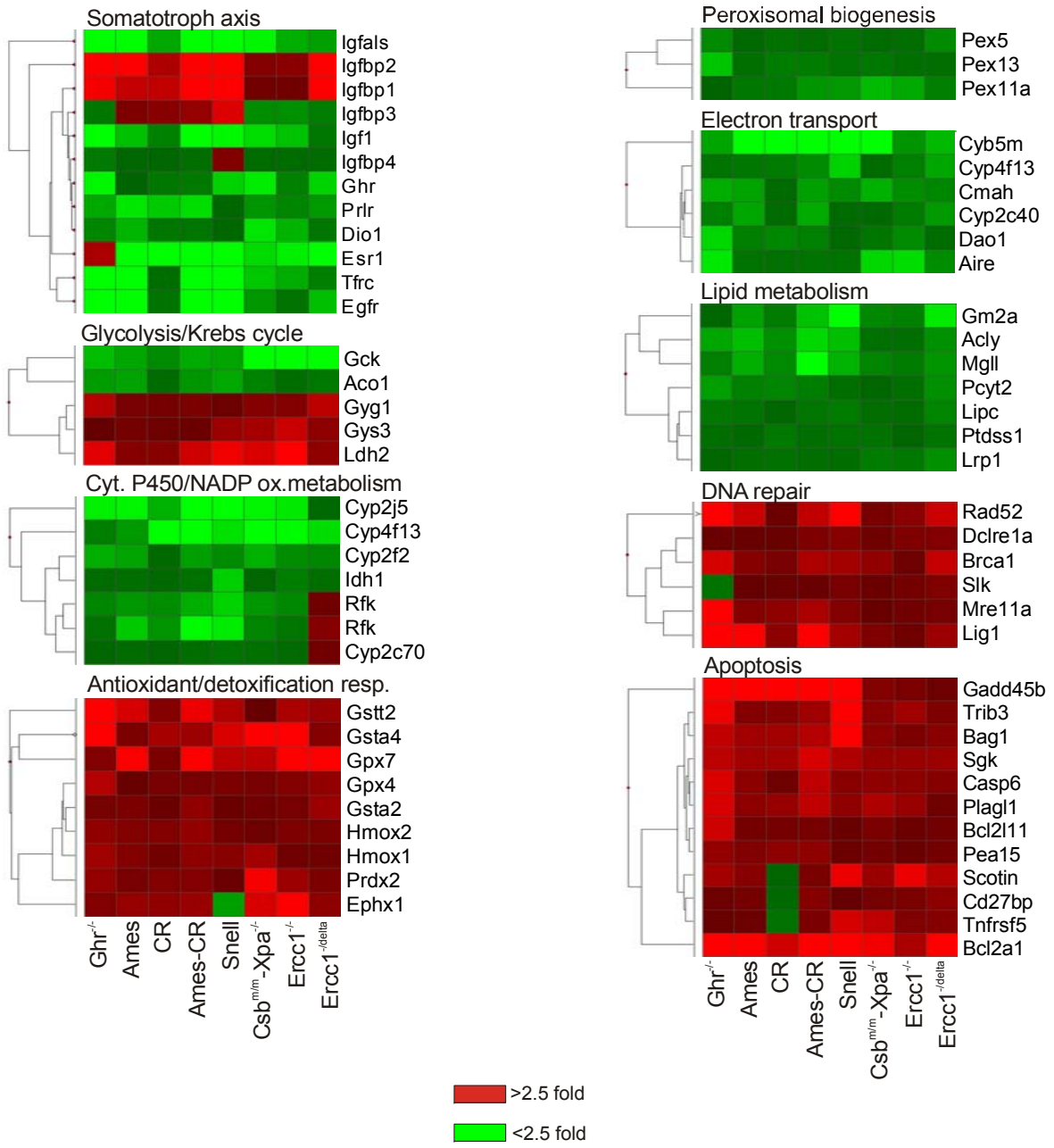


PLoS Genetics 2008

Common processes between NER progeria, long-lived dwarfism and CR



Similar expression profiles between NER progeroid and long-lived mice



Substantial genome-wide expression parallels between NER progeroid and long-lived mice

DNA repair mutants

Long-lived mice

Ercc1$^{-/-}$

Csb$^{m/m}$/Xpa$^{-/-}$

Ercc1$^{-/\Delta}$

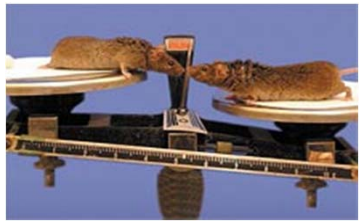
GH/IGF1 suppression

Ghr$^{-/-}$

Snell

Ames

CR mice



130W mice

DNA repair mutants

↓

conserved metabolic response

↓

Resources from growth to somatic preservation

↓

Extension of life span (by limiting the deleterious effects of arrested transcription, cellular senescence and death)

Rationale of the GH/IGF1 response in NER progeroid mice

Why do *Csb*$^{m/m}$/*Xpa*$^{-/-}$ or *Ercc1*$^{-/-}$ mice display a caloric restricted-like response associated with long life span, whereas they live extremely short?

Rationale of the GH/IGF1 response in normal aging

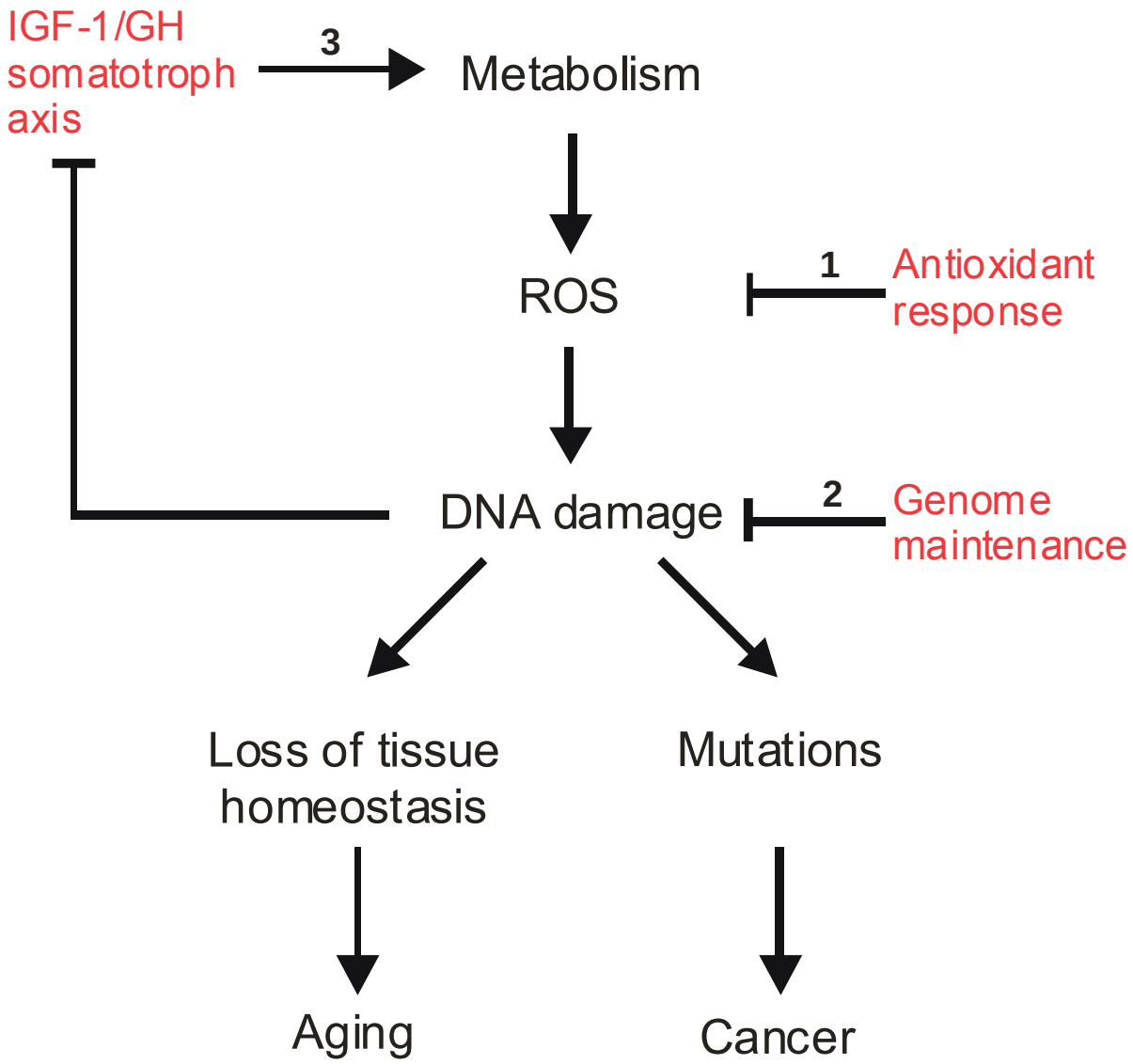
Early in life, development to adulthood is priority:

- resources are used for growing and to generate progeny
- GH/IGF-1 and metabolism are high,
- however, at the expense of more DNA damage

When this goal is reached, priorities shift:
"now it is important to switch from growth to maintenance"

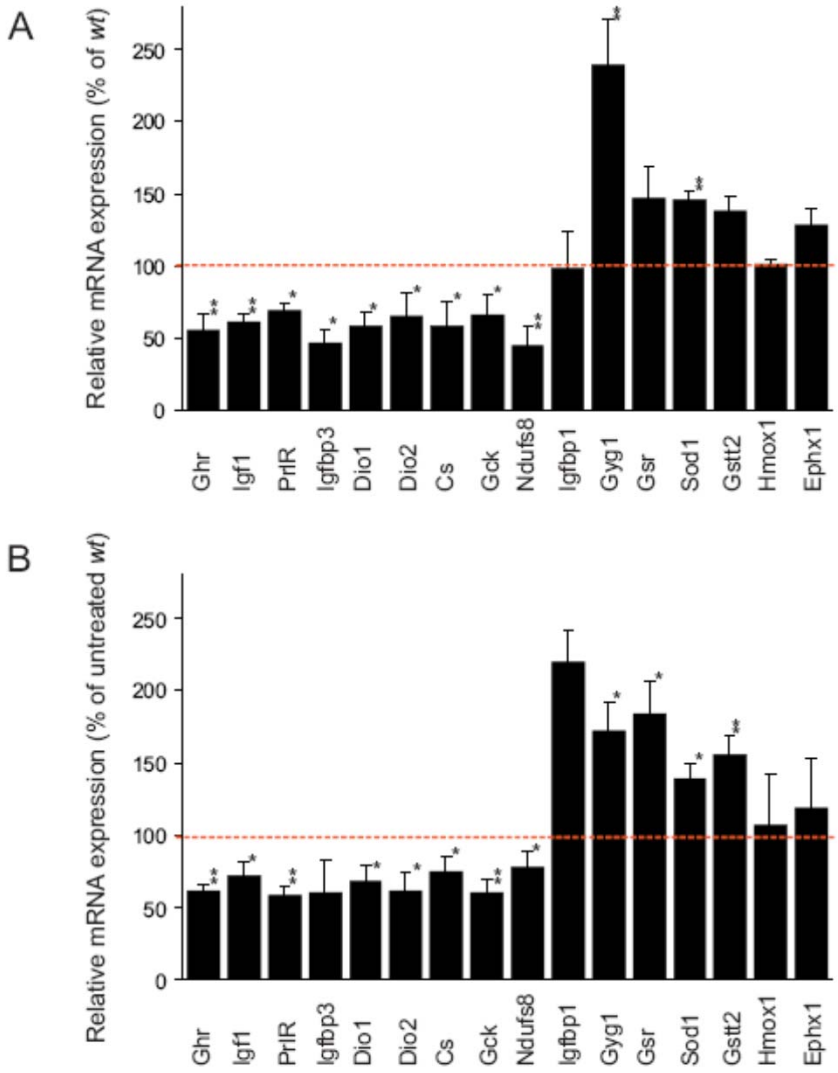
- remaining resources are used to extend life span
- GH/IGF-1 and rate of metabolism are turned down
- will reduce the DNA damage load

