Translational Regulation of the Human PERK by Upstream Open Reading Frames

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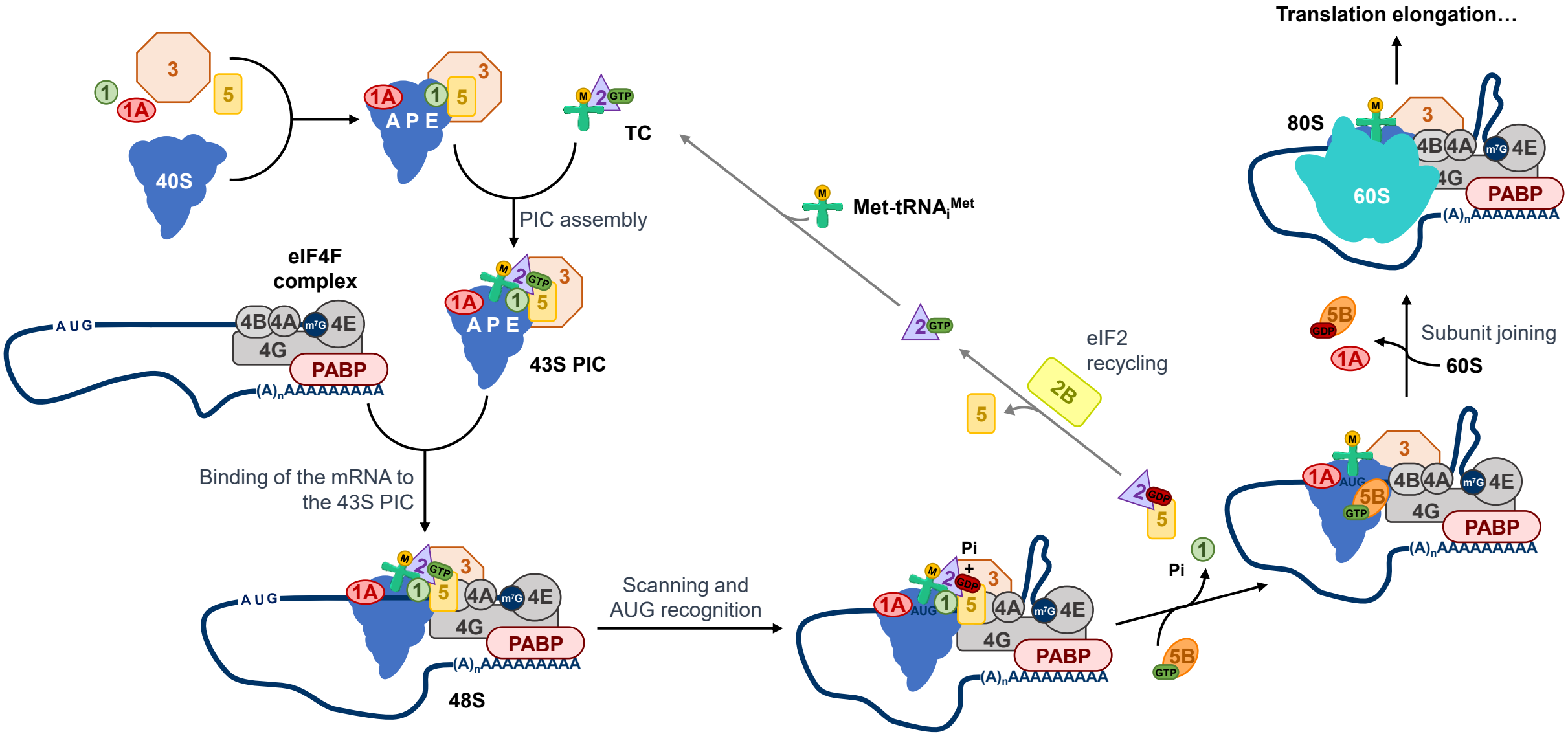
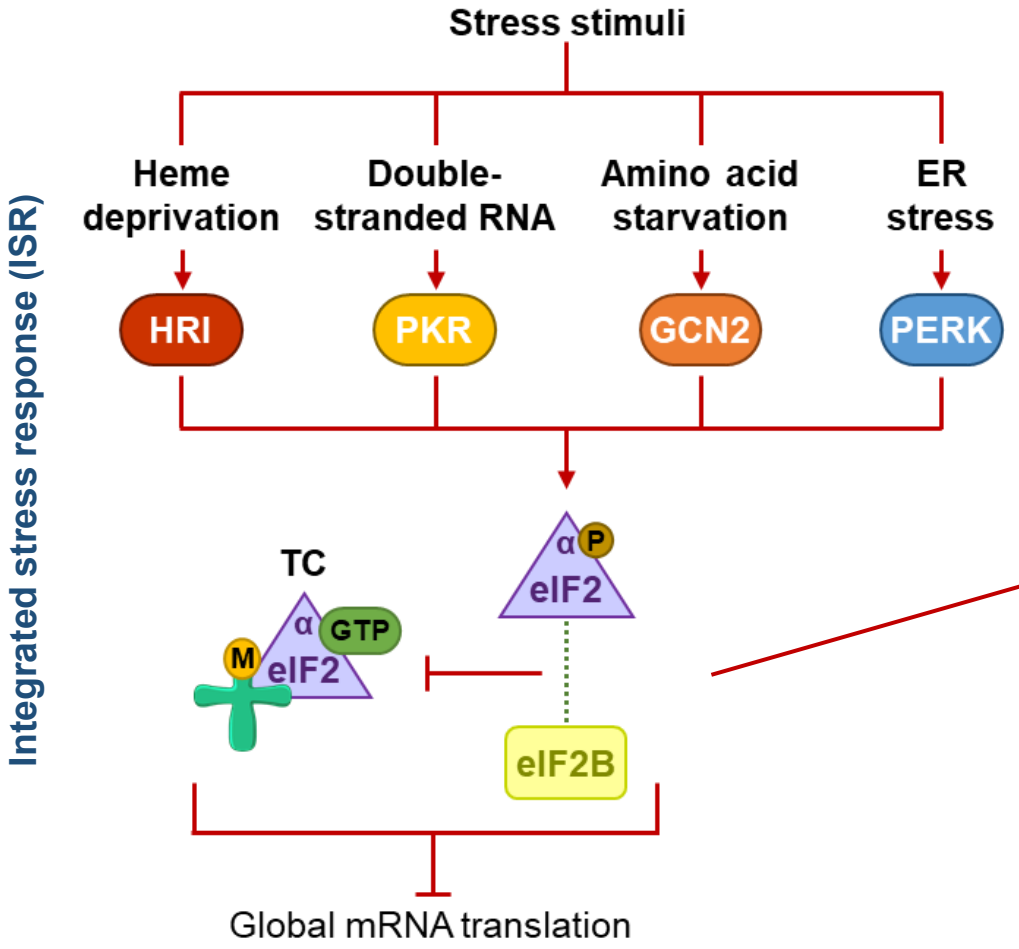
https://www.fc.ul.pt/pt/unidade/bioisi

