VI SPG Conference

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Translational activation of Δ160p53 is triggered during the Integrated Stress Response to promote survival

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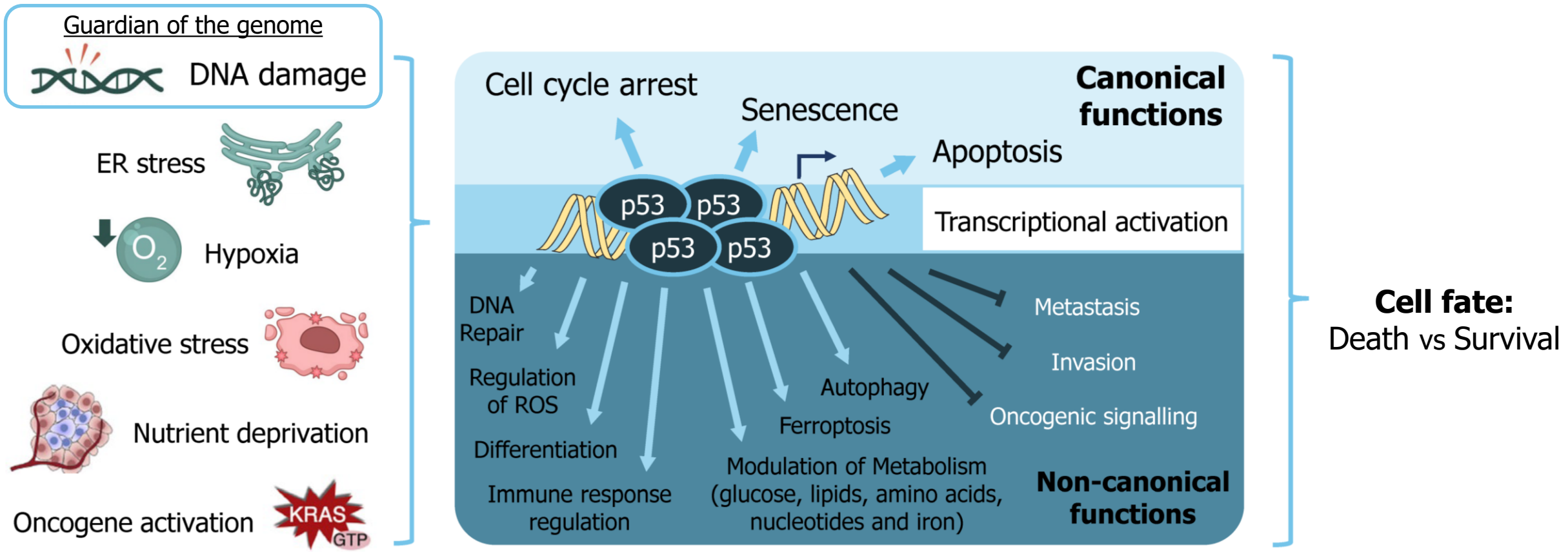
Introduction: p53 is a key player in stress response

p53 is a transcription factor activated by different **stresses** that can modulate multiple **cellular responses**.



Tumour suppressor gene — Avoids the propagation of potentially tumorigenic cells

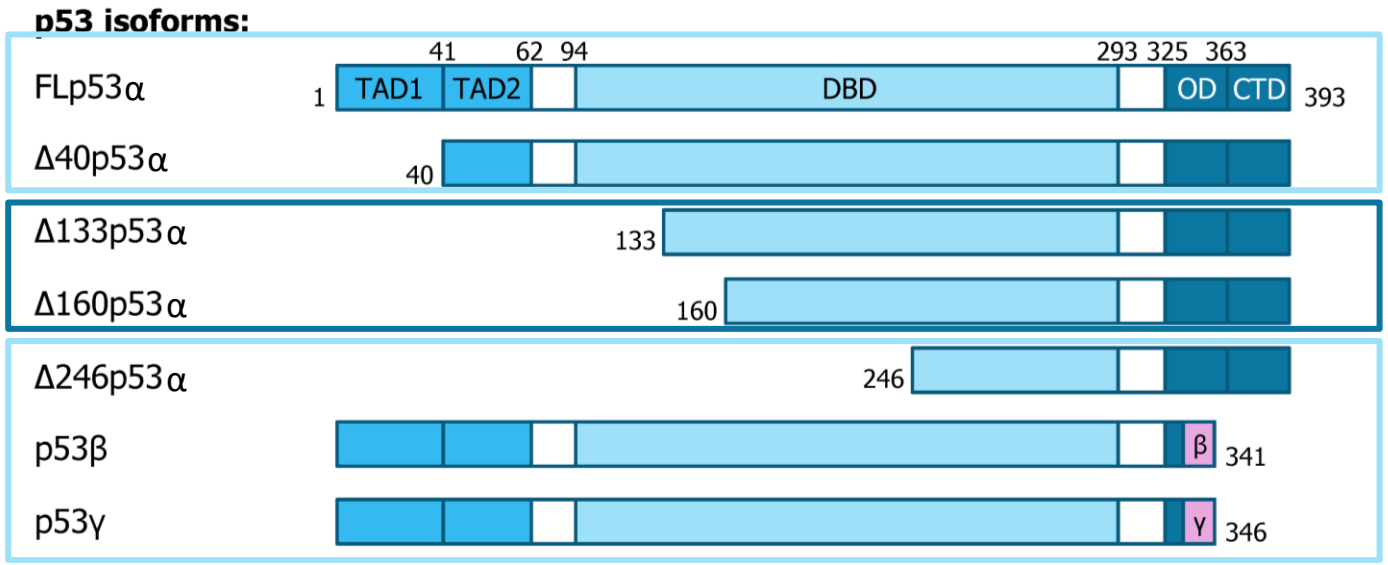
The *p53* gene is mutated in over 50% of all human cancers.

Illustrations adapted from: Mello SS, Attardi LD. Deciphering p53 signaling in tumor suppression. *Curr Opin Cell Biol.* **51**:65–72 (2018).
Lior C et al. Mapping the tumor stress network reveals dynamic shifts in the stromal oxidative stress response. *Cell Reports*. **5**(43):114236 (2024).

Introduction: the *p53* gene and its protein isoforms

The *p53* gene encodes at least **13 protein isoforms** resulting from transcription from 2 promoters, alternative splicing and internal initiation of translation.

The isoforms have been extensively studied and shown to modulate the tumour suppressor functions of FLp53.



Tumour suppressor

Oncogenic

Promote DNA repair, proliferation, invasion and survival.