Scenario for *NER* progeria and natural aging

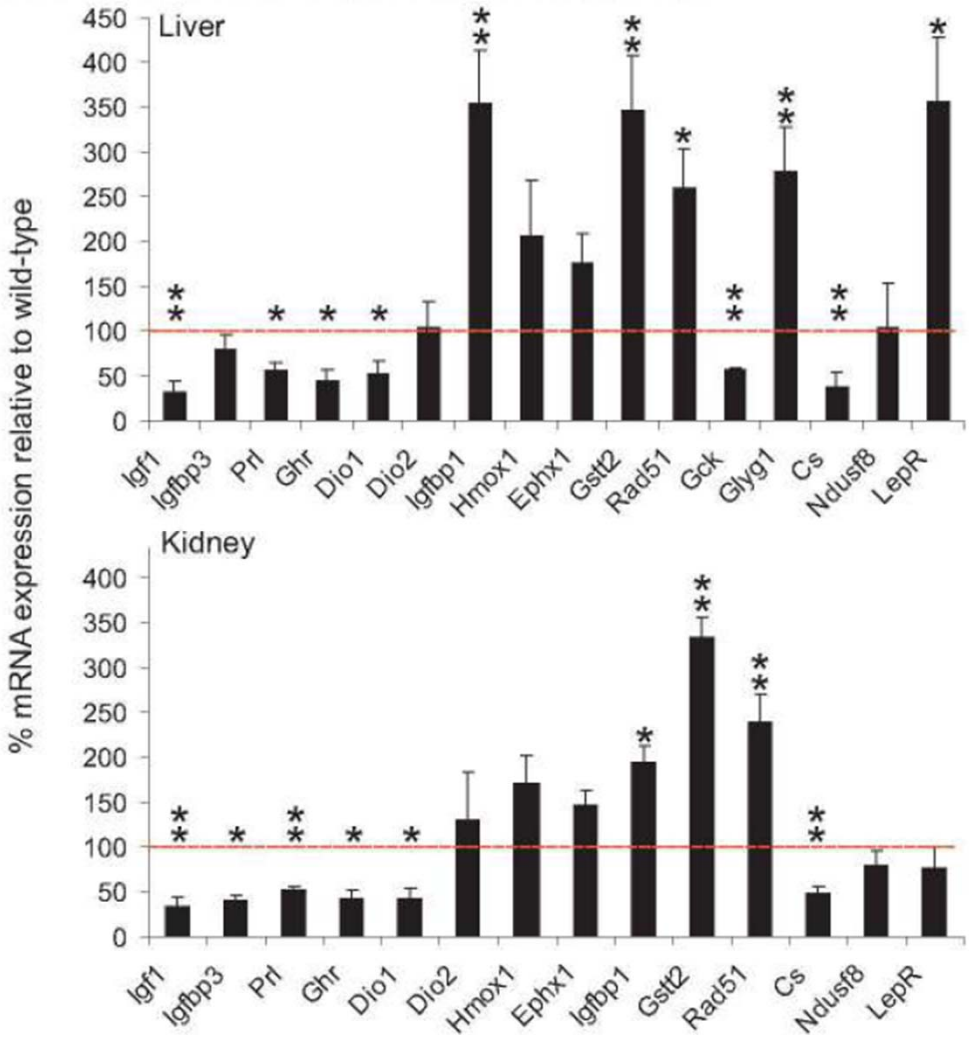


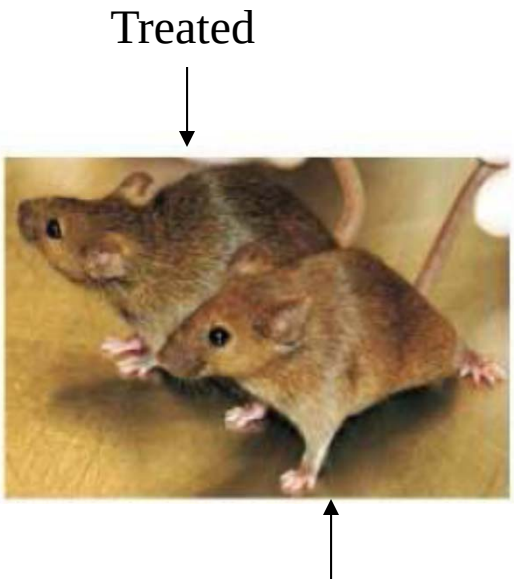
A. 13-week old *Csb*$^{m/m}$ mice

B. Wt mice chronically (4 weeks) exposed to a low dose (1500ppm) of pro-oxidant DEHP



Wt mice chronically exposed to the crosslinking agent mitomycin C

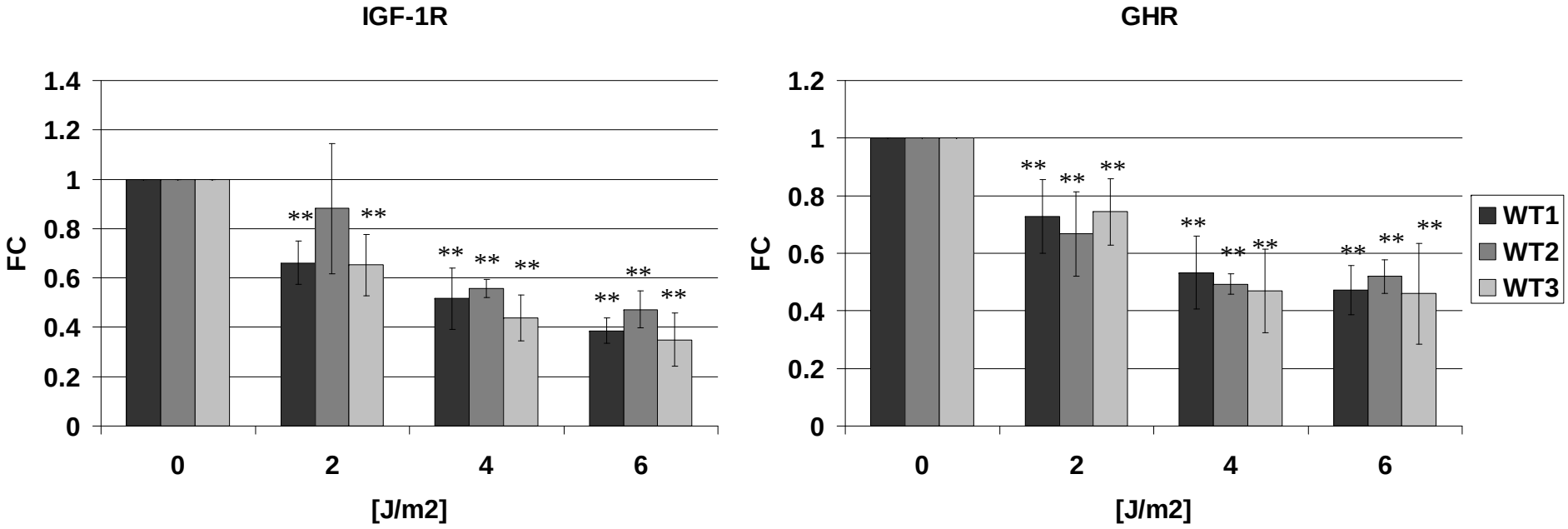




How does DNA damage lead to the suppression of the GH/IGF1 axis?

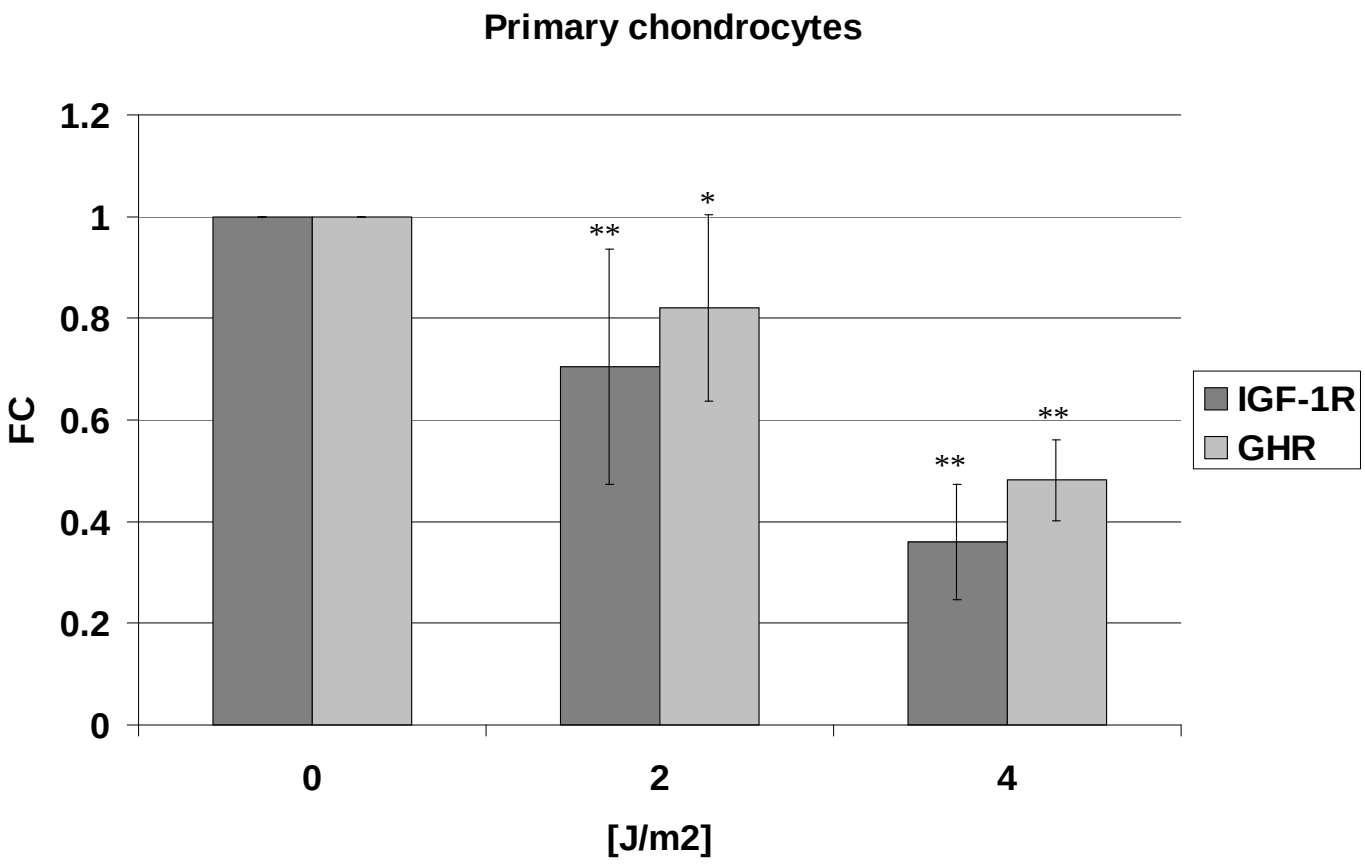
- Is the repression of IGF-1R/GHR a direct response to DNA damage?
- Is the repression of IGF-1R/GHR a cell autonomous response?

Dose-dependent UV-induced suppression of IGF-1R/GHR expression in primary MDFs

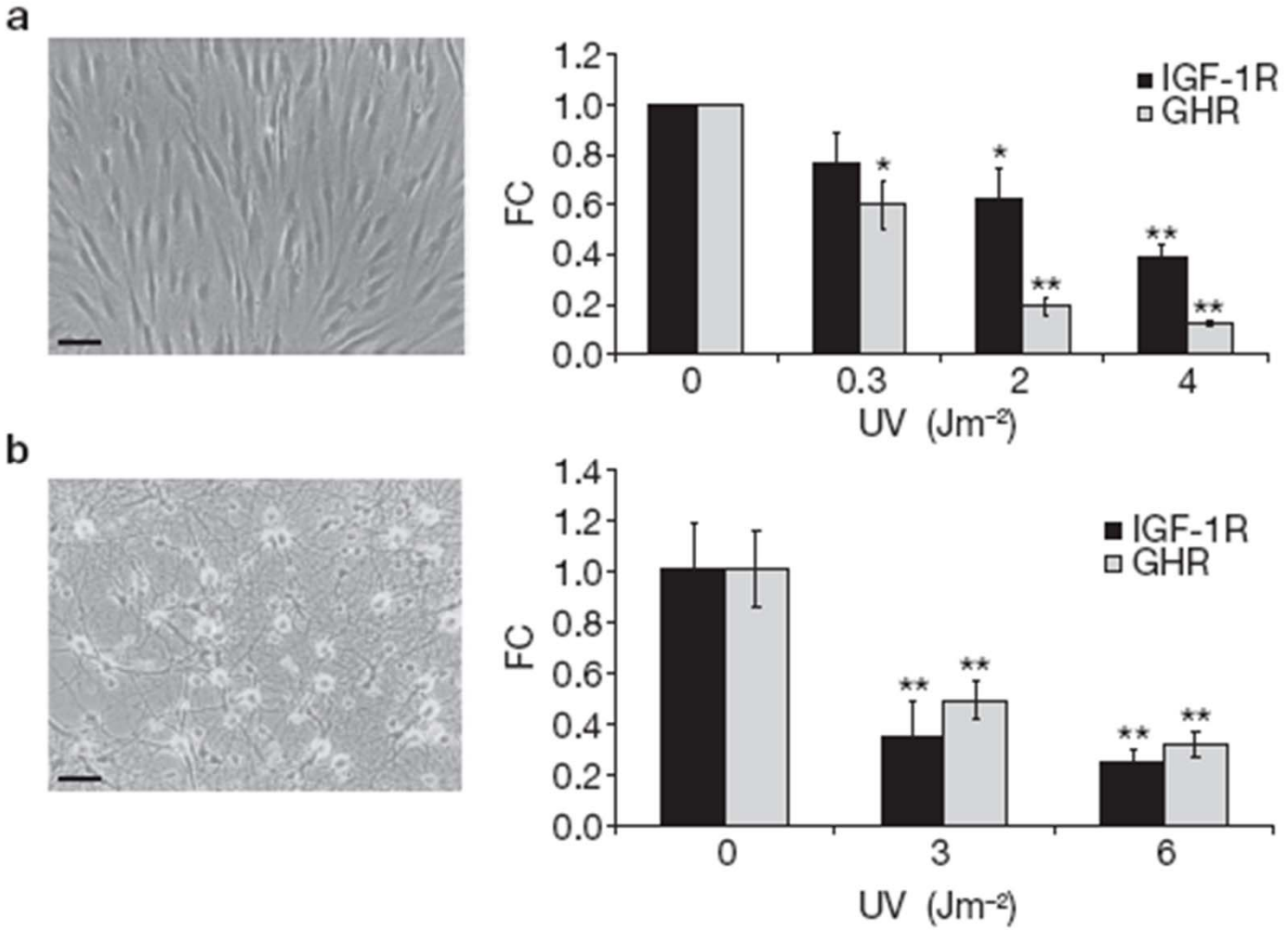


Nature Cell Biology 2009

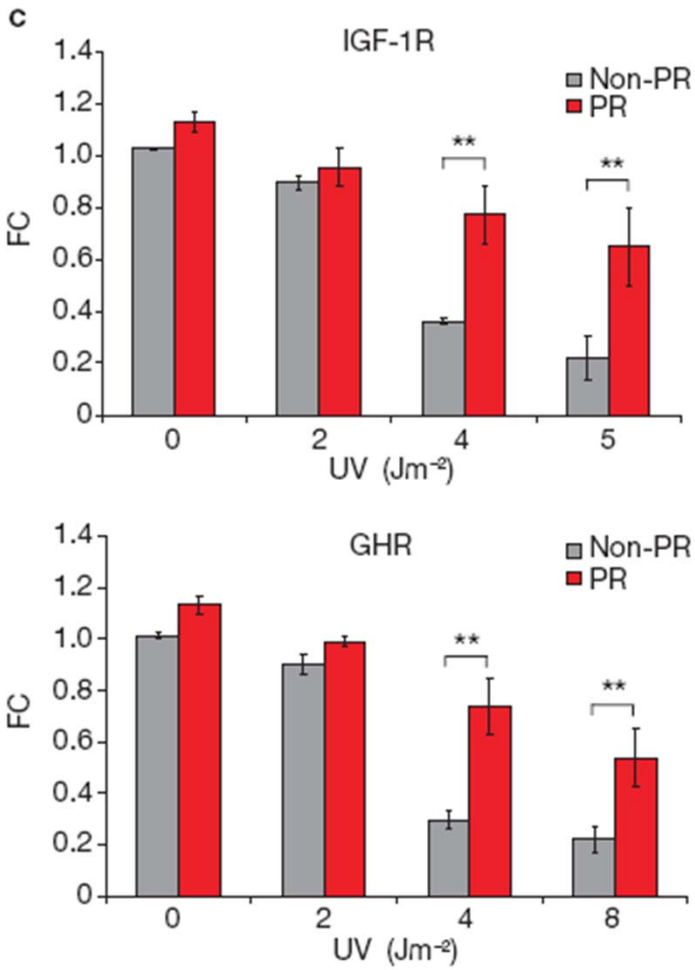
IGF-1R/GHR suppression in primary chondrocytes