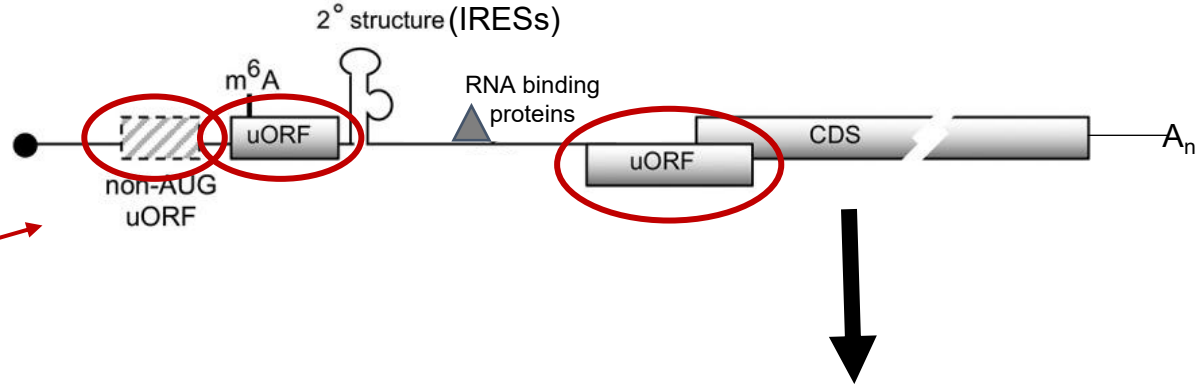
The canonical translation initiation process in eukaryotes



Fernandes R, Romão L (2020)

Translational regulation during stress: eIF2α phosphorylation and transcript-specific translation



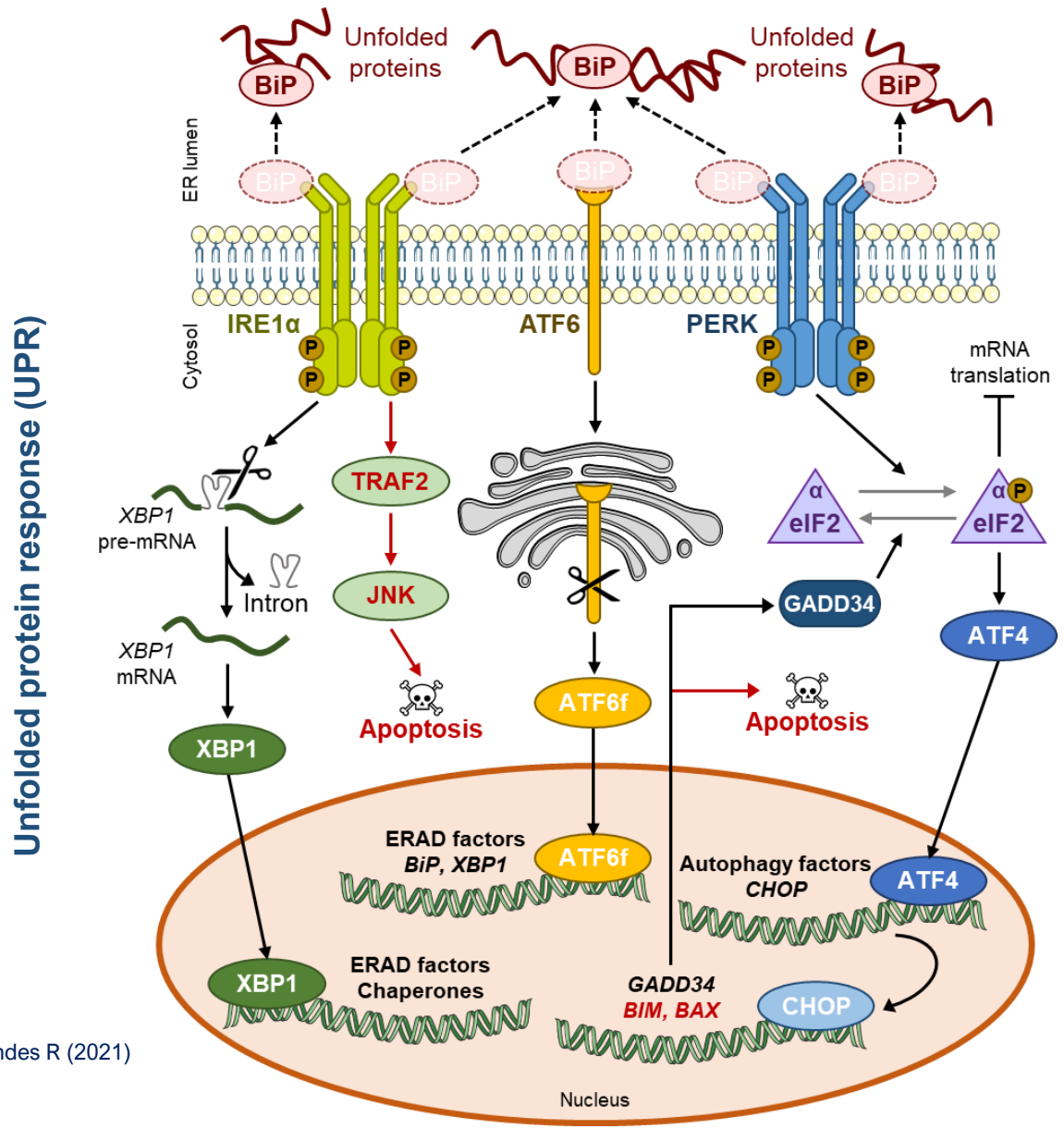


Selective translation of proteins that are vital for cell survival and stress recovery

Fernandes R, Romão L (2020)
Silva J, Fernandes R, Romão L (2019)

PERK: protein kinase double-stranded RNA-dependent (PKR)-like endoplasmic reticulum kinase

The PERK kinase and its paradoxical roles in cell physiology and disease

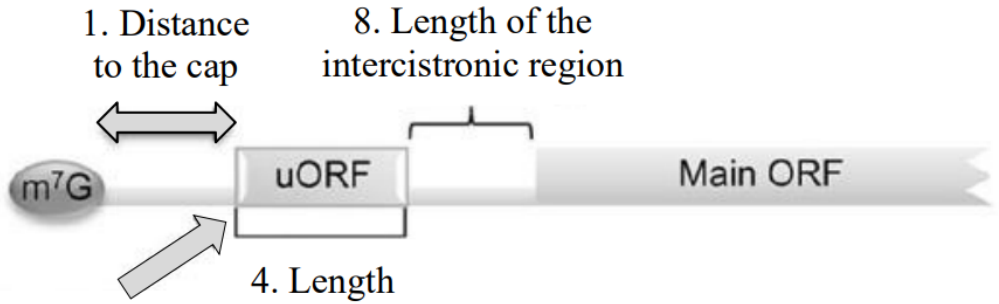


Fernandes R (2021)

- Key role in maintaining cell homeostasis and controlling cell fate
- Mutations in the *EIF2AK3* gene were implicated in the **Wolcott-Rallison Syndrome (WRS)**
  - ✓ Rare autosomal recessive disease
  - ✓ Neonatal insulin-dependent diabetes, non-autoimmune-mediated destruction of pancreatic β-cells, skeletal dysplasia, hepatic dysfunction and growth retardation
- PERK activity has context-dependent effects, being both protective and detrimental. Impairing β-cell function and influencing diabetes progression, showing neuroprotective or neurotoxic roles in different neurodegenerative diseases, and acting as either tumor-suppressive or tumor-promoting in cancer.