Δ160p53
Overexpressed in cancer cells
Conserved among mammals

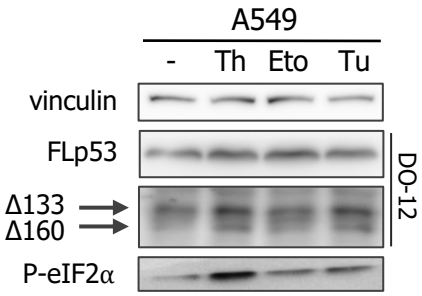
Aims of this work

- Explore the mechanisms and factors that regulate Δ160p53 translation

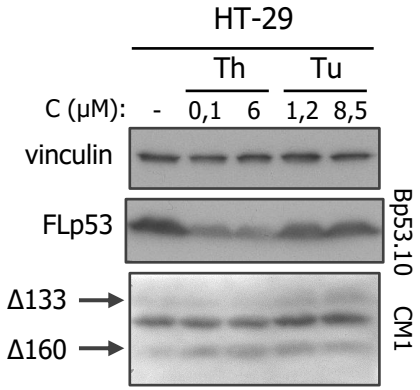
- Investigate how Δ160p53 exerts its pro-survival functions

Δ160p53 is stimulated by the Integrated Stress Response (ISR)

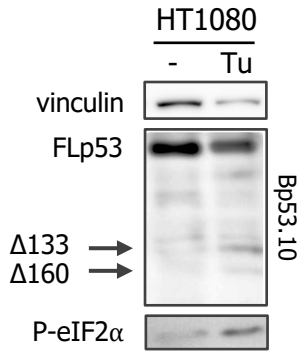
lung adenocarcinoma



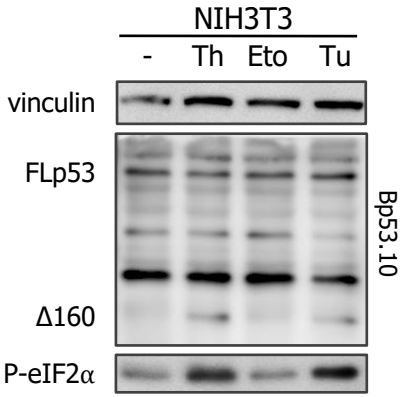
colon carcinoma



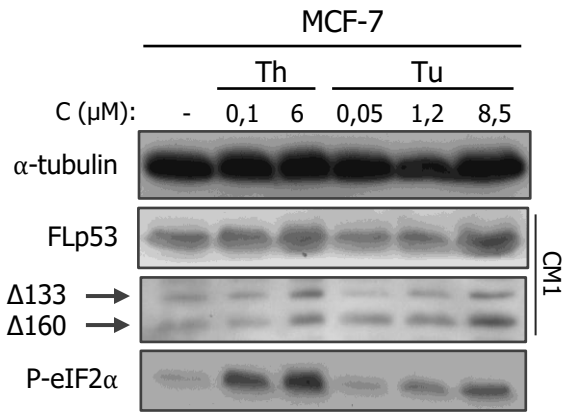
fibrosarcoma



mouse embryonic fibroblast

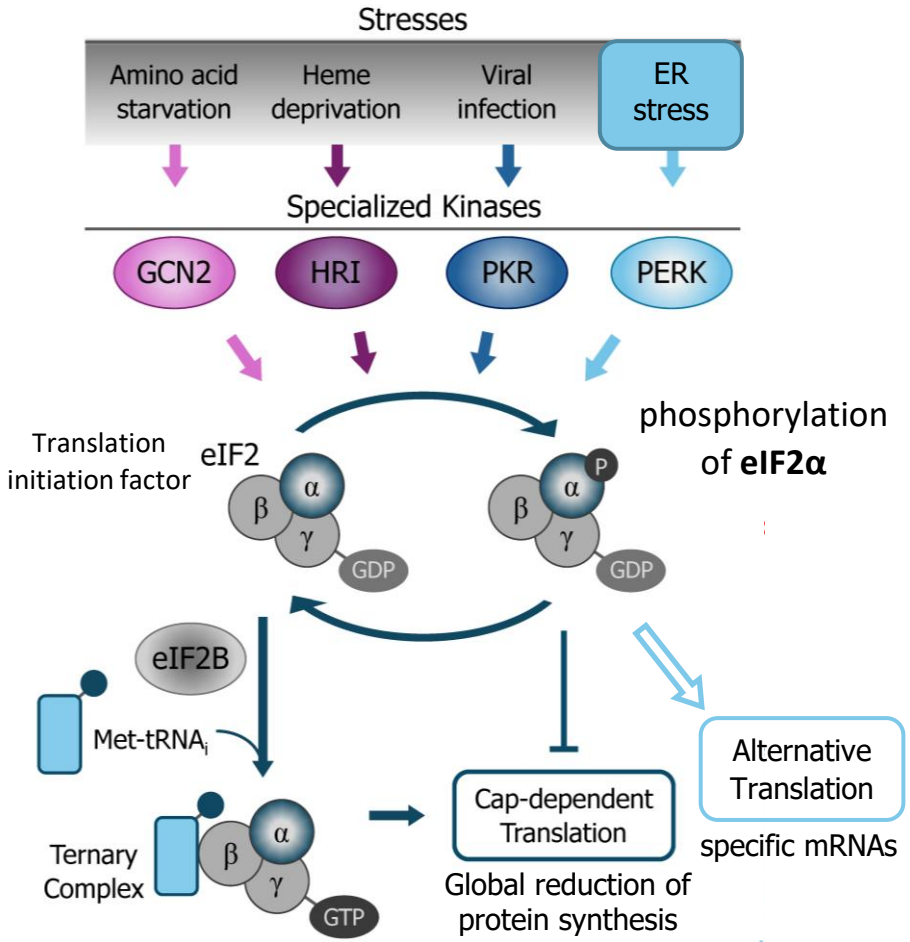


breast adenocarcinoma



ISR

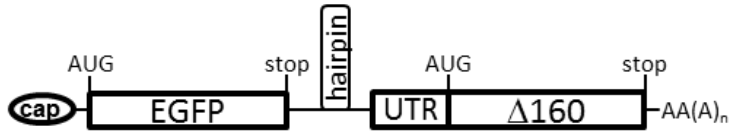
Translational reprogramming to promote survival and re-establishment of homeostasis

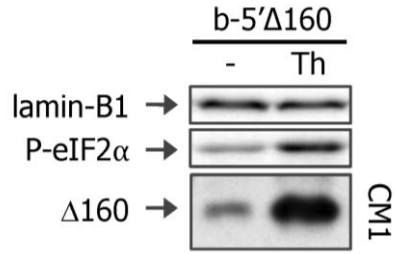


Endoplasmic reticulum (ER) stress was induced by tunicamycin and thapsigargin 16h treatments.

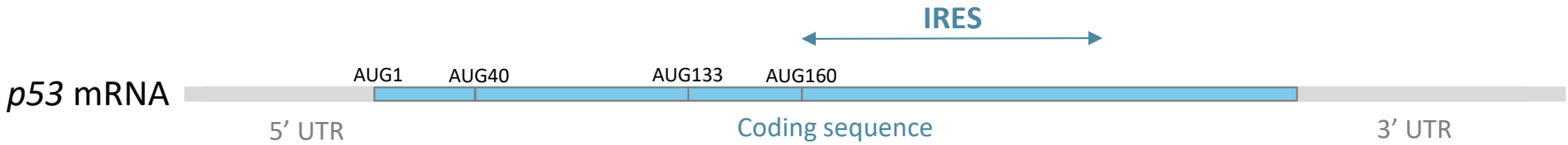
Internal Translation of Δ160p53 is mediated by an Internal Ribosome Entry Site (IRES)

bicistronic 5'UTR-Δ160
(b-5'Δ160)

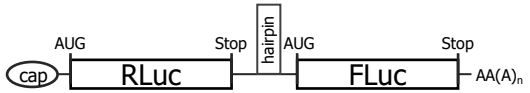




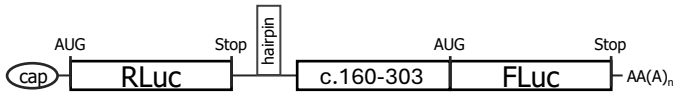
- **Δ160p53** is translated via internal initiation.