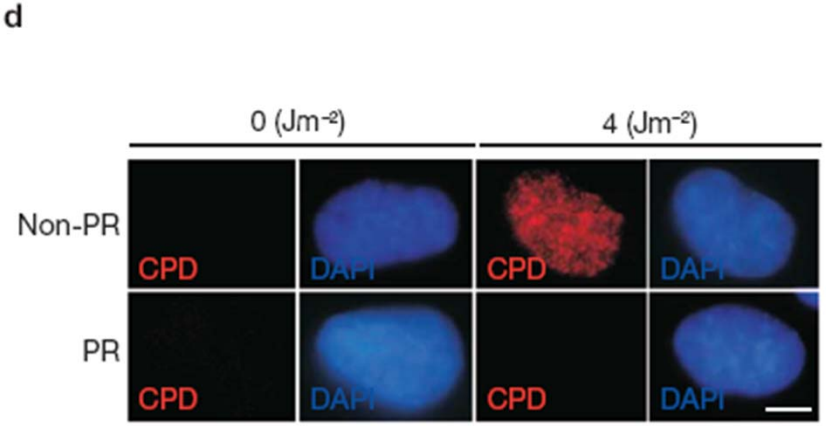


UV irradiation leads to IGF-1R and GHR attenuation in quiescent (a) and terminally differentiated (b) cells *(primary rat neurons)*



Repair of persistent CPD lesions alleviates IGF-1R and GHR repression

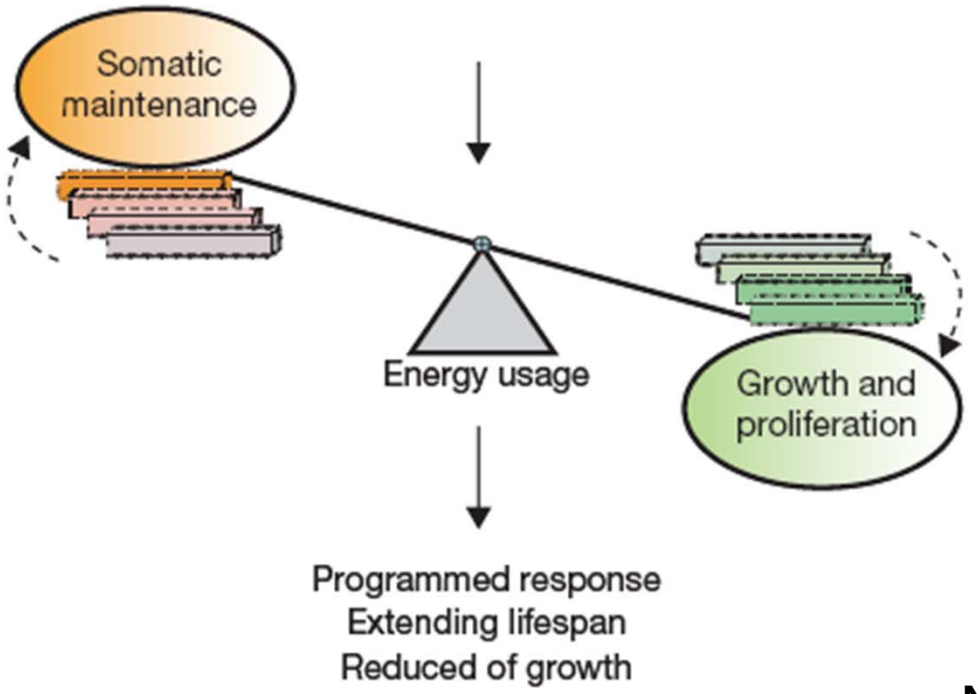




Photolyases can repair UV-induced DNA lesions in a light-dependent manner

CPD and 6-4PP photolyase transgenic MEFs

Somatic maintenance vs. growth

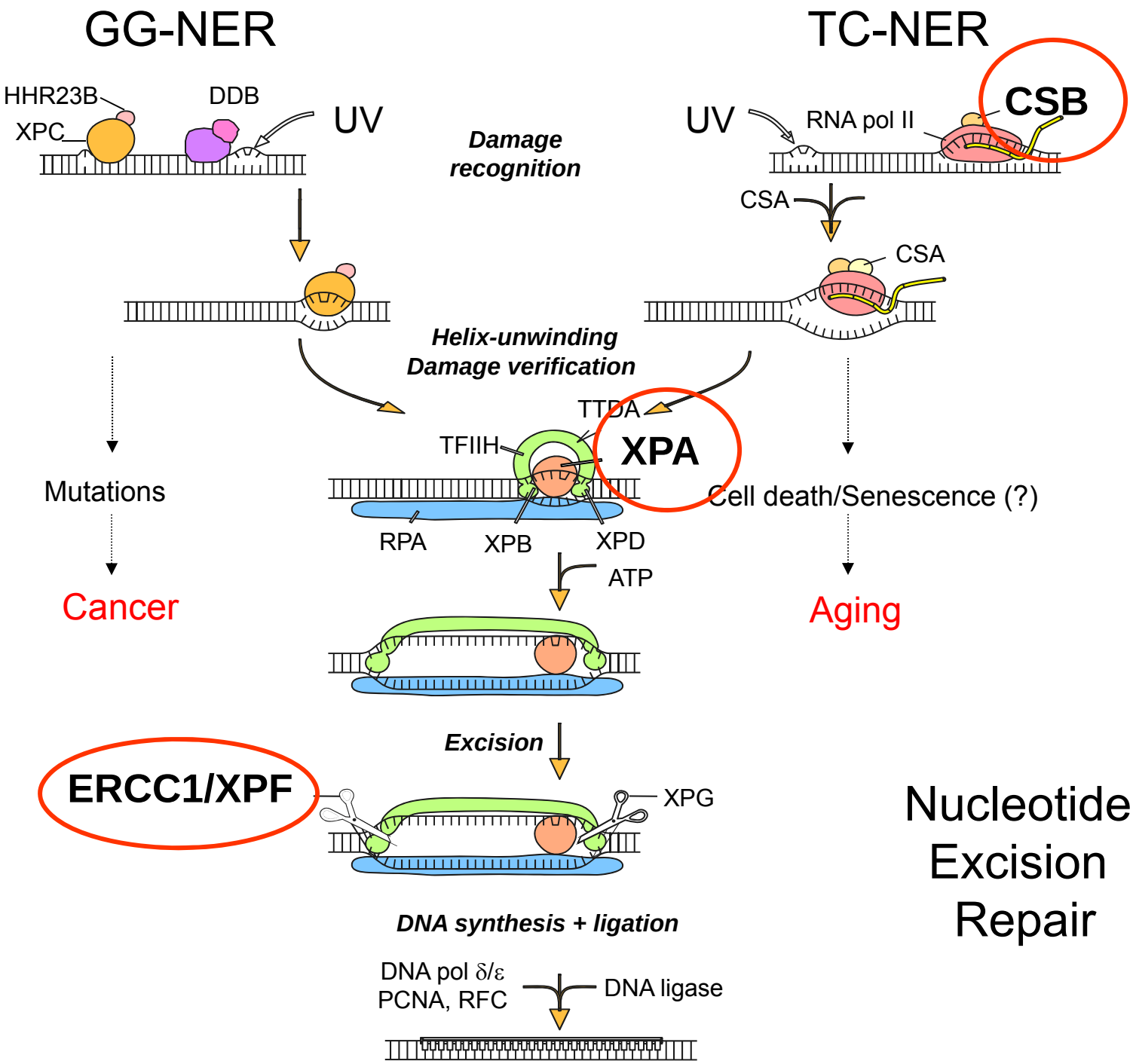


-Long-lived pituitary dwarfs
-Calorie-restricted mice
-DNA repair mutants
-Naturally aged mice

Nature Cell Biology 2008

Is there a role for ERCC1-XPF complex in transcription during development ?

…and perhaps a role beyond NER?


GG-NER
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Damage recognition
UV
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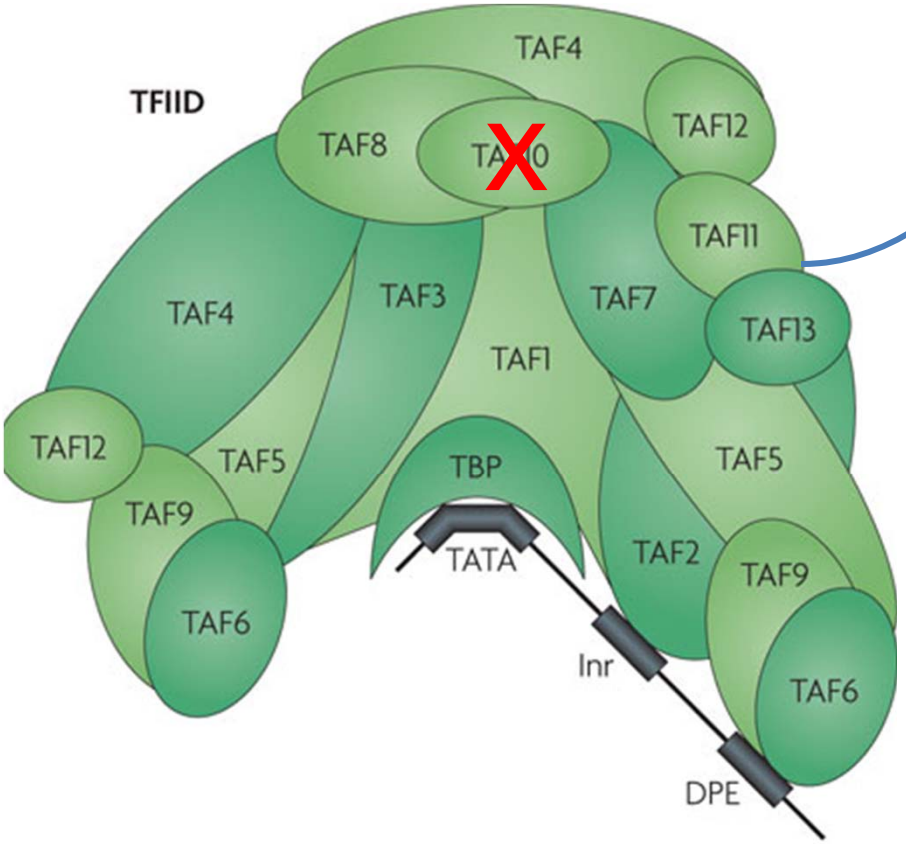
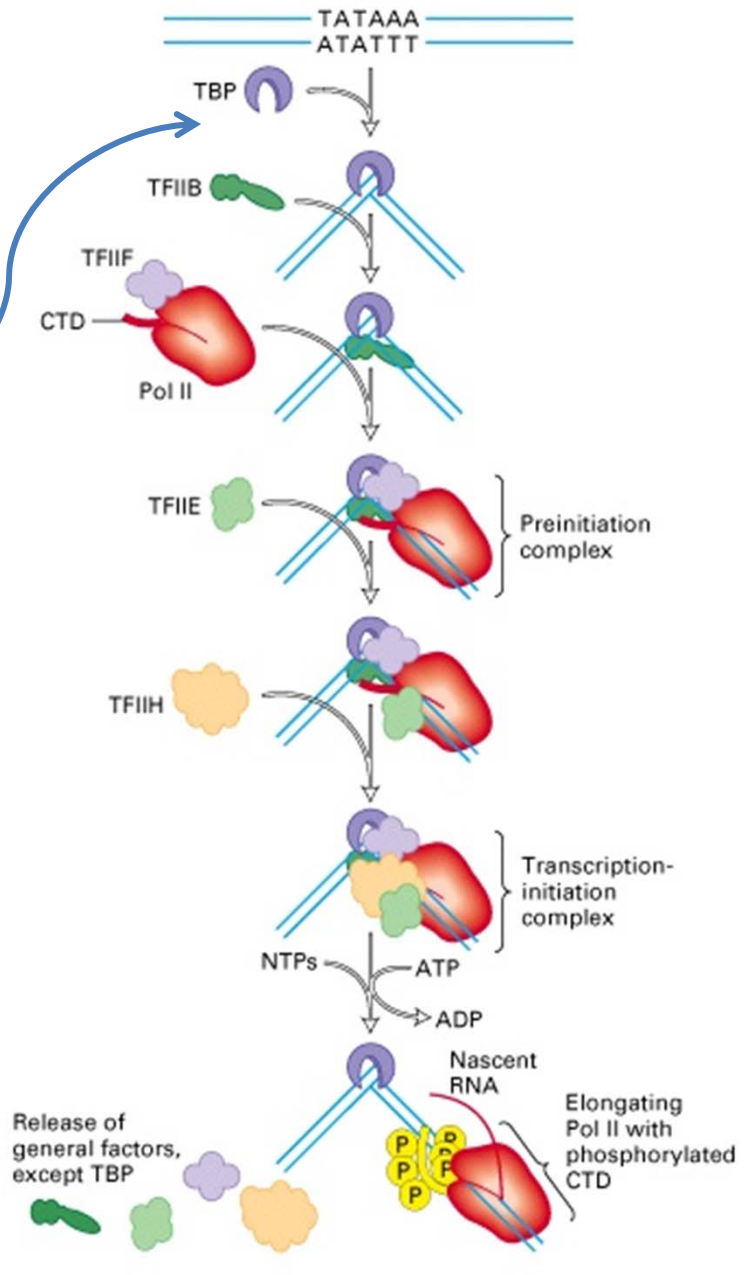
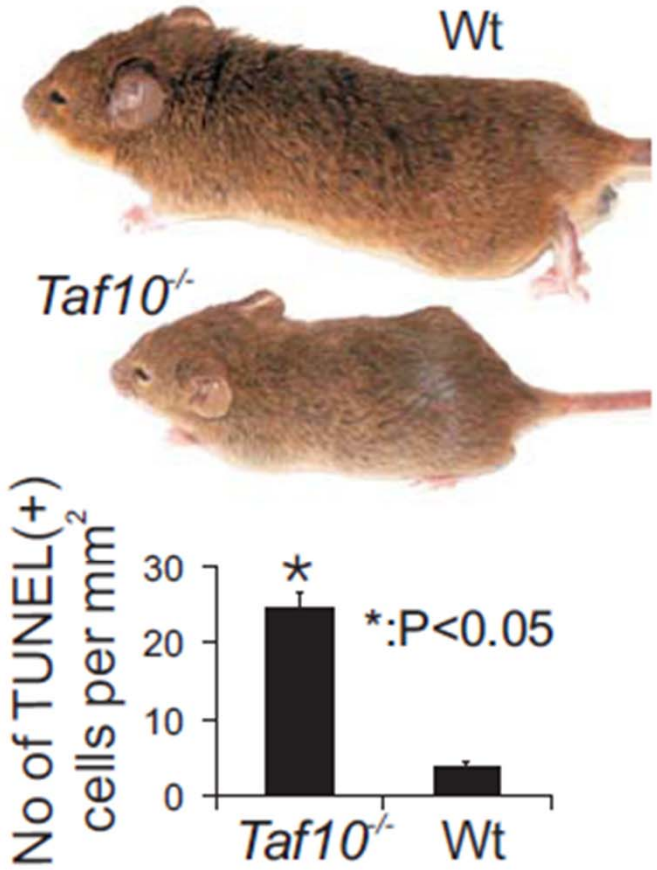
CSA
CSA
Helix-unwinding
Damage verification
TTDA
TFIIH
XPA
Mutations
Cell death/Senescence (?)
RPA
XPB
XPD
ATP
Cancer
Aging
Excision
ERCC1/XPF
XPG
DNA synthesis + ligation
DNA pol δ/ε
PCNA, RFC
DNA ligase
Nucleotide Excision Repair