PERK: protein kinase double-stranded RNA-dependent (PKR)-like endoplasmic reticulum kinase

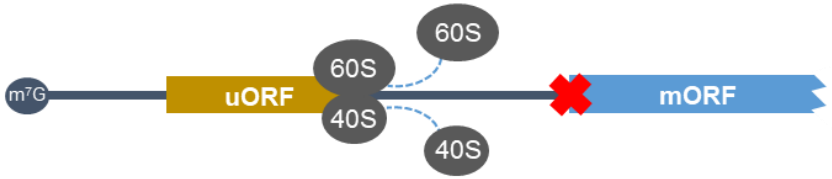
upstream Open Reading Frames (uORFs)



2. Initiation codon type
3. Initiation codon context
Kozak consensus sequence
(GCCA/GCC**AUG**G)

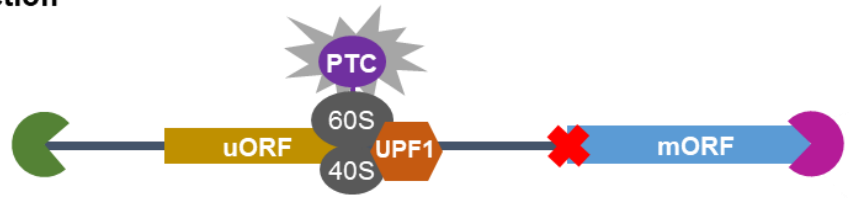
A) uORF translation represses mORF expression by:

1) Ribosome dissociation

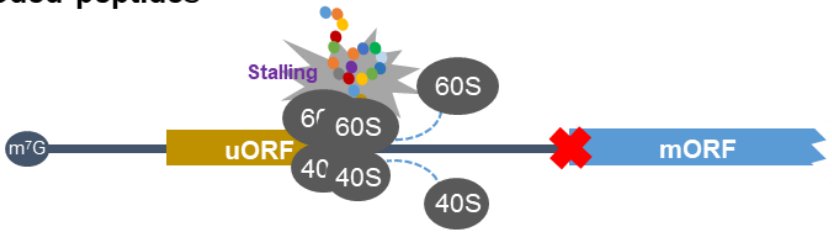


2) Ribosome stalling

3) NMD induction



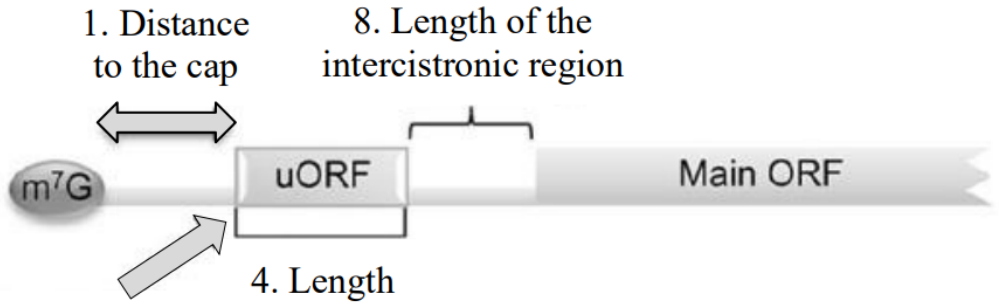
4) uORF-encoded peptides



Barbosa C, Peixeiro I, Romão L (2013)

Fernandes R (2021)

upstream Open Reading Frames (uORFs)

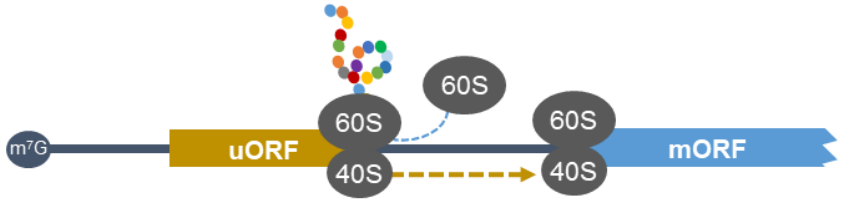


2. Initiation codon type
3. Initiation codon context
Kozak consensus sequence
(GCCA/GCC**AUG**G)

4. Length
5. Secondary/tertiary structures
6. Number
7. Position of stop codon

B) uORF facilitates mORF expression by:

1) Reinitiation

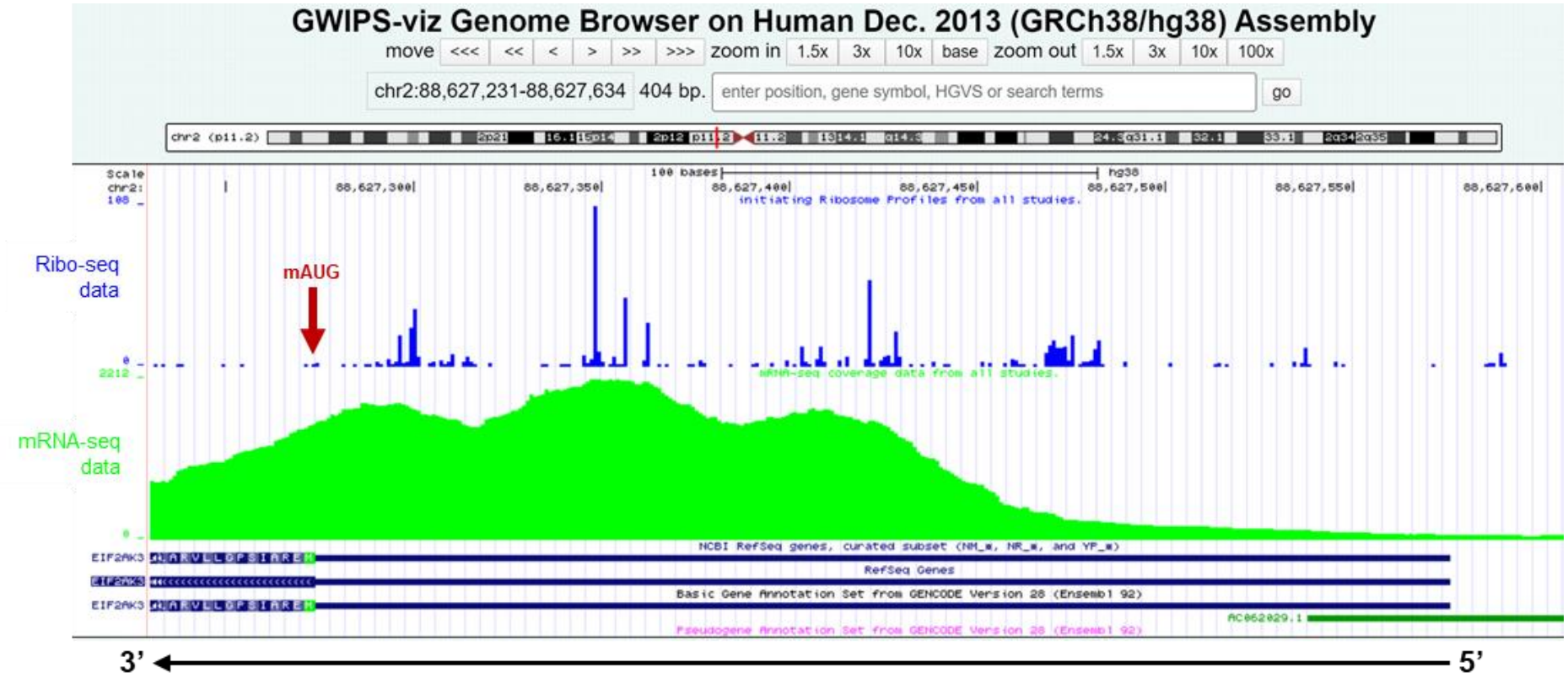


2) Ribosomal bypass or ribosomal leaky scanning

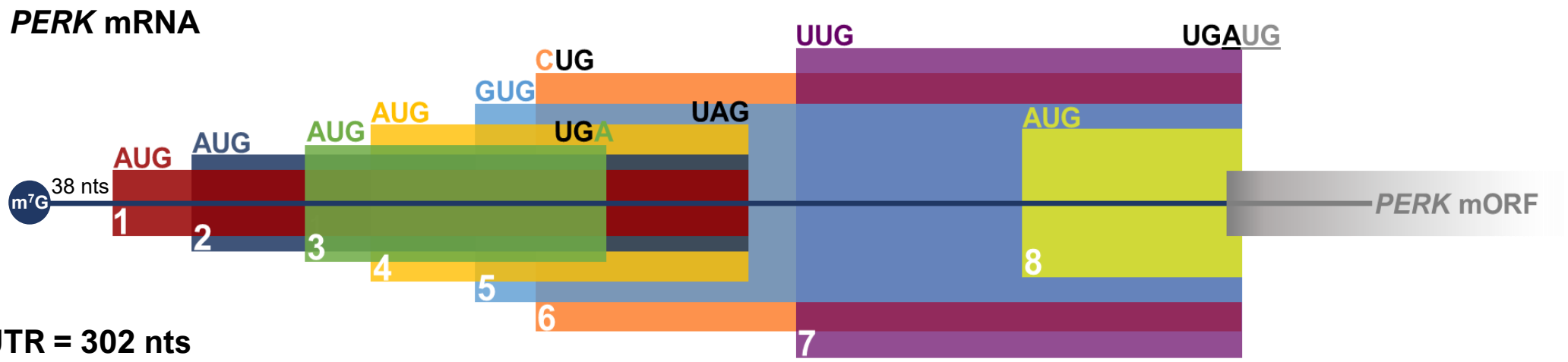
Barbosa C, Peixeiro I, Romão L (2013)

Fernandes R (2021)

Ribosome-profiling studies suggest that the *PERK* mRNA contains uORFs potentially translated



The *PERK* mRNA contains potentially translated uORFs



***PERK* 5'UTR = 302 nts**

A **Empirical analysis**

ExPASy Bioinformatics Resource Portal

B **Data from Ribo-seq studies**

SORFS.ORG
A REPOSITORY OF SMALL ORFS IDENTIFIED BY RIBOSOME PROFILING

5 AUG-uORFs

3 non-AUG-uORFs: GUG, CUG, UUG

Some of these uORFs are **likely to be translated**, as suggested by the Kozak sequence context strength of their start codons.

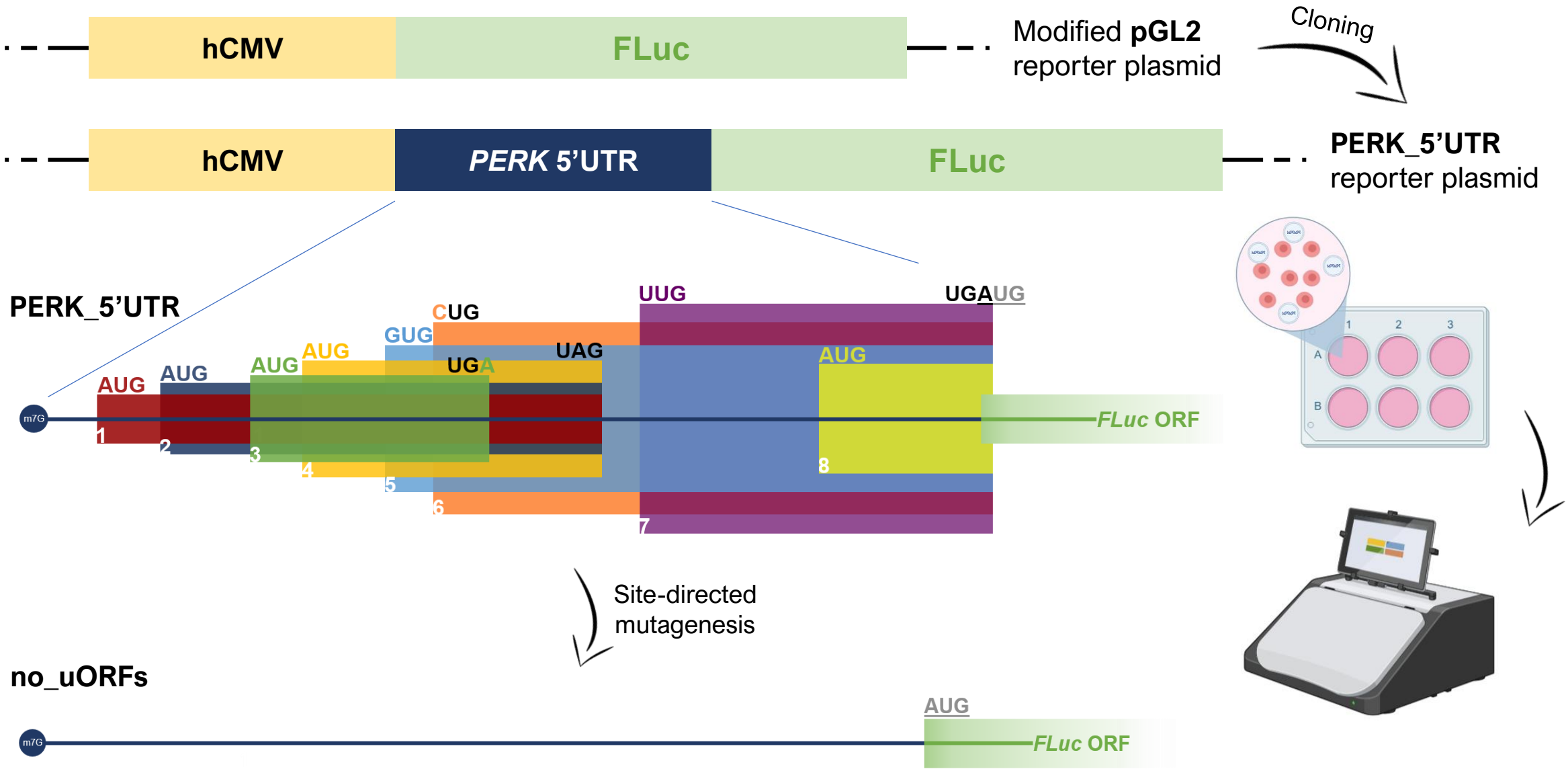
uORF ID	Length (nts)	Start codon strength
uORF1	165	++
uORF2	141	-
uORF3	57	+
uORF4	102	+
uORF5	177	+
uORF6	156	-
uORF7	84	-
uORF8	27	-