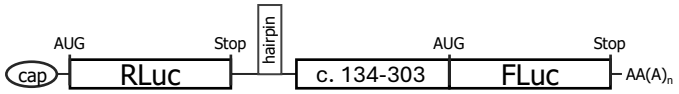
bicistronic Luc (b-Luc)

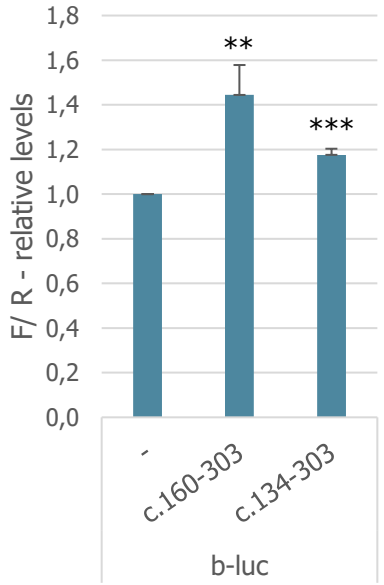


b-Luc c.160-303



b-Luc c.134-303



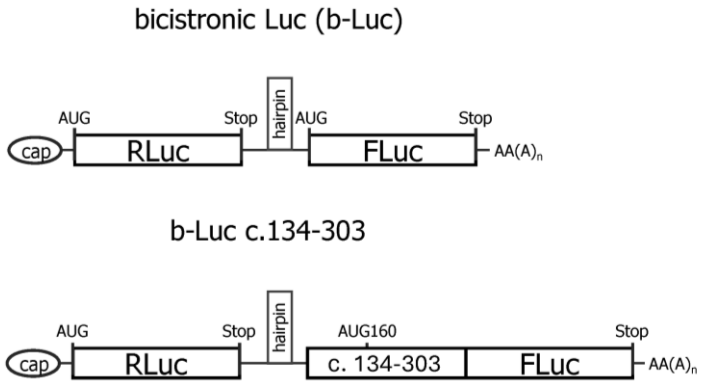


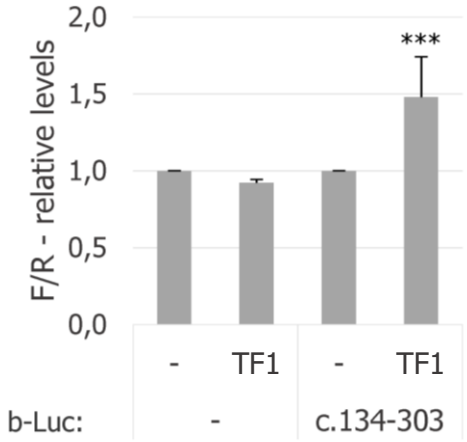
- The region spanning codons 160 – 303 is sufficient to support internal initiation of translation: **Δ160p53 IRES** identified.

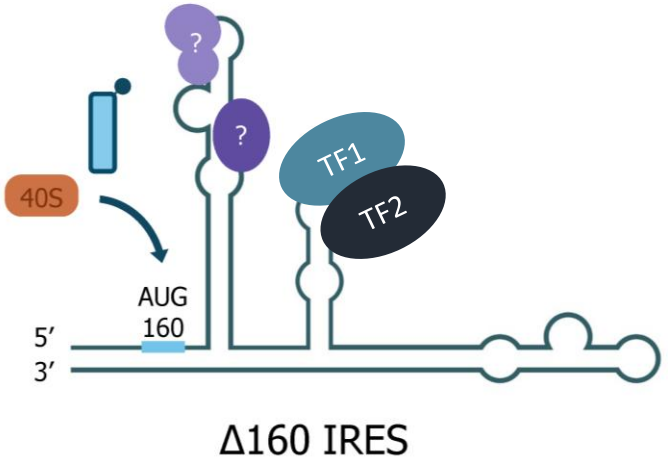
H1299 cells: p53-null

The translation of Δ160p53 is regulated by stress-responsive factors

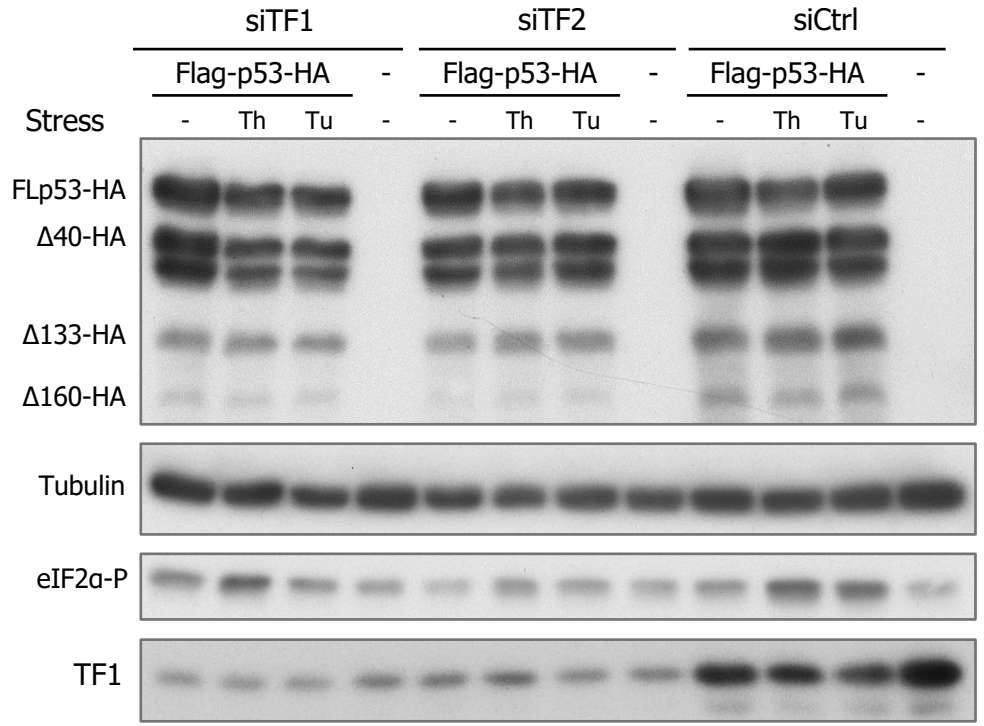
Overexpression of an ISR Translation Factor