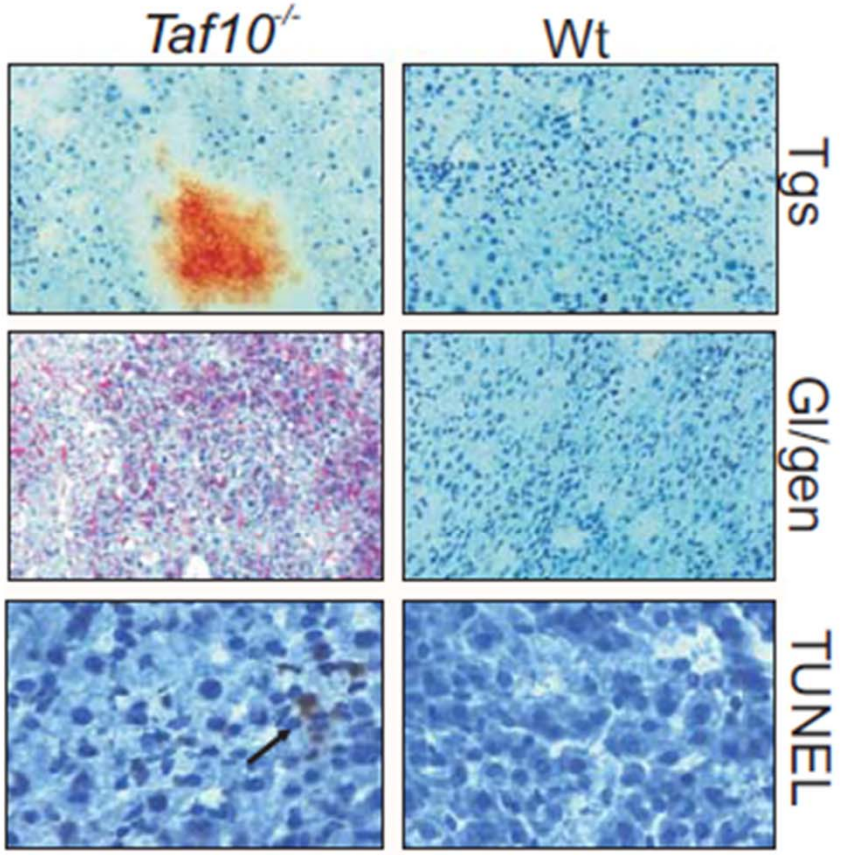
The TFIID connection …



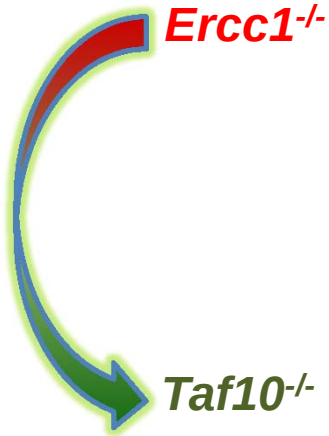


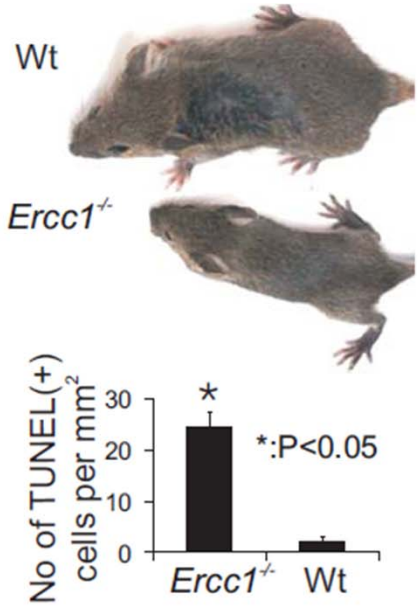
The physiologic & metabolic defects in liver-specific *Taf10*$^{-/-}$ mice

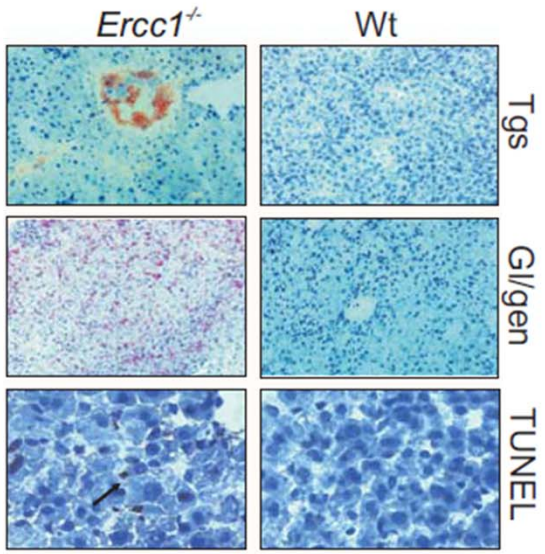


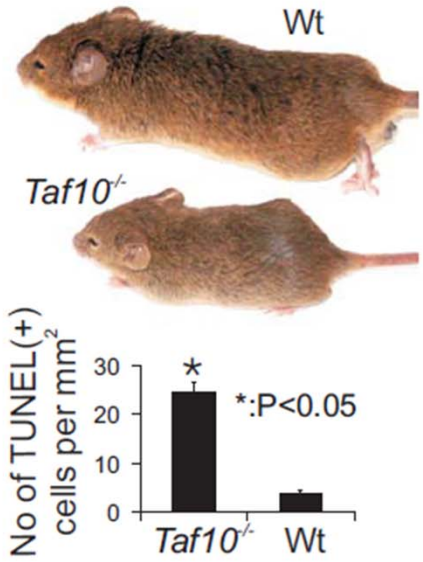


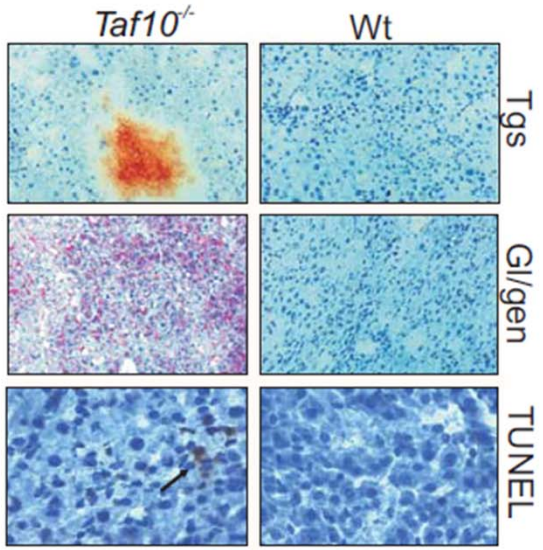
… are so similar to those seen in *Ercc1*$^{-/-}$ mice

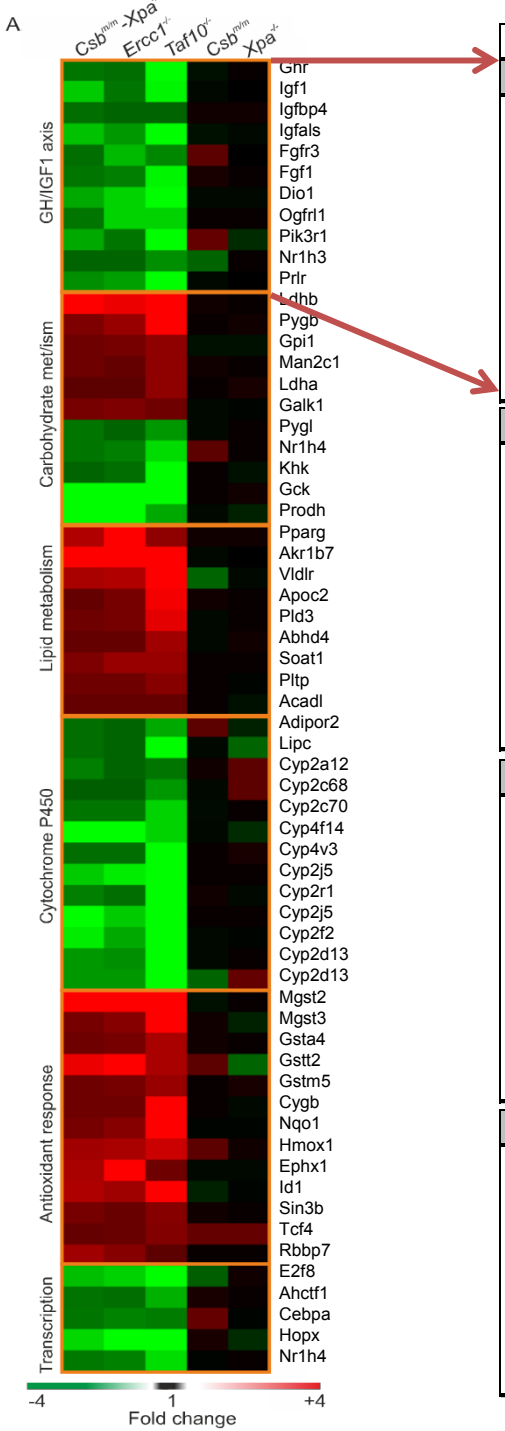












		FC	p	FC	p	FC	p
	Symbol	Csb m/m /Xpa -/-		Ercc1 -/-		Taf10 -/-	
1417962_s_at	Ghr	-1.4	0.002	-1.3	0.047	-4.1	0.027
1419519_at	Igf1	-2.4	0.002	-1.4	0.035	-2.9	0.001
1421992_a_at	Igfbp4	-1.3	0.001	-1.2	0.003	-1.2	0.017
1422826_at	Igfals	-2.3	0.000	-1.8	0.013	-3.8	0.007
1421841_at	Fgfr3	-1.3	0.022	-2.2	0.000	-1.6	0.035
1450869_at	Fgf1	-1.4	0.025	-1.5	0.000	-2.9	0.002
1417991_at	Dio1	-2.0	0.002	-2.5	0.000	-109.3	0.002
1424414_at	Ogfrl1	-1.4	0.043	-2.5	0.000	-2.5	0.008
1451737_at	Pik3r1	-2.0	0.032	-1.4	0.037	-8.8	0.003
1450444_a_at	Nr1h3	-1.2	0.014	-1.2	0.003	-1.7	0.029
1437397_at	Prlr	-1.7	0.000	-1.9	0.013	-3.2	0.000

GH/IGF1 axis

	Symbol	Csb m/m /Xpa -/-		Ercc1 -/-		Taf10 -/-	
1455235_x_at	Ldhb	3.0	0.035	2.8	0.006	6.8	0.022
1433504_at	Pygb	1.5	0.006	1.8	0.000	4.2	0.001
1420997_a_at	Gpi1	1.3	0.011	1.4	0.000	1.7	0.008
1423687_a_at	Man2c1	1.3	0.003	1.2	0.027	1.7	0.003
1419737_a_at	Ldha	1.1	0.002	1.1	0.031	1.7	0.002
1417177_at	Galk1	1.4	0.009	1.5	0.000	1.3	0.010
1417741_at	Pygl	-1.4	0.000	-1.2	0.039	-1.8	0.002
1419105_at	Nr1h4	-1.4	0.010	-1.5	0.000	-2.6	0.019
1449062_at	Khk	-1.2	0.011	-1.3	0.005	-3.0	0.003
1425303_at	Gck	-3.7	0.011	-8.9	0.000	-6.2	0.006
1417629_at	Prodh	-4.4	0.004	-5.1	0.000	-2.0	0.010

Glucose metabolism

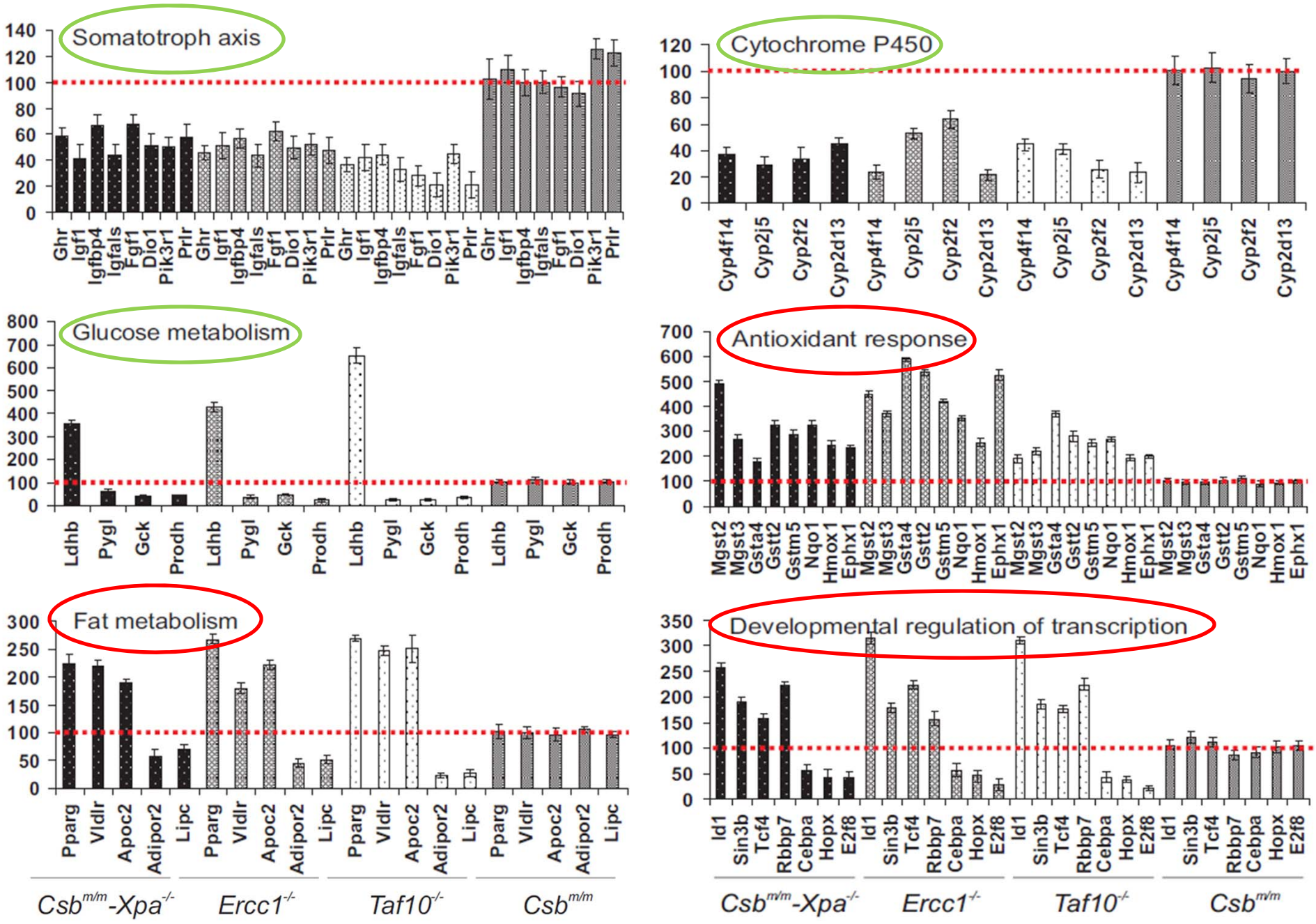
	Symbol	Csb m/m /Xpa -/-		Ercc1 -/-		Taf10 -/-	
1420715_a_at	Pparg	2.1	0.003	3.5	0.000	1.7	0.026
1423556_at	Akr1b7	5.7	0.002	5.7	0.000	24.4	0.000
1417900_a_at	Vldlr	2.0	0.022	2.1	0.000	4.9	0.009
1418069_at	Apoc2	1.2	0.010	1.4	0.000	2.9	0.001
1416013_at	Pld3	1.3	0.004	1.4	0.006	2.7	0.001
1439259_x_at	Abhd4	1.2	0.014	1.2	0.002	1.9	0.004
1417697_at	Soat1	1.5	0.025	1.8	0.022	1.8	0.021
1456424_s_at	Pltp	1.3	0.008	1.3	0.021	1.6	0.032
1448987_at	Acadl	1.2	0.003	1.2	0.010	1.2	0.026
1434329_s_at	Adipor2	-1.3	0.008	-1.2	0.039	-2.0	0.002
1419560_at	Lipc	-1.3	0.000	-1.2	0.013	-5.1	0.000