Objectives

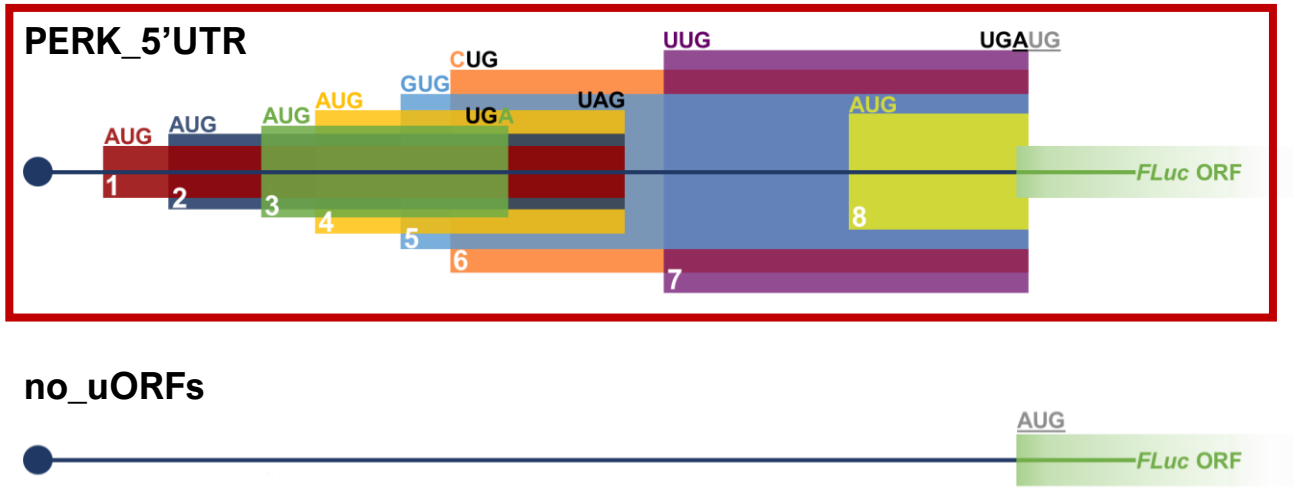
Study the functional role of uORFs in PERK expression and evaluate their biological role

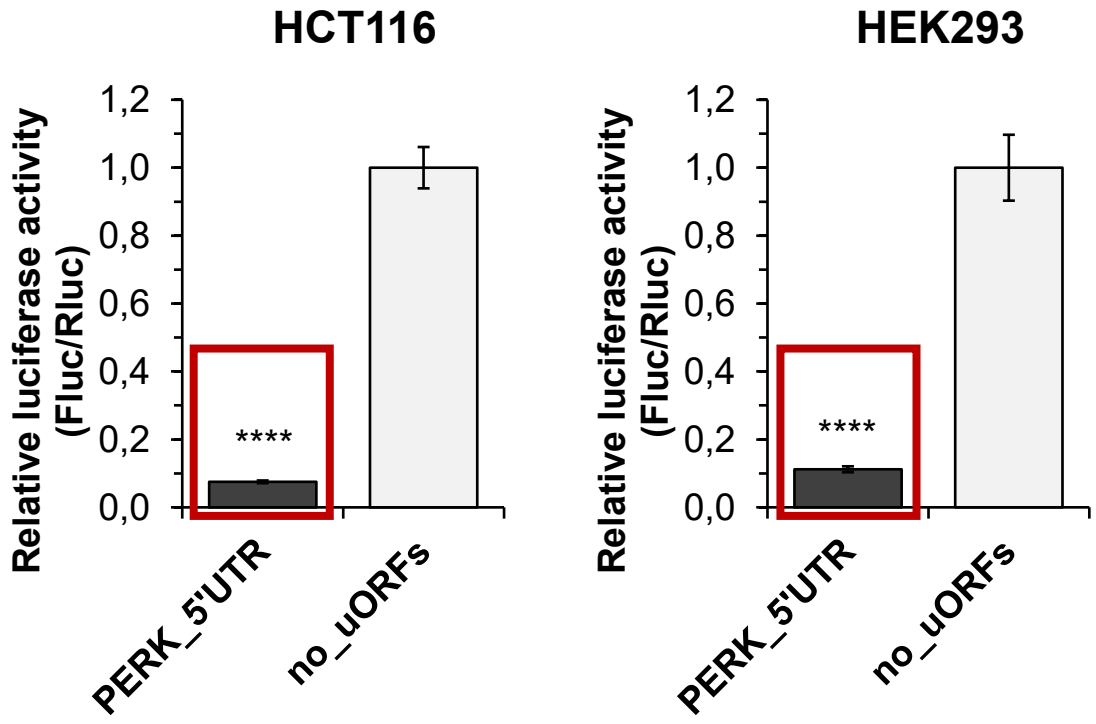
1. Explore the uORF-mediated regulatory mechanisms acting on *PERK* mORF translation
2. Assess the impact of uORF dysregulation in PERK expression
3. Study the role of ER stress in the uORF-mediated translational regulation of PERK
4. Determine the consequences of dysregulated PERK expression in cell physiology

Studying the regulatory potential of the *PERK* uORFs



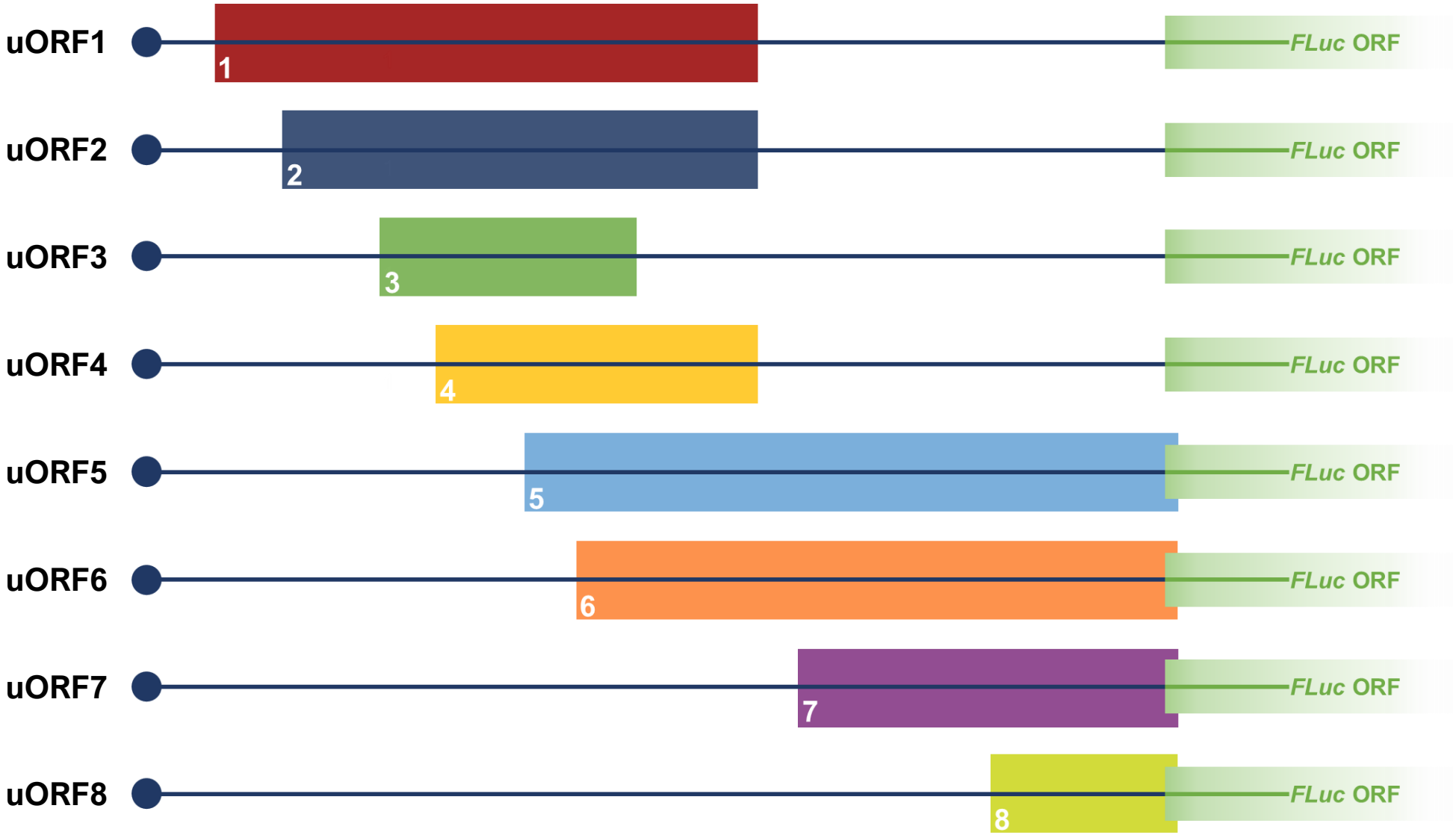
PERK uORFs repress mORF translation in basal conditions