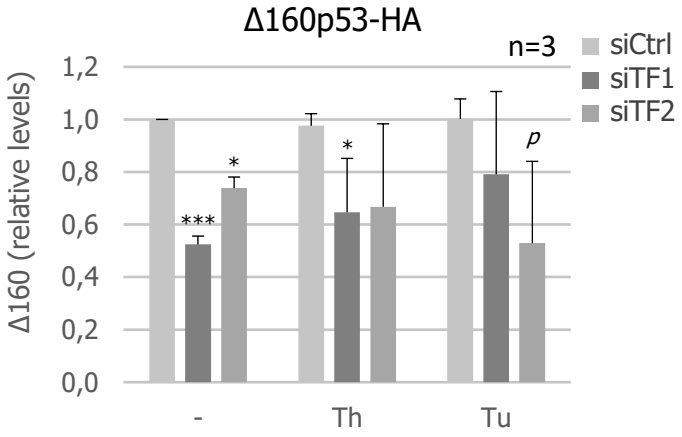






Knockdown of ISR Translation Factors



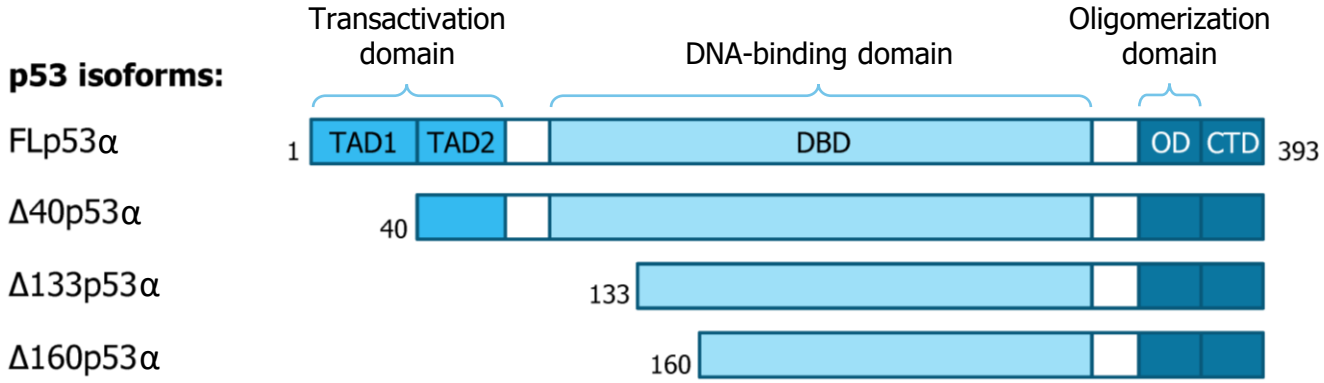


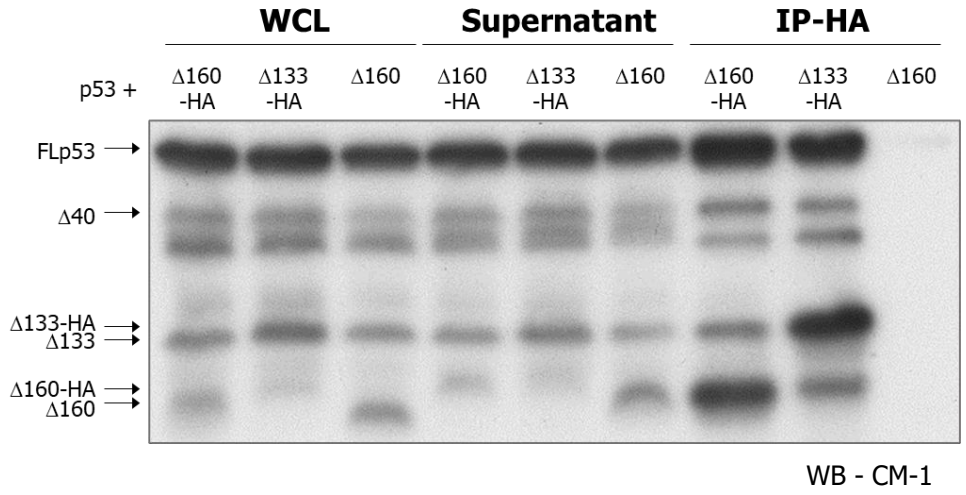
Aims of this work

- Explore the mechanisms and factors that regulate Δ160p53 translation

- Investigate how Δ160p53 exerts its pro-survival functions

Δ160p53 interacts with FLp53





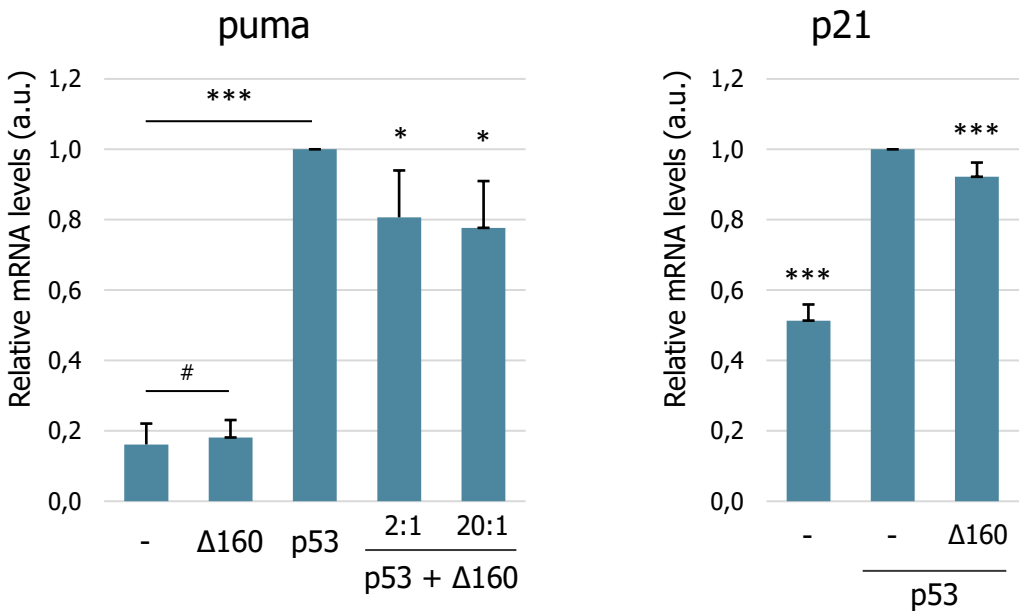
WB - CM-1

Co-transfection of **H1299** cells with:

- **Δ160p53-HA**, **Δ133p53-HA** or untagged **Δ160p53** plasmid;
- untagged **p53** plasmid.

Δ160p53 modulates the transcriptional activity of FLp53

Δ160p53 hampers the activation of classical p53 target genes:

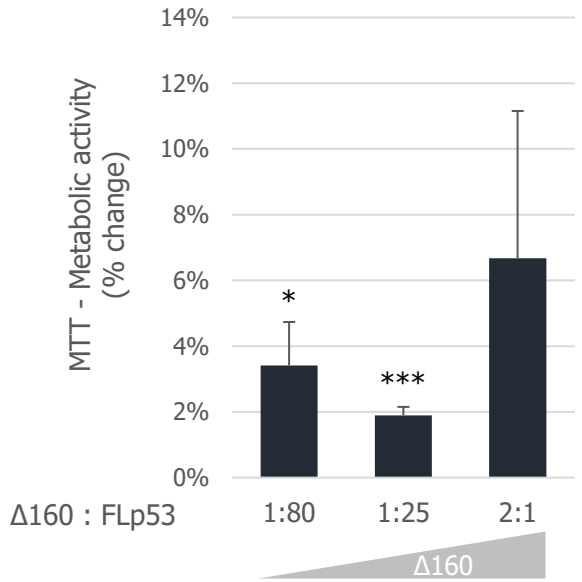


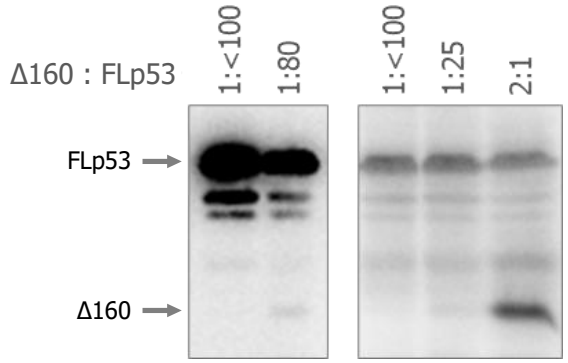
puma (*BBC3*)
promotes apoptosis

p21 (*CDKN1A*)
promotes cell cycle arrest

- puma mRNA levels are not affected by **Δ160p53** alone.
- **Δ160p53** counteracts the p53-mediated increase of puma and p21 mRNA levels.