Fat metabolism

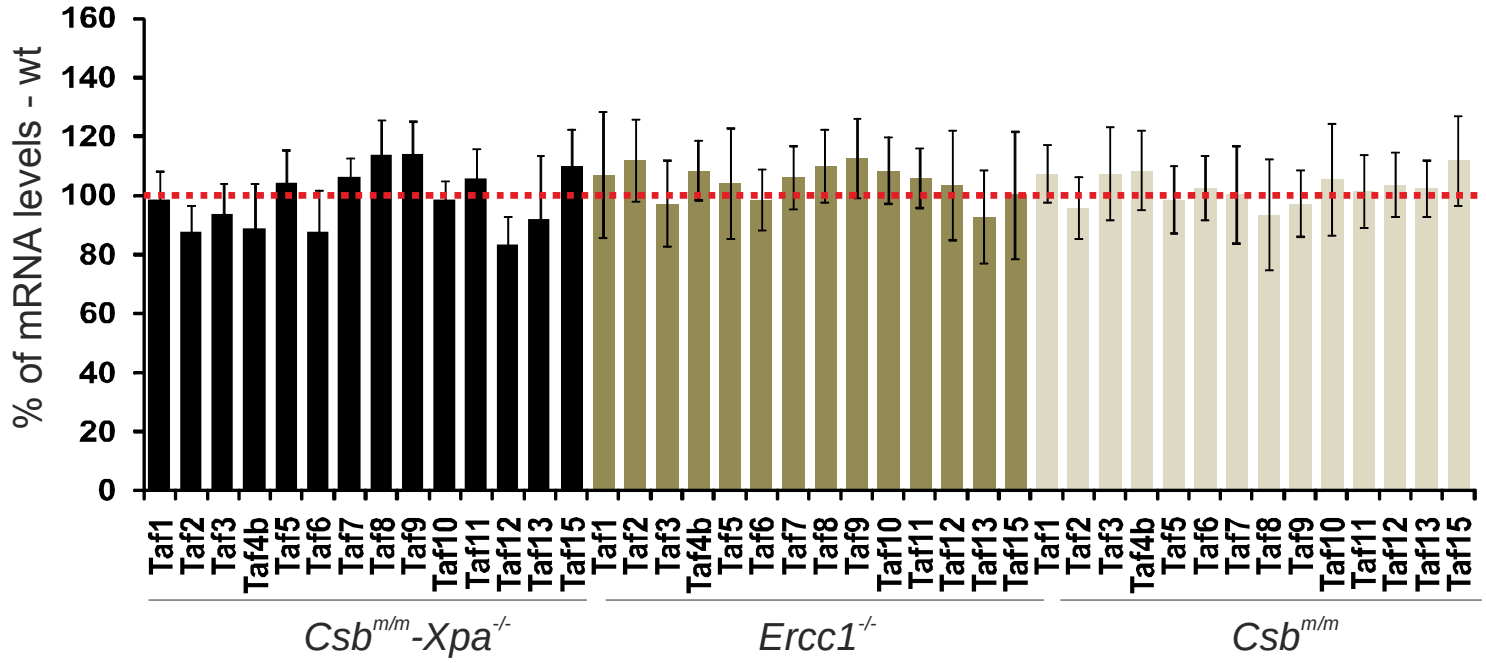
	Symbol	Csb m/m /Xpa -/-		Ercc1 -/-		Taf10 -/-	
1452592_at	Mgst2	5.9	0.005	5.3	0.001	4.6	0.003
1448300_at	Mgst3	1.4	0.012	1.6	0.002	4.2	0.002
1416368_at	Gsta4	1.3	0.043	1.4	0.000	2.0	0.006
1417883_at	Gstt2	2.8	0.002	4.3	0.000	2.0	0.027
1416842_at	Gstm5	1.3	0.001	1.4	0.000	1.8	0.006
1423630_at	Cygb	1.3	0.015	1.3	0.002	7.6	0.003
1423627_at	Nqo1	1.4	0.024	1.6	0.002	6.0	0.049
1448239_at	Hmox1	1.9	0.001	2.0	0.002	2.4	0.008
1422438_at	Ephx1	2.0	0.004	3.0	0.000	1.3	0.001

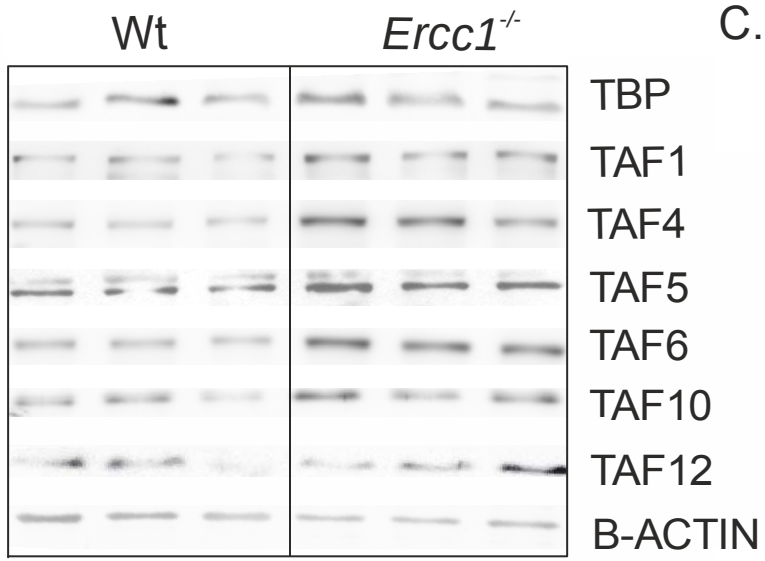
Antioxidant response

Ercc1$^{-/-}$ and *Taf10*$^{-/-}$ livers share common over-represented biological processes

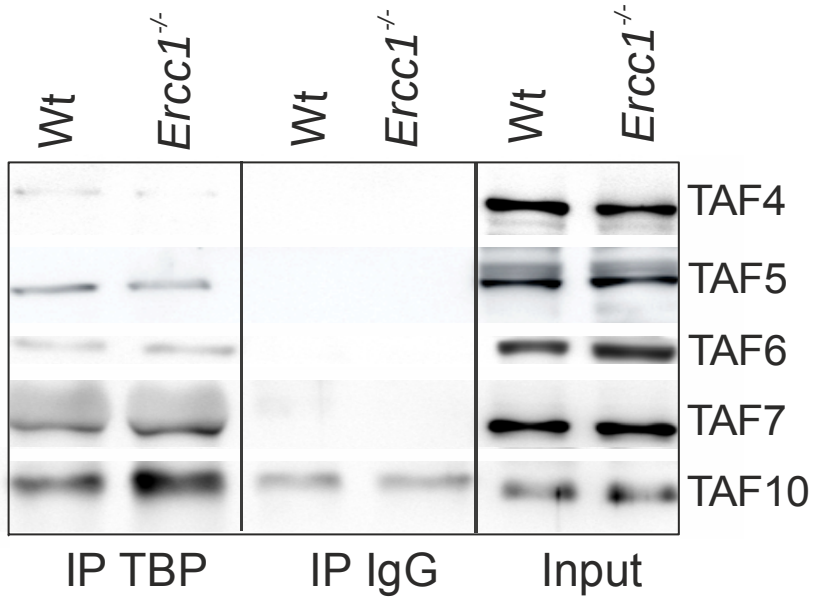


TFIID is assembled in *Ercc1*$^{-/-}$ livers

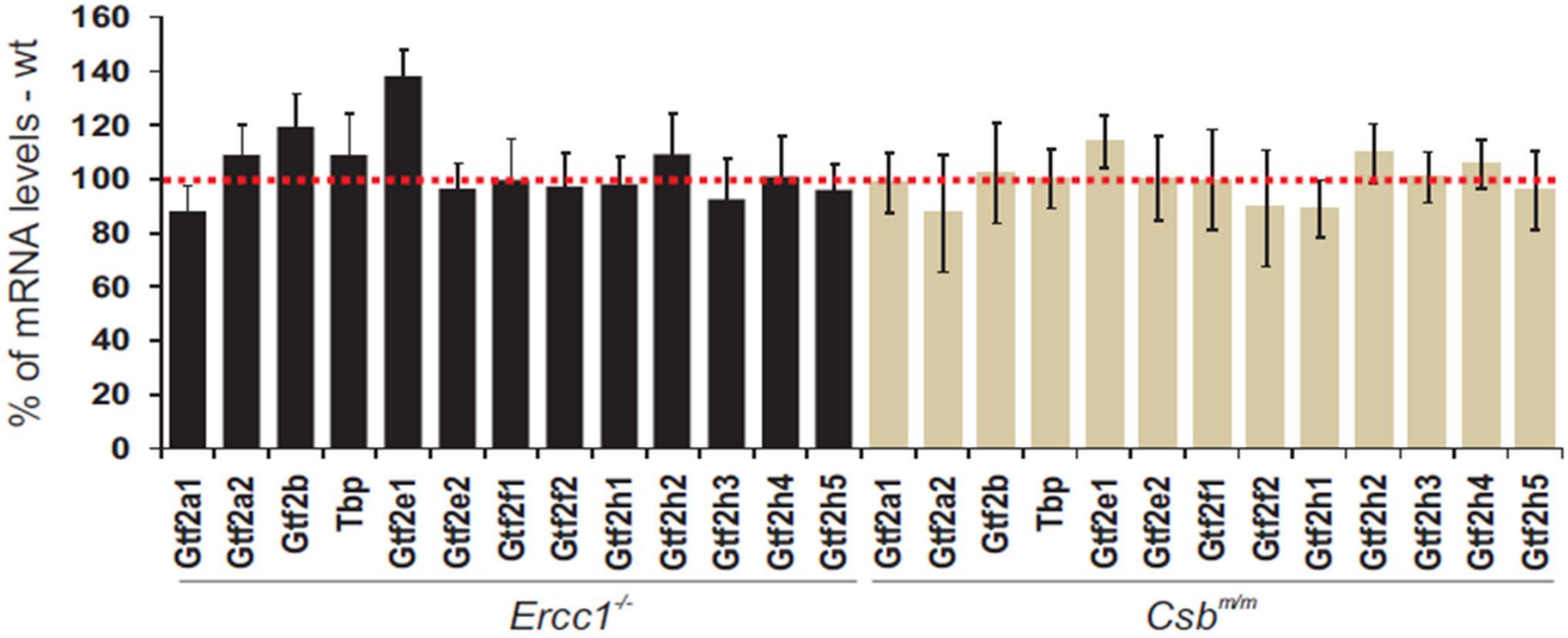


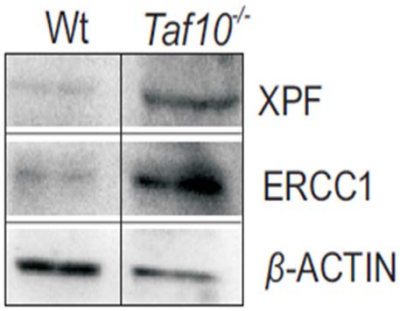


C.