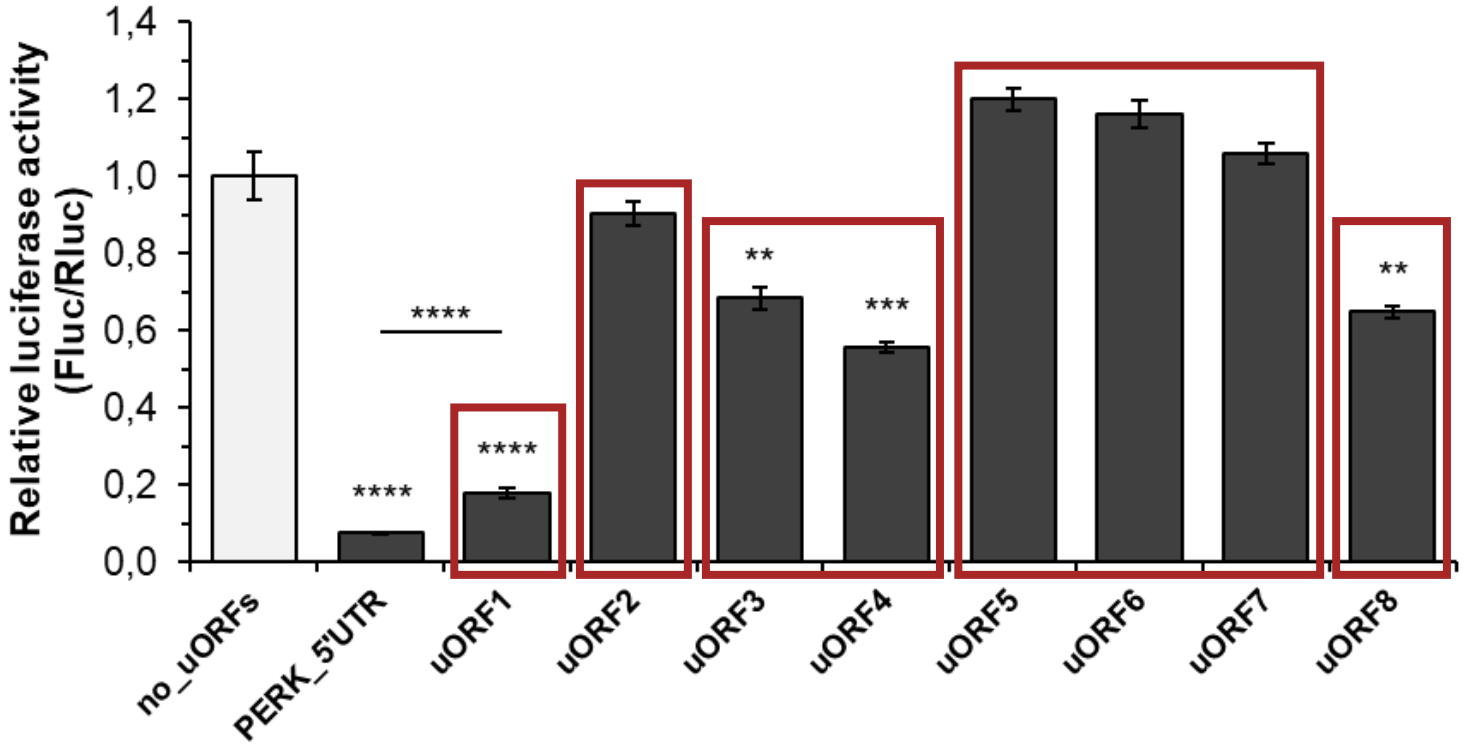
PERK uORFs **repress** mORF translation in:
92% in HCT116 cells and **89%** in HEK293 cells