Cell viability is improved by Δ160p53 across a range of Δ160:FLp53 ratios:

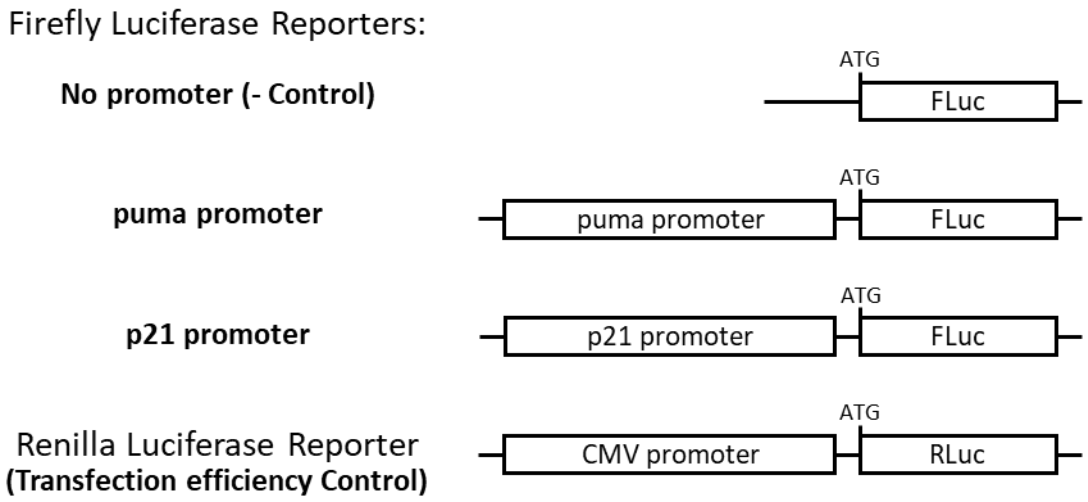


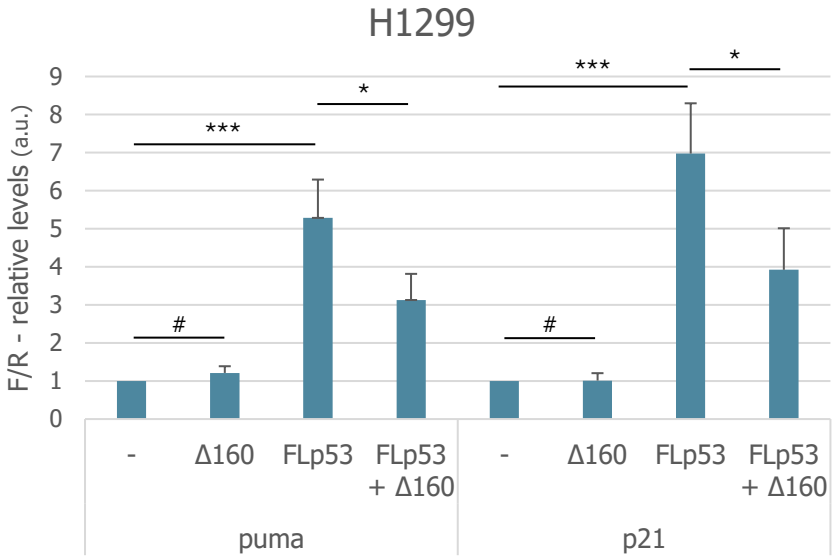


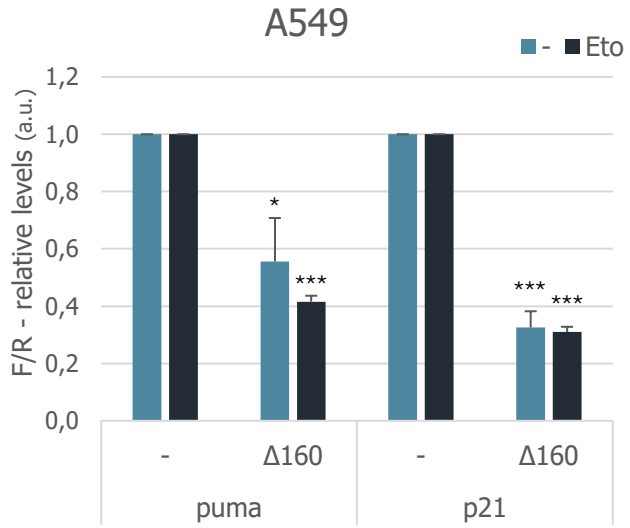
- **Δ160p53** favors the viability of H1299 cells even when its levels are 25 or 80x lower than FLp53.

RT-qPCR assays conducted with H1299 cells

Δ160p53 modulates the transcriptional activity of FLp53







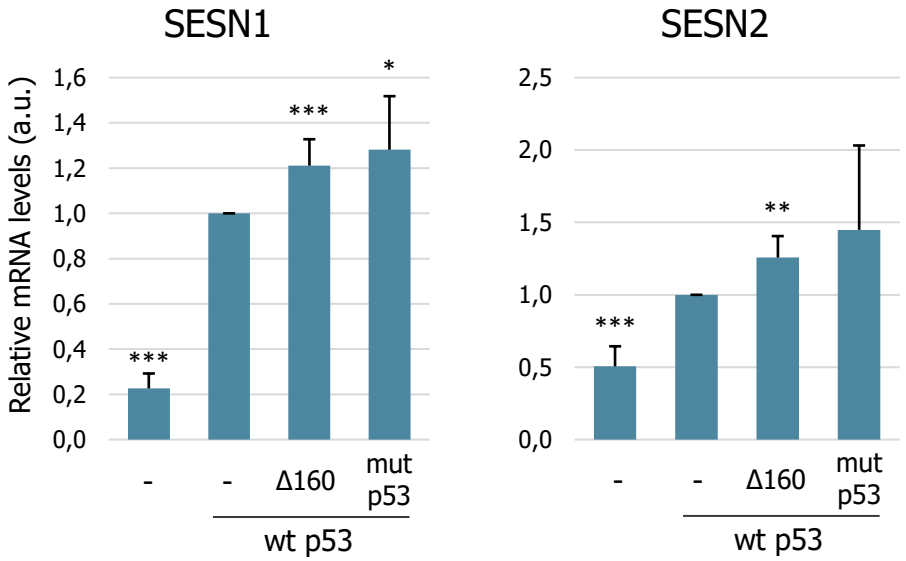
- **Δ160p53** inhibits their transcription in A549 cells expressing wild-type p53.

- Transcription from puma and p21 promoters is not affected by **Δ160p53** alone in H1299 cells.
- **Δ160p53** counteracts FLp53-mediated transcription from the 2 promoters.