General TFs are expressed normally in *Ercc1*$^{-/-}$ livers

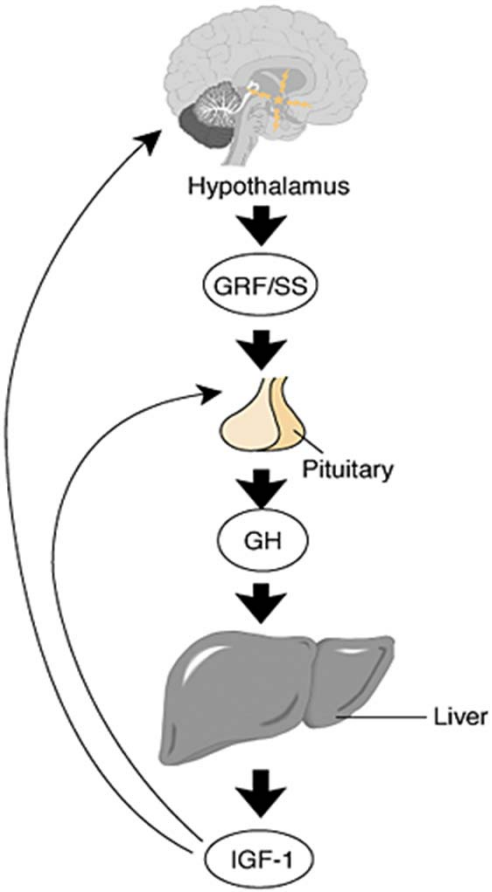


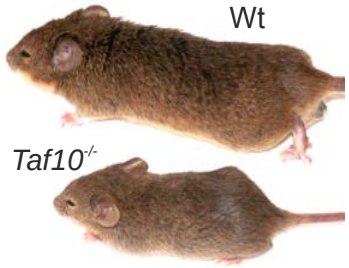


ERCC1-XPF is expressed in *Taf10*$^{-/-}$ livers

Suppression of GH/IGF1 axis leads to growth defect

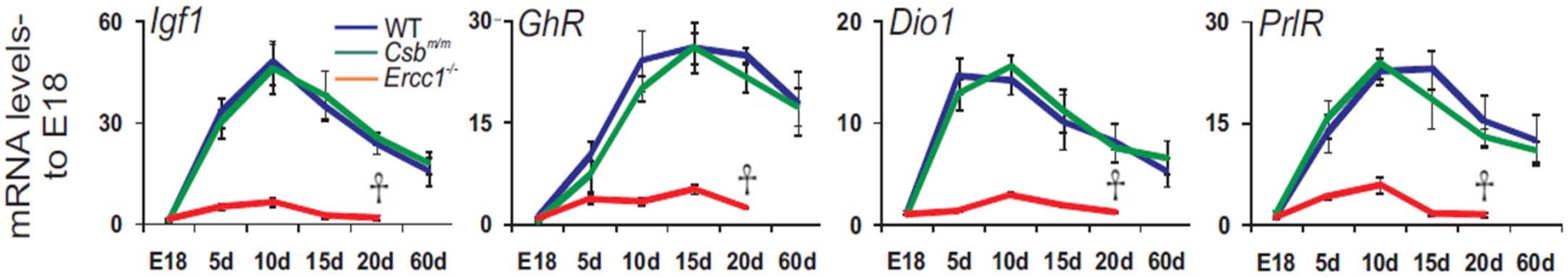
↓IGF-1
↓GH-R
↓PRL-R
↓DIO1



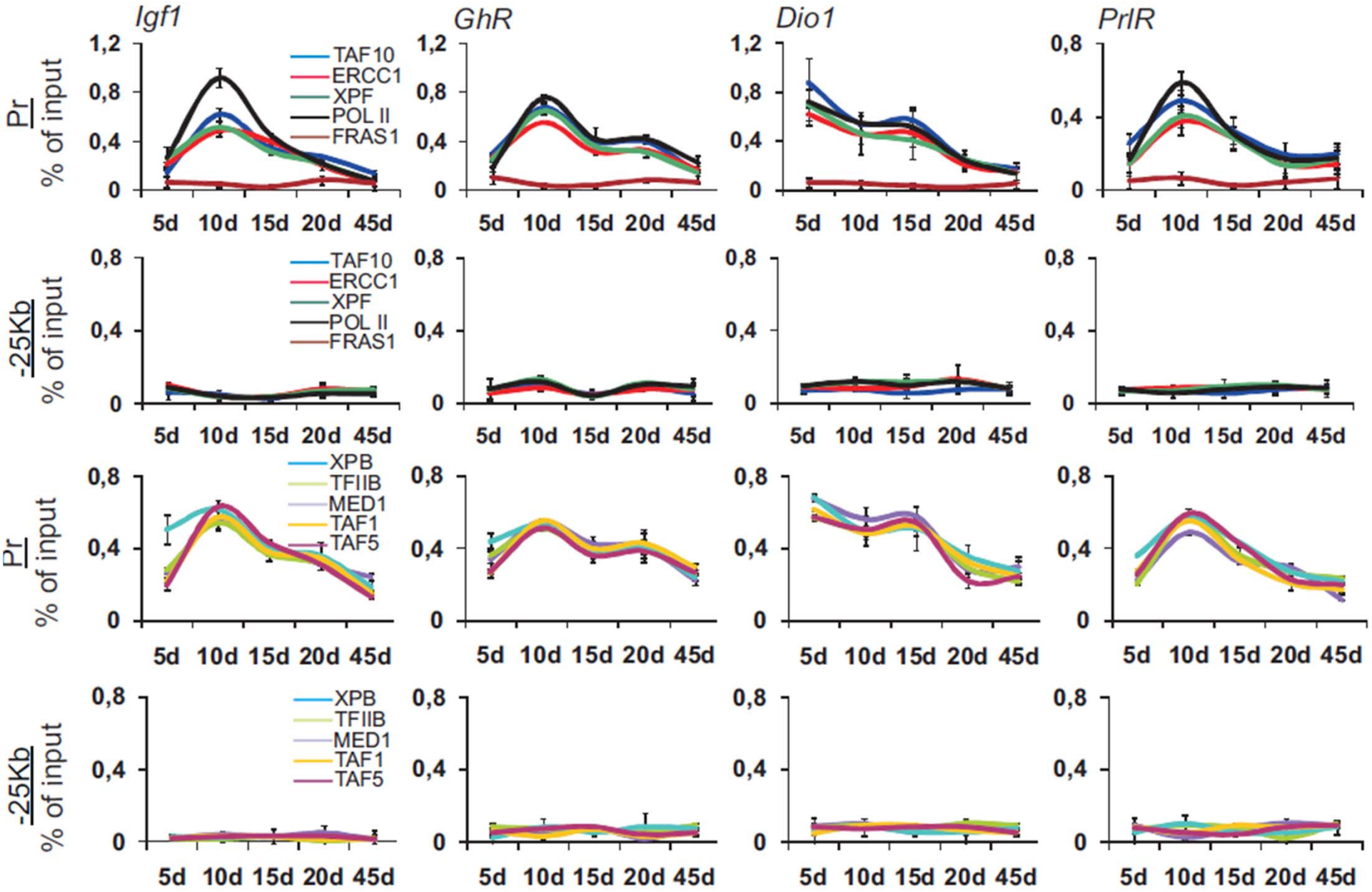


↓IGF-1
↓GH-R
↓PRL-R
↓DIO1

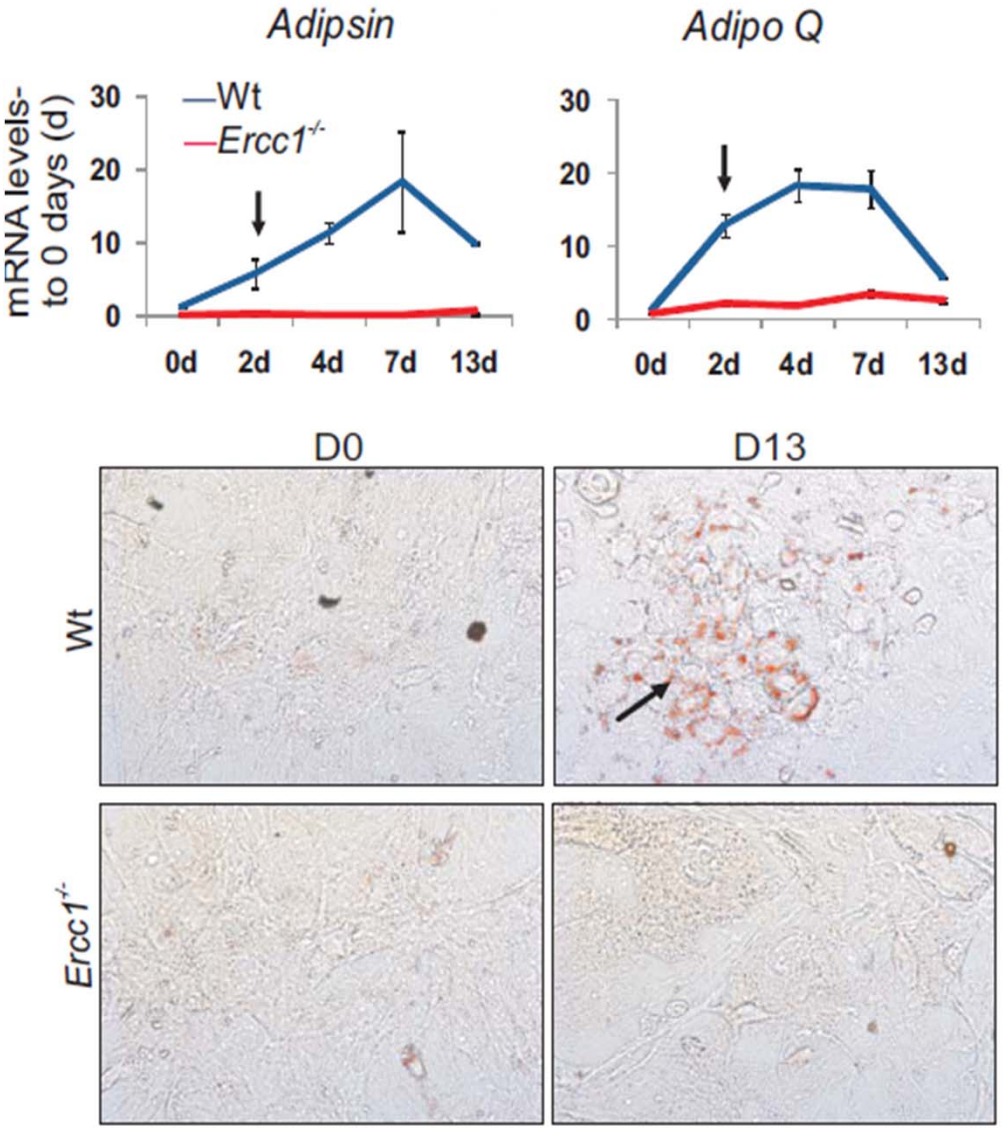
E18 → P9

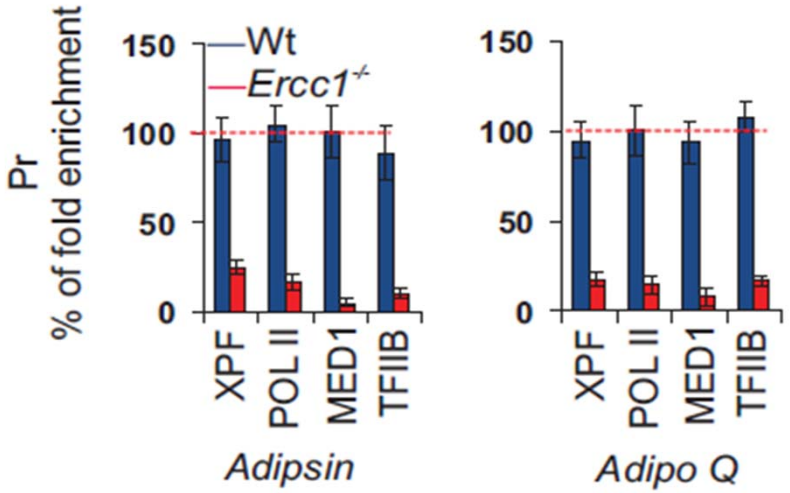


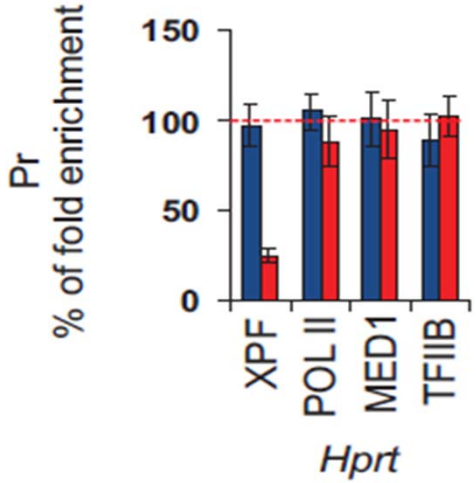
ERCC1-XPF is recruited on the promoters of genes associated with postnatal murine growth

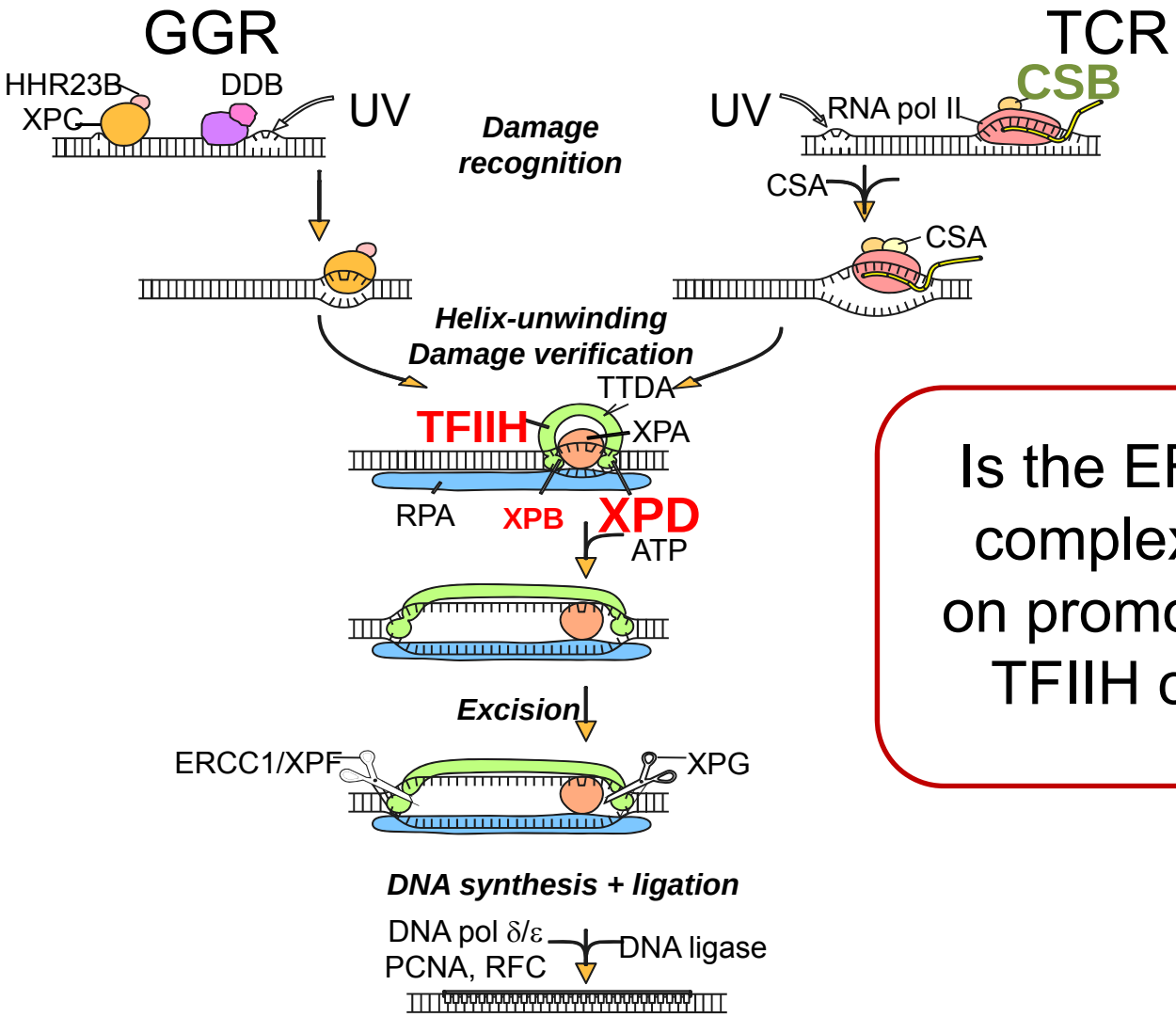


ERCC1-XPF is required for the transcriptional activation of genes promoting adipogenesis