PERK uORFs contribute differentially to mORF regulation



Study the **regulatory activity** of each uORF, **individually.**

PERK uORFs contribute differentially to mORF regulation

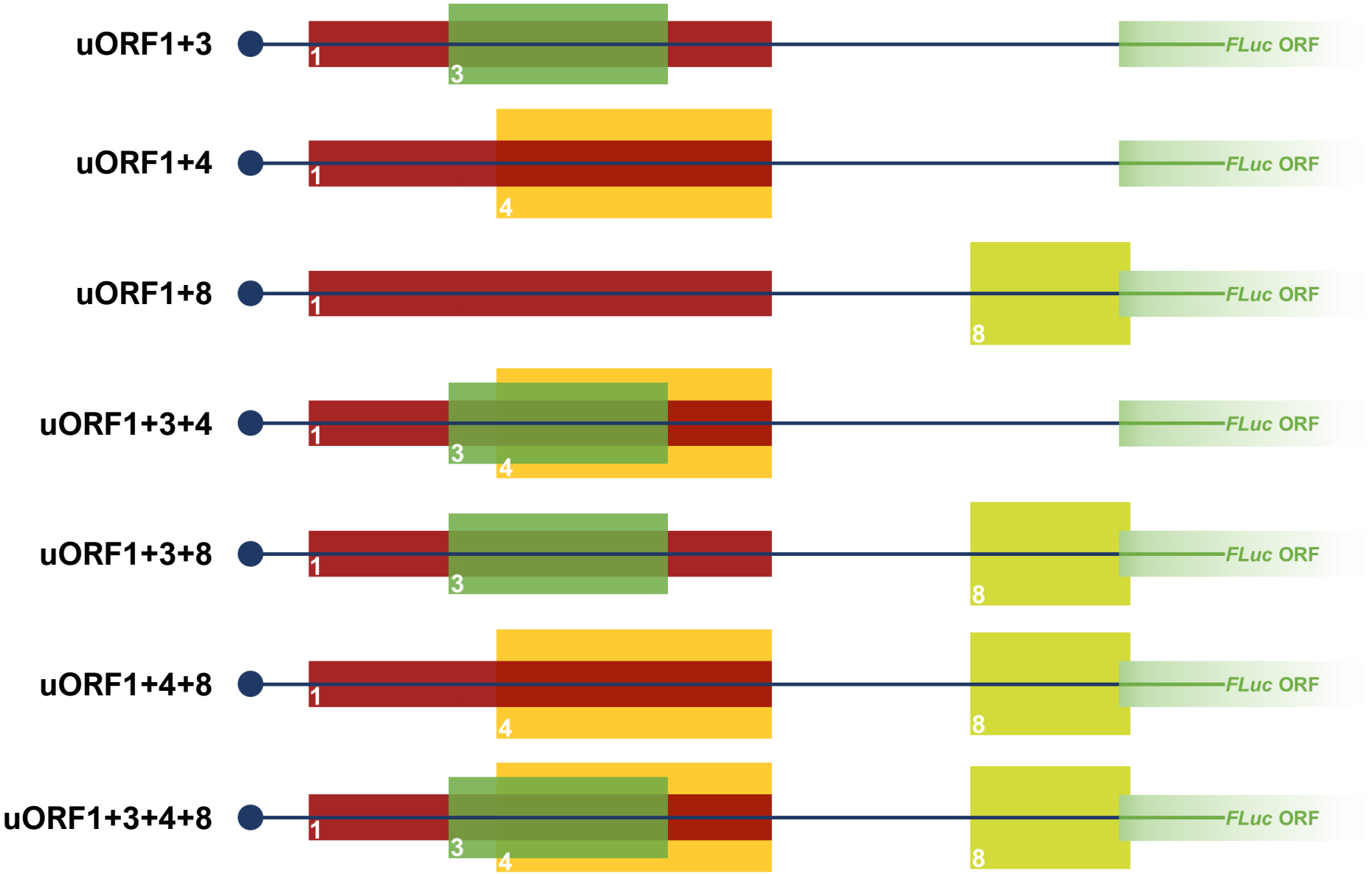


When individually present:

- **uORF 1** is a **major repressor** of mORF translation
- **uORFs 3**, **4** and **8** are **mild repressors** of mORF translation
- **uORFs 2**, **5**, **6** and **7** do not have regulatory activity

uORF1 may act in combination with the other repressive uORFs to achieve the **maximum translational repression**

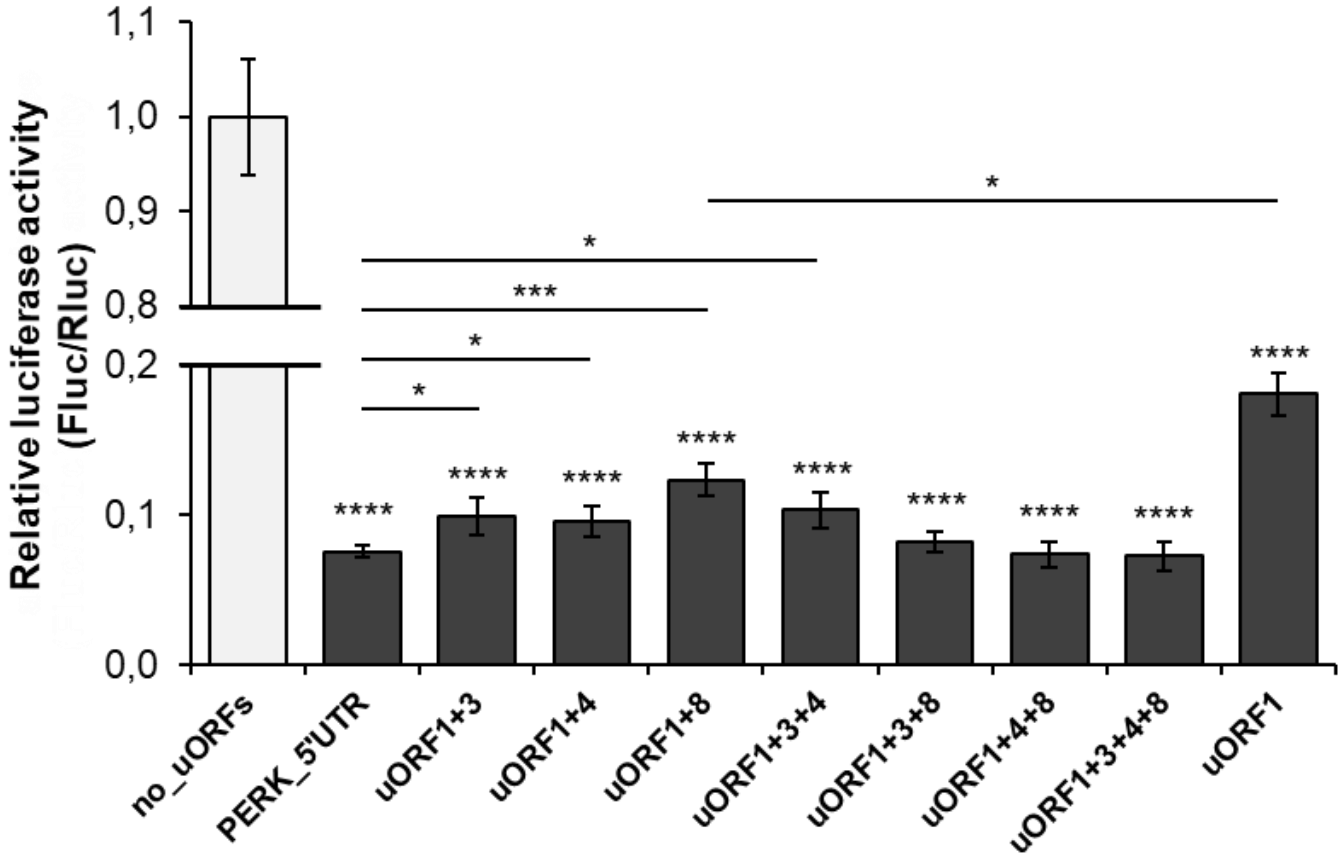
The repressive uORFs cooperate to repress mORF translation



Study the **regulatory activity** of the repressive uORFs, **combined.**

The repressive uORFs cooperate to repress mORF translation

uORF1, **uORF3**, **uORF4** and **uORF8** are **repressive** and **enough** to **regulate mORF translation.**



uORF3, uORF4 and **uORF 8** are **equivalent** and work in a **fail-safe manner**:

when the ribosomes fail to recognize the first one, the other may be translated with the same regulatory outcome