Δ160p53 directly affects the transcriptional activity of FLp53

Δ160p53 modulates the transcriptional activity of FLp53

Δ160p53 enhances the activation of p53 target genes:

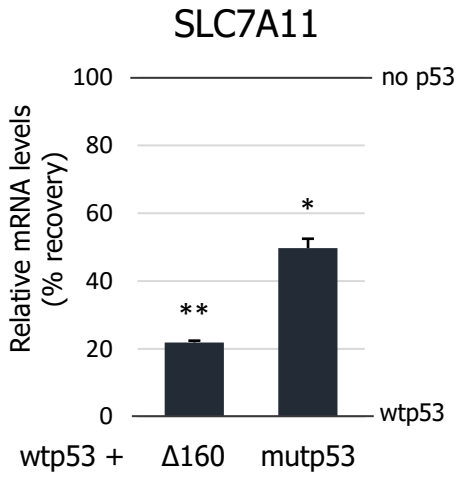


Sestrins

ER stress-responsive genes

Promote autophagy and repress ferroptosis

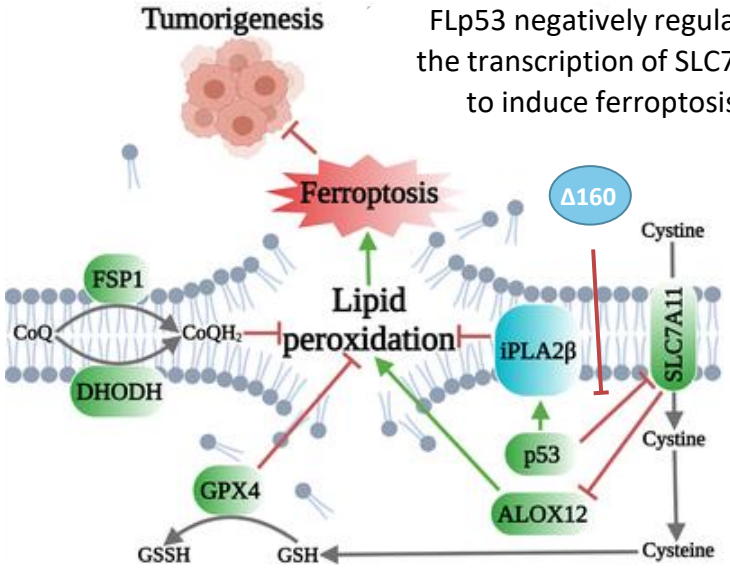
- **Δ160p53** enhances the FLp53-mediated increase of *SESN1* and *SESN2* mRNA levels.



SLC7A11

Represses ferroptosis

- **Δ160p53** counteracts the FLp53-mediated suppression of *SLC7A11* mRNA levels.



FLp53 negatively regulates the transcription of SLC7A11 to induce ferroptosis

Mao, C. *et al*. Phospholipase iPLA2β acts as a guardian against ferroptosis. *Cancer Communications*. 41: 11 (2021).

Δ160p53 protects from FLp53-induced cell death pathways (apoptosis, ferroptosis) and cell cycle arrest, while favouring autophagy.

mutp53 = R248Q hotspot mutant

RT-qPCR assays conducted with H1299 cells

Δ160p53 binds to different p53 Response Elements (RE)

REs described in the literature →

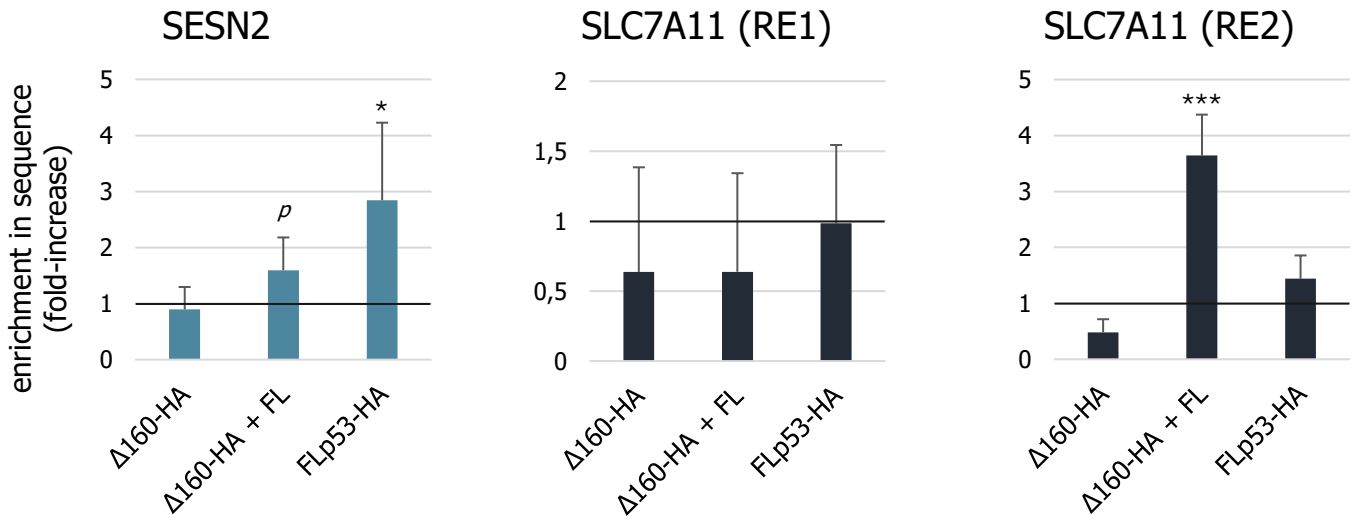
New RE identified →

Consensus	RRR**CWWG**YYY		RRR**CWWG**YYY
SESN2	CTT**CAAG**TCC		AGG**CAAA**TCC
SLC7A11 RE1	AGG**CAGG**CGC	*TTAA*	ATA**CAAG**CCC
SLC7A11 RE2	AAG**CTTG**CTT	*TCTGGGA*	AAG**CAAA**TGG

R = purine, Y = pyrimidine and W = A / T

p53 RE – DNA sequence specifically bound by p53

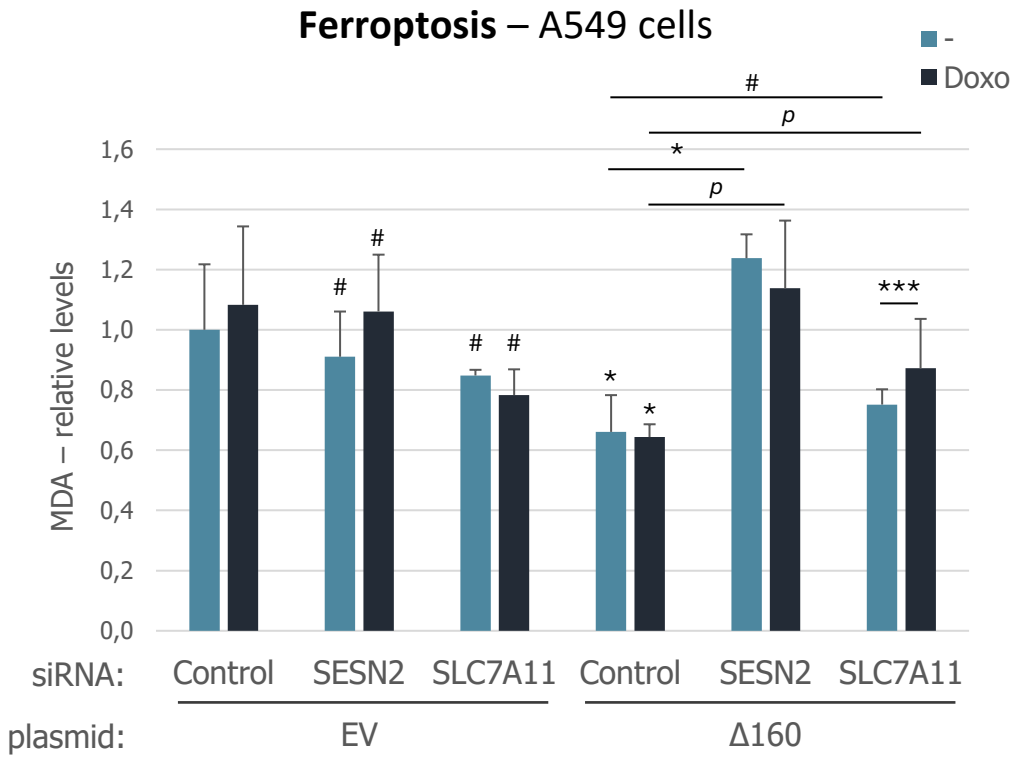
Chromatin Immunoprecipitation (ChIP)-PCR