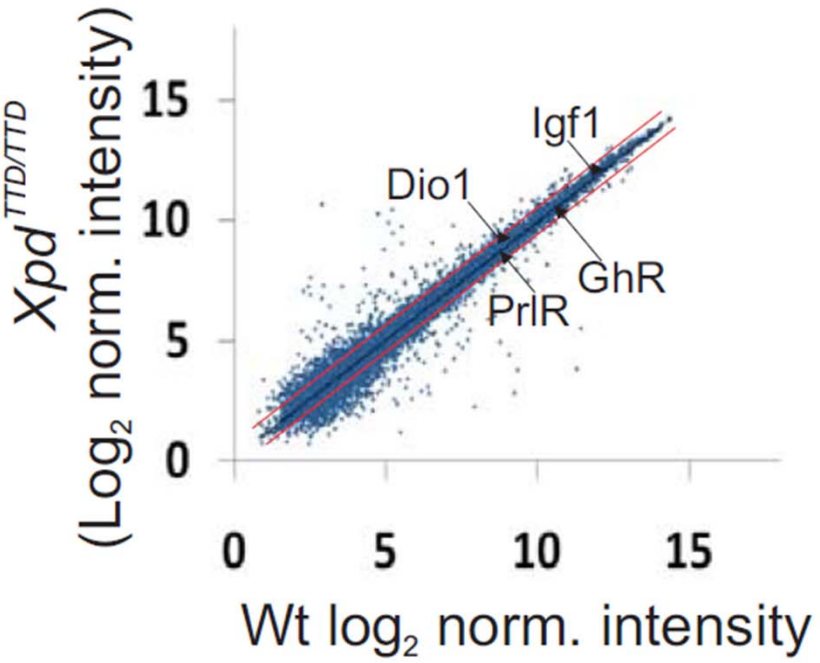


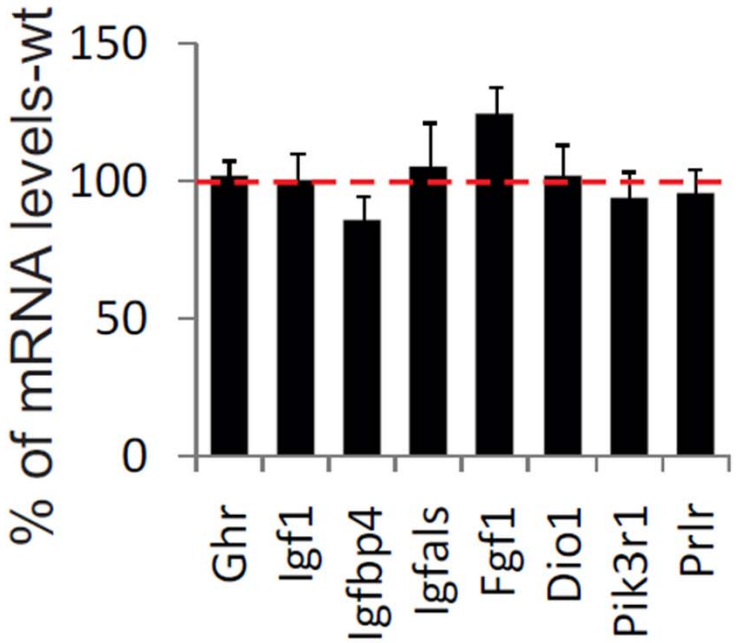
Is the ERCC1-XPF complex recruited on promoters due to TFIIH complex ?

ERCC1-XPF has a role in transcription distinct from that of other NER factors.

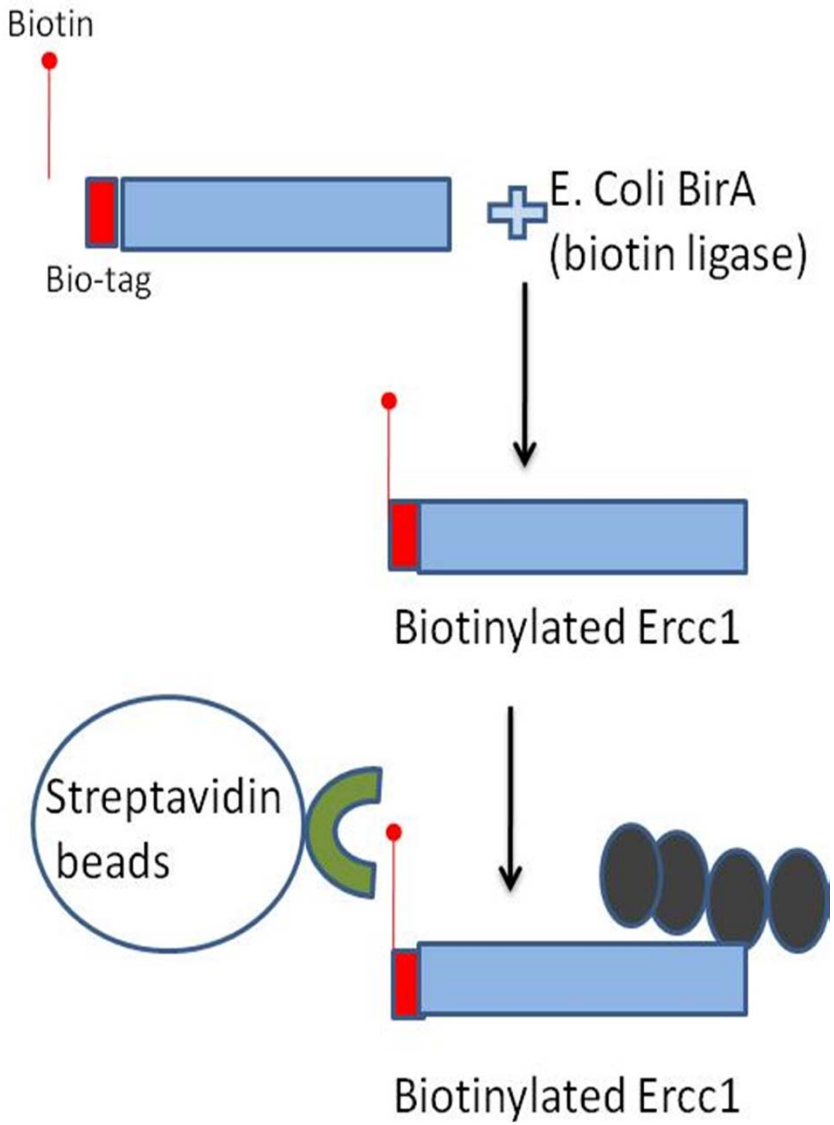
WT

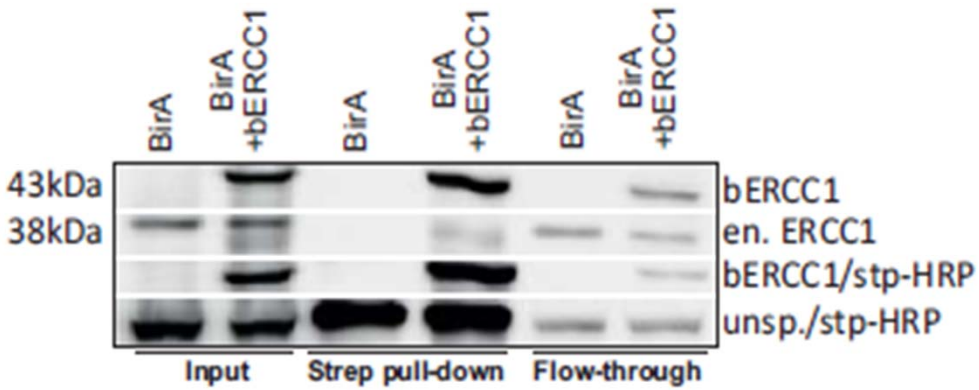
$Xpd^{TTD/TTD}$

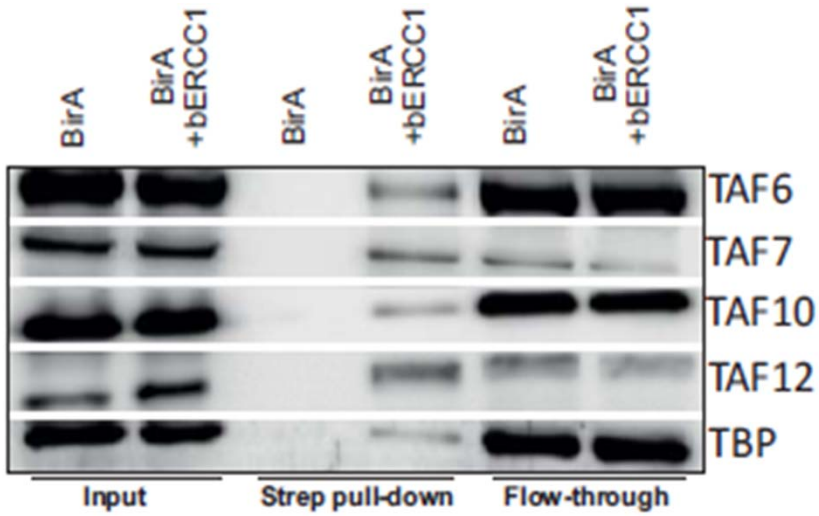




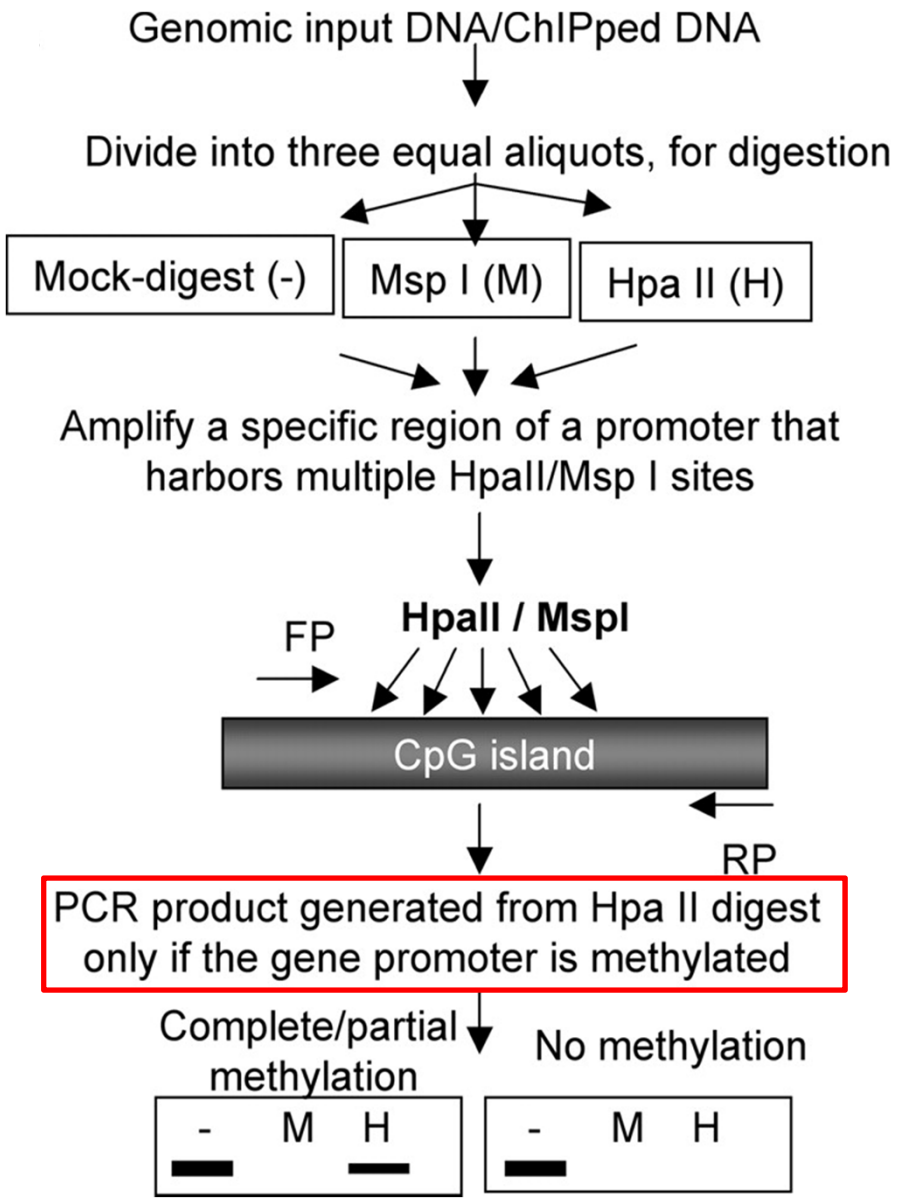
ERCC1-XPF interacts with TFIID subunits

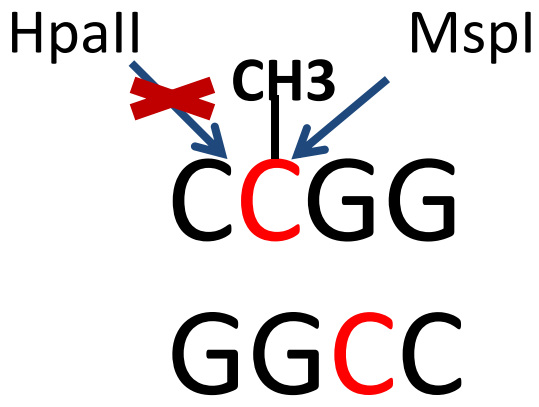




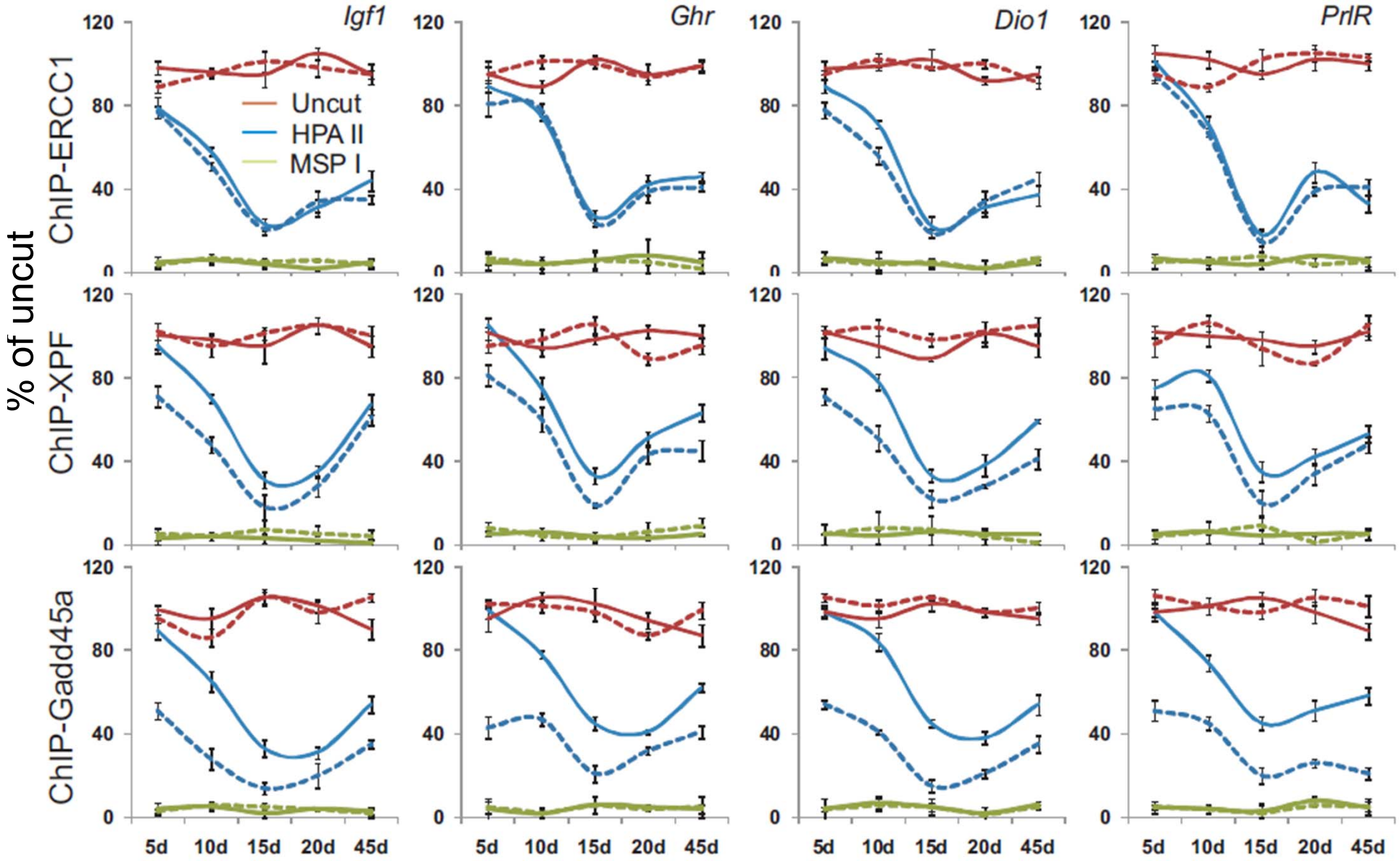


The “Chip/chop” approach

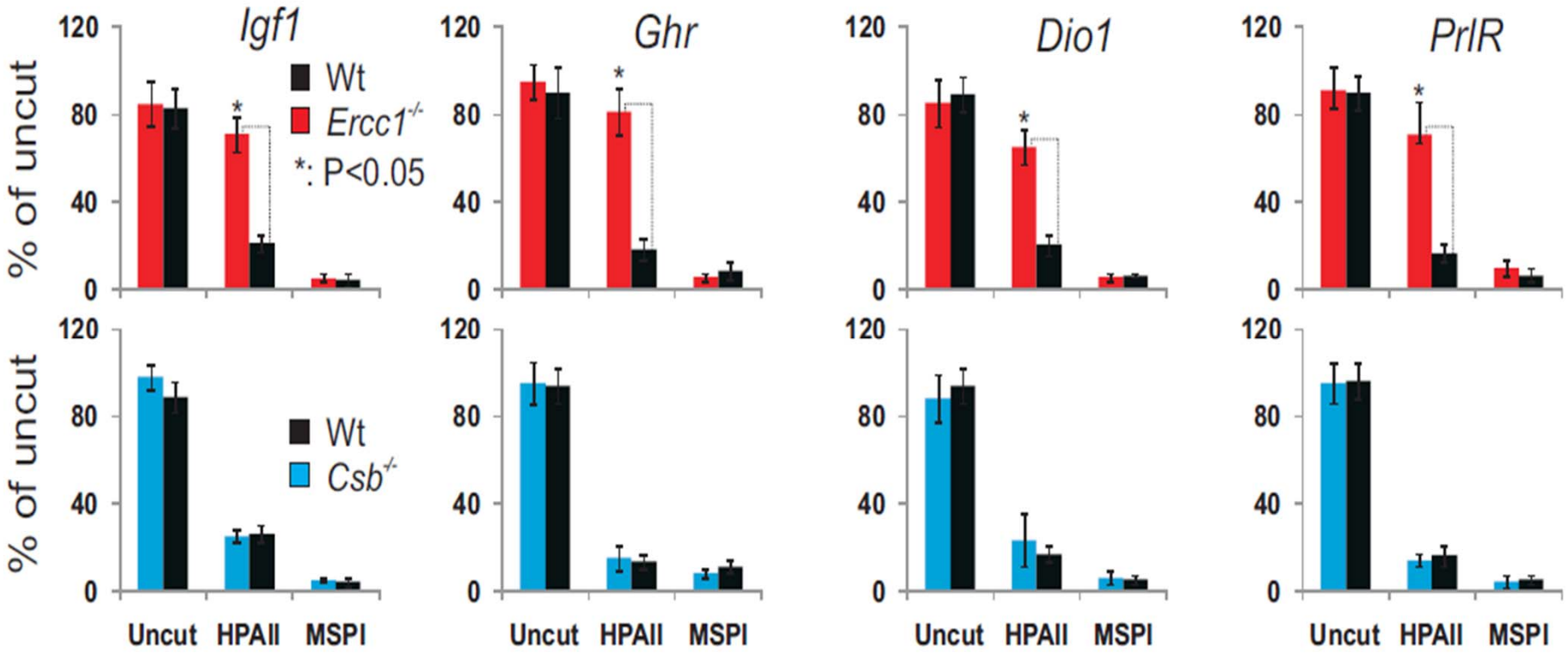




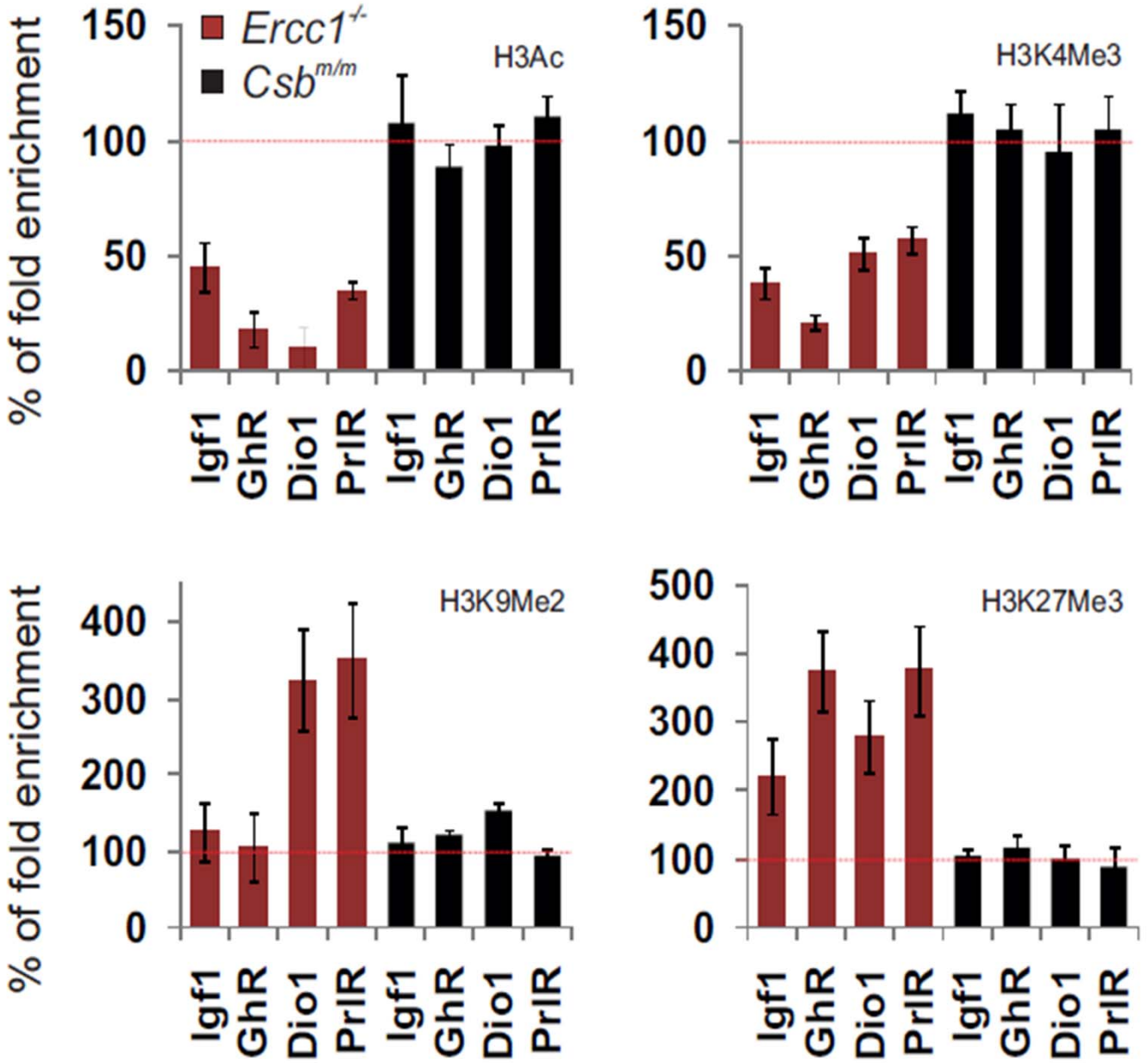
ERCC1-XPF promotes active DNA demethylation on promoters associated with hepatic development



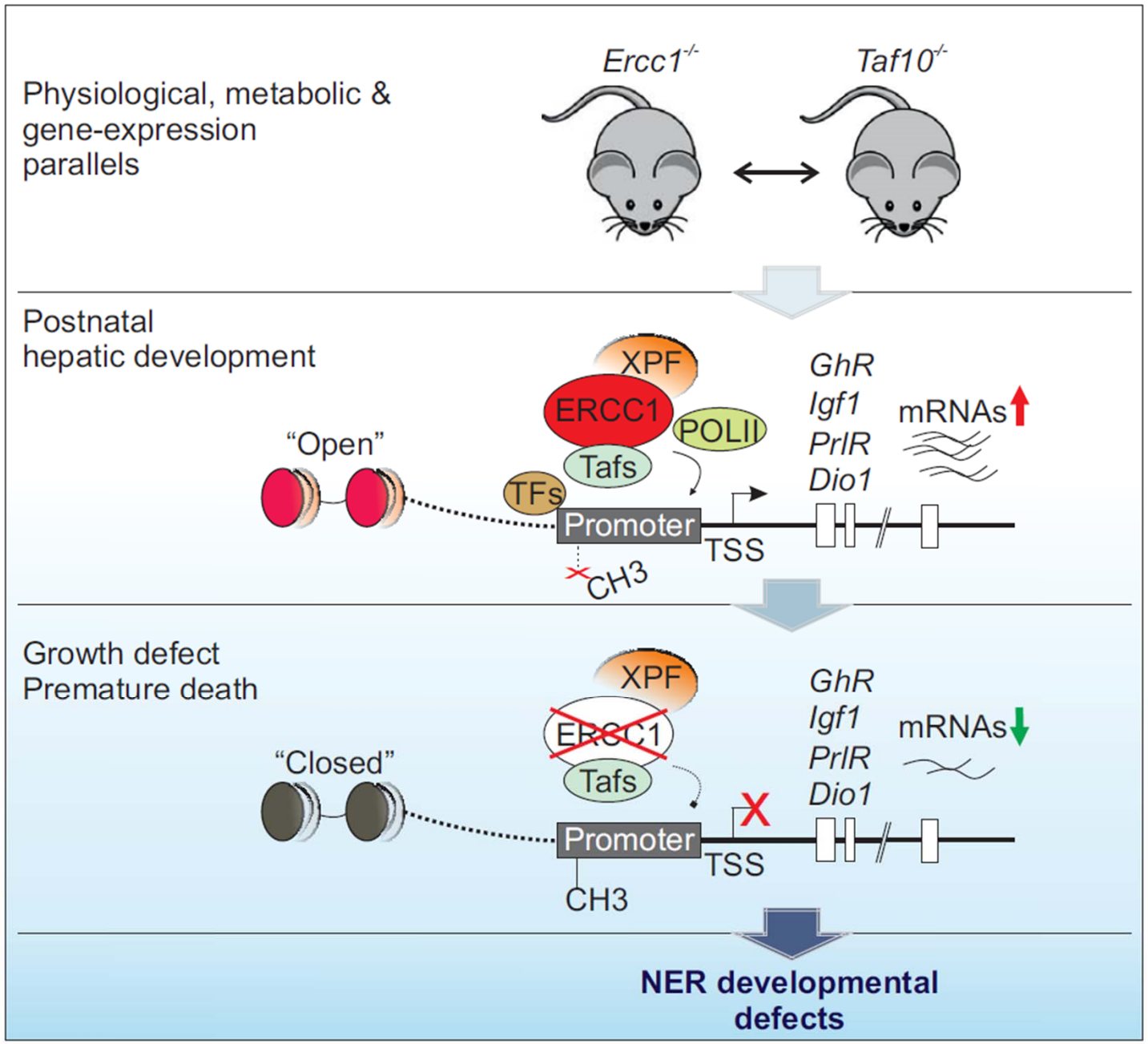
Disruption of *Ercc1* -but not of *Csb*- gene leads to the aberrant DNA methylation on promoters



Histone PTMs in *Ercc1*$^{-/-}$ and *Csb*$^{m/m}$ livers



Summary


Physiological, metabolic & gene-expression parallels
Ercc1-/-
Taf10-/-
Postnatal hepatic development
XPF
ERCC1
POLII
Tafs
TFs
"Open"
Promoter
TSS
CH3
GhR
Igf1
PrlR
Dio1
mRNAs
Growth defect Premature death
XPF
ERCC1
Tafs
"Closed"
Promoter
TSS
CH3
GhR
Igf1
PrlR
Dio1
mRNAs
NER developmental defects

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Marie Curie Actions
Human Resources and Mobility Activity

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FP7-Capacities

NSRF 2007-2013
programme for development

EMBO