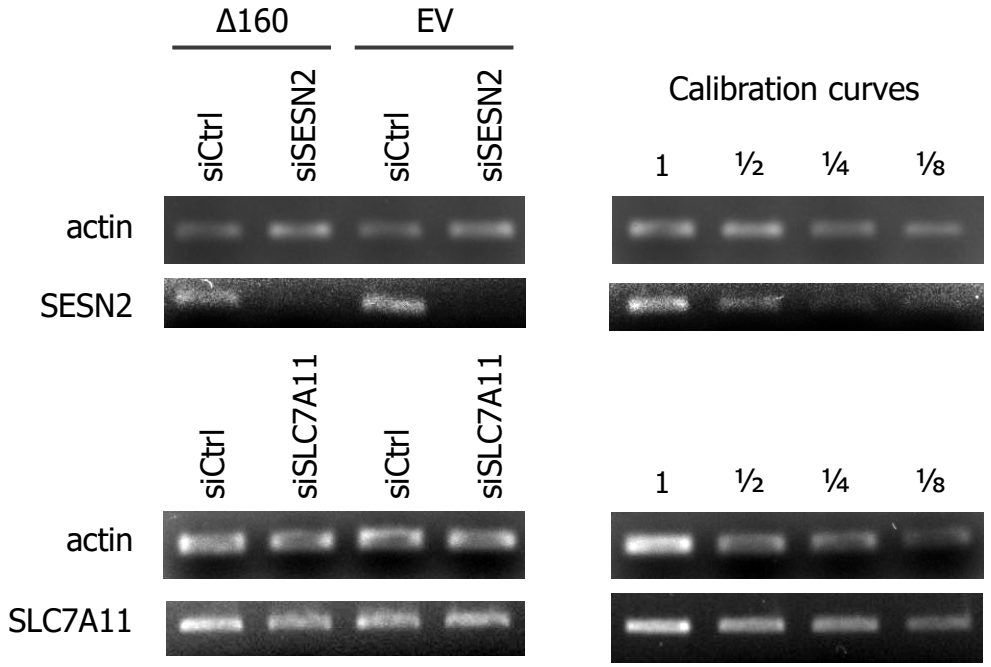


- FLp53 binds the SESN2 RE described, but not the SLC7A11 RE1.
- In the absence of FLp53, **Δ160p53** could not bind the REs tested.
- When FLp53 is present, **Δ160p53** can bind a newly identified SLC7A11 RE2 better than FLp53 alone.

*p*P < 0,1; *P < 0,05; ***P < 0,005; n = 3

Δ160p53 protects cells from ferroptosis in a SESN2-dependent manner

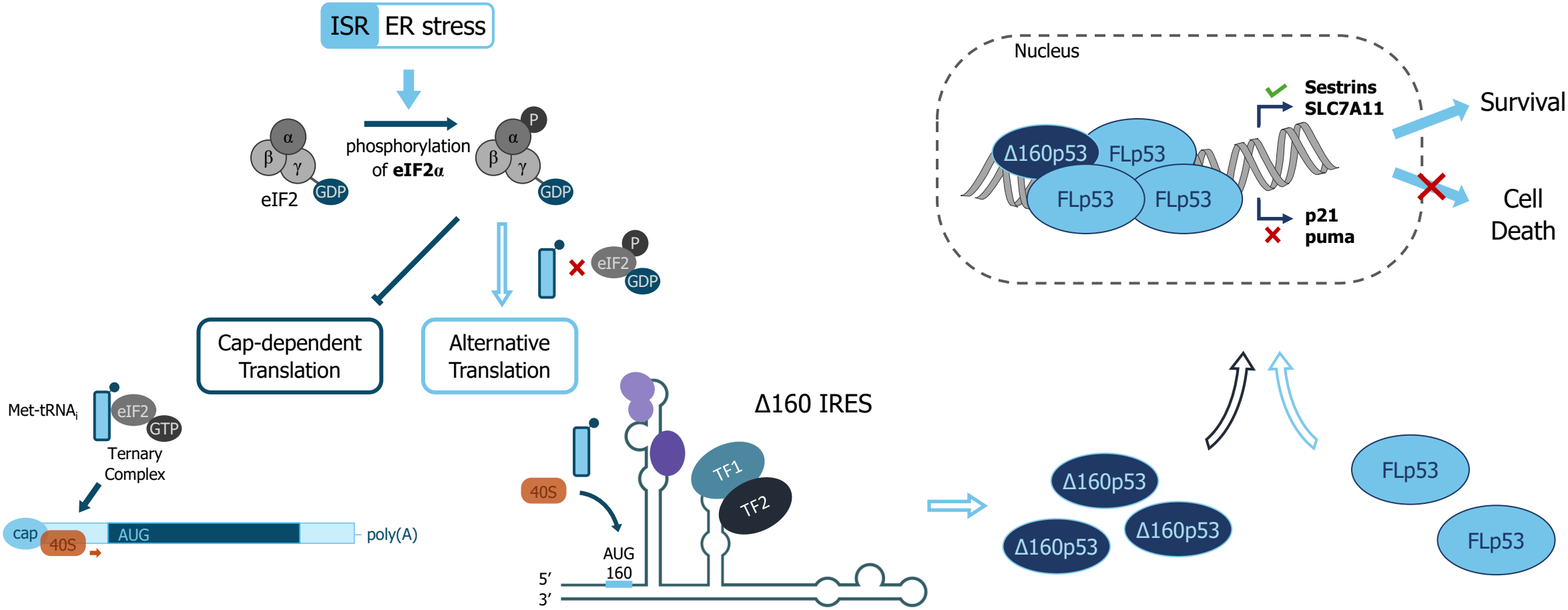




- **Δ160p53** inhibits ferroptosis in A549 cells expressing wtp53.
- Depletion of SESN2 completely abrogates the decrease in ferroptosis levels mediated by **Δ160p53**.

EV = empty vector

Conclusions



- The activation of **Δ160p53** during the ISR counteracts the effect of FLp53 to suppress cell death and support survival.

Acknowledgments

MARCO Lab

mRNA Metabolism Lab

Supervisors:
Dr Marco Candeias
Dr Luísa Romão
Dr Rafaela Lacerda Santos

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MolecularAgeing RNAChemistry Oncogenesis MARCO

MIA Portugal Multidisciplinary Institute of Ageing

KYOTO UNIVERSITY FOUNDED 1897 KYOTO JAPAN

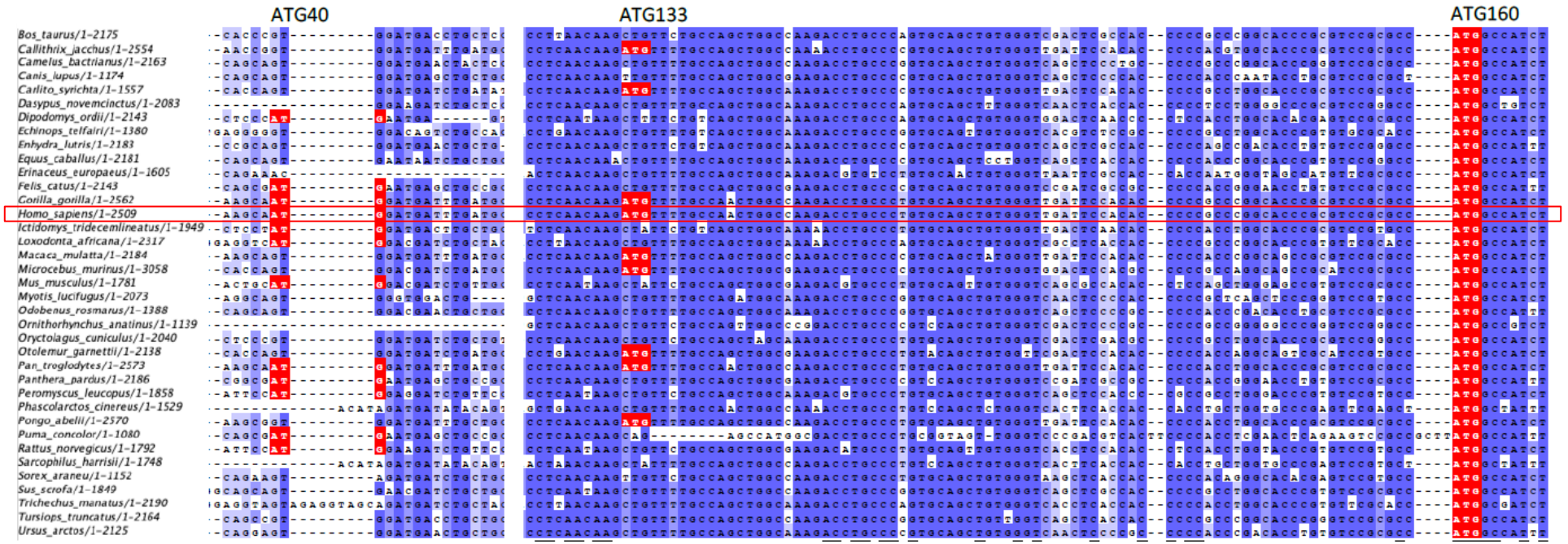
BioISI

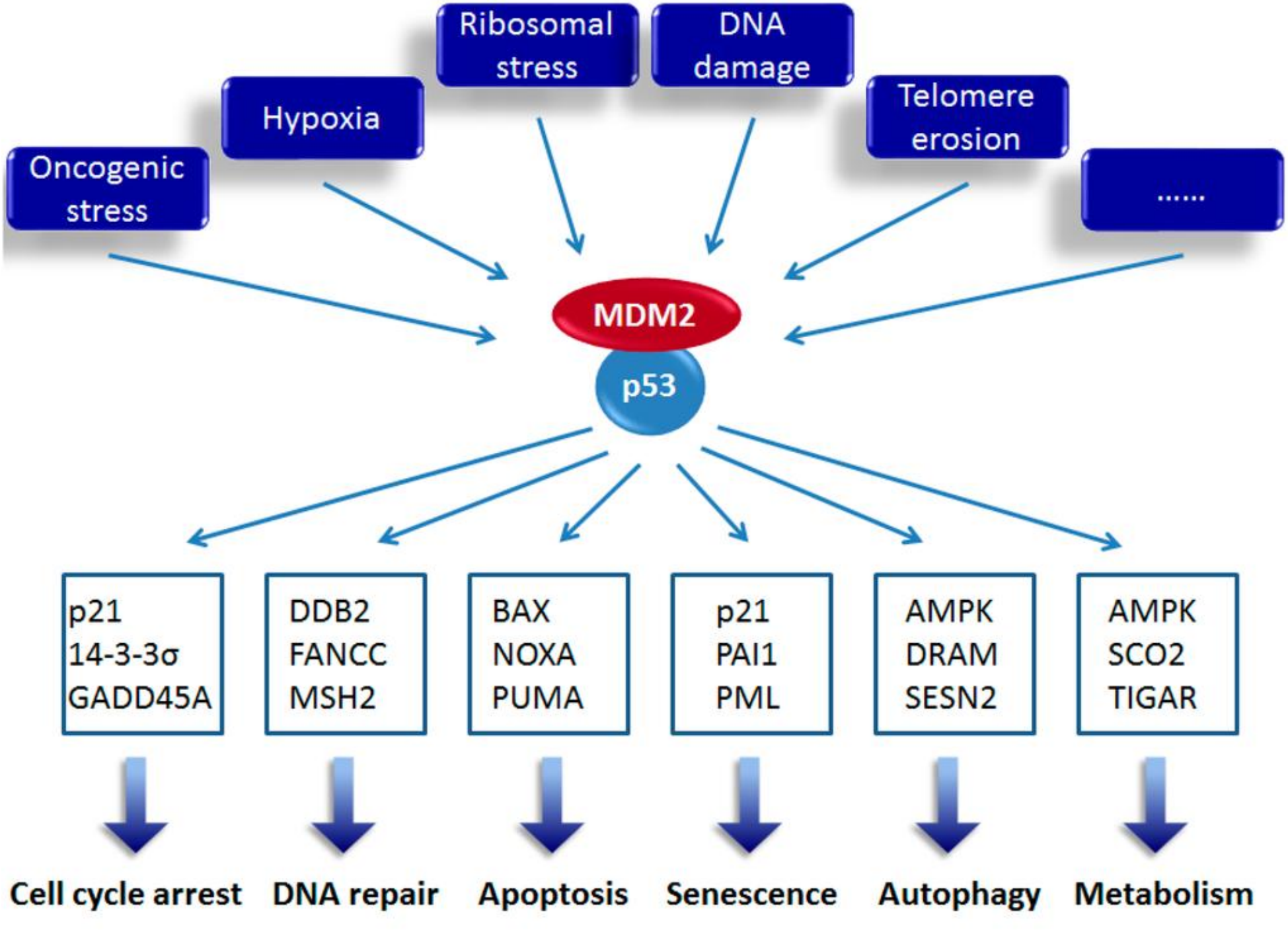
Instituto_Nacional de Saúde Doutor Ricardo Jorge

REPÚBLICA PORTUGUESA SAÚDE

June 6th, 2025

Conservation among species from different Orders within the Mammal Class:




Ribosomal stress
DNA damage
Hypoxia
Telomere erosion
Oncogenic stress
.....
MDM2
p53
p21
14-3-3σ
GADD45A
DDB2
FANCC
MSH2
BAX
NOXA
PUMA
p21
PAI1
PML
AMPK
DRAM
SESN2
AMPK
SCO2
TIGAR
Cell cycle arrest
DNA repair
Apoptosis
Senescence
Autophagy
Metabolism