PERK uORF-altering mutations affect mORF expression

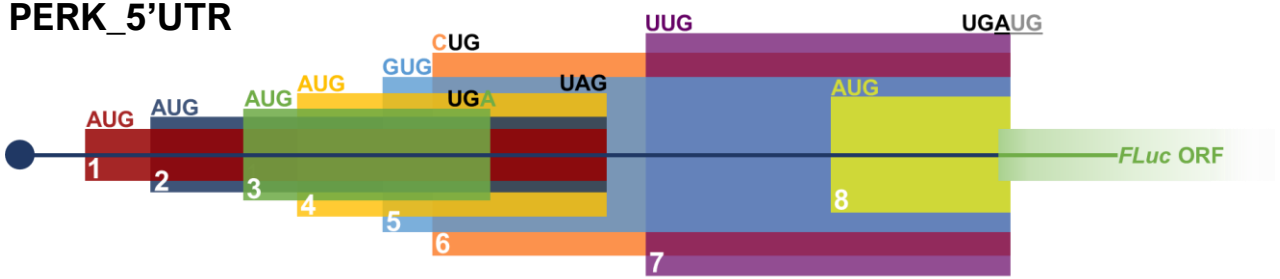
ClinVar

	Variation *Location*	Gene(s)	Protein change	Condition(s)	Clinical significance (Last reviewed)	Review status	Accession
8.	NM_004836.6(EIF2AK3):c.-162G>A *GRCh37:* Chr2:88926954 *GRCh38:* Chr2:88627436	EIF2AK3		Wolcott-Rallison dysplasia	Uncertain significance (Jun 14, 2016)	criteria provided, single submitter	VCV000337423
9.	NM_004836.6(EIF2AK3):c.-172C>T *GRCh37:* Chr2:88926964 *GRCh38:* Chr2:88627446	EIF2AK3		Wolcott-Rallison dysplasia	Likely benign (Jun 14, 2016)	criteria provided, single submitter	VCV000337424
10.	NM_004836.6(EIF2AK3):c.-195G>T *GRCh37:* Chr2:88926987 *GRCh38:* Chr2:88627469	EIF2AK3		Wolcott-Rallison dysplasia	Uncertain significance (Jun 14, 2016)	criteria provided, single submitter	VCV000337425
11.	NM_004836.6(EIF2AK3):c.-201A>G *GRCh37:* Chr2:88926993 *GRCh38:* Chr2:88627475	EIF2AK3		not specified, Wolcott-Rallison dysplasia	Uncertain significance (Jun 14, 2016)	criteria provided, multiple submitters, no conflicts	VCV000337426
12.	NM_004836.6(EIF2AK3):c.-263T>C *GRCh37:* Chr2:88927055 *GRCh38:* Chr2:88627537	EIF2AK3		Wolcott-Rallison dysplasia	Uncertain significance (Jun 14, 2016)	criteria provided, single submitter	VCV000337427

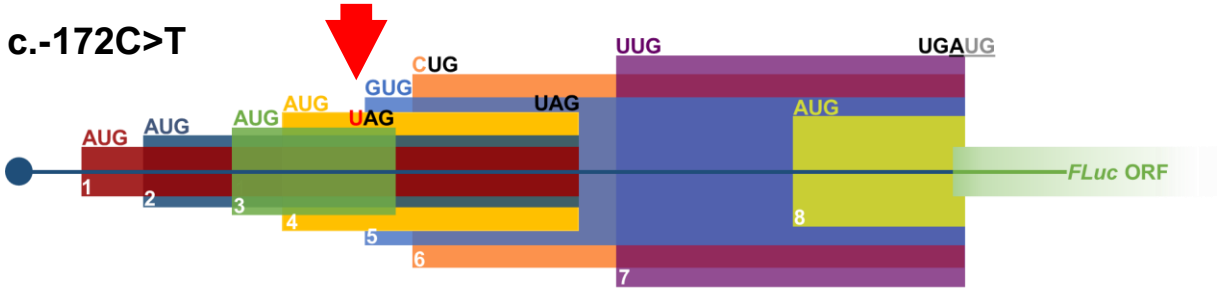
Affect *PERK* uORFs

PERK uORF-altering mutations affect mORF expression

PERK_5'UTR

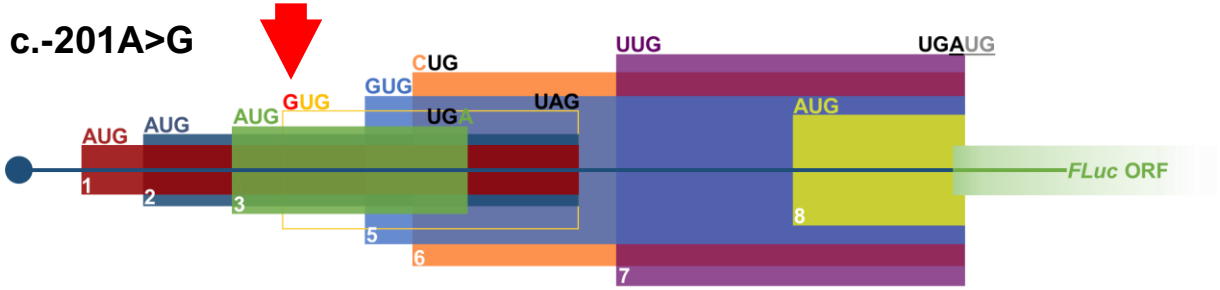


c.-172C>T



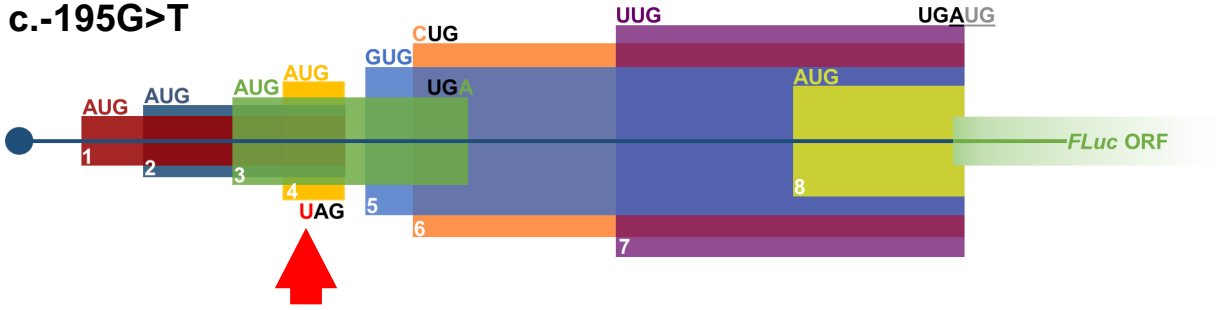
Introduces a premature stop codon in uORF3

c.-201A>G



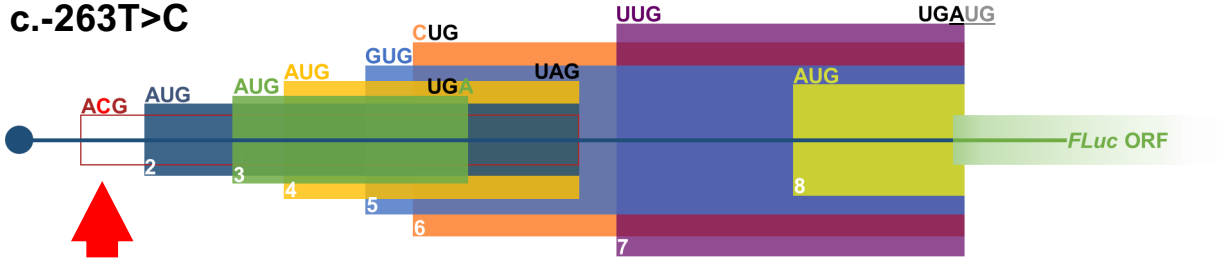
Start codon mutation that eliminates uORF4

c.-195G>T



Introduces a premature stop codon in uORF1, uORF2 and uORF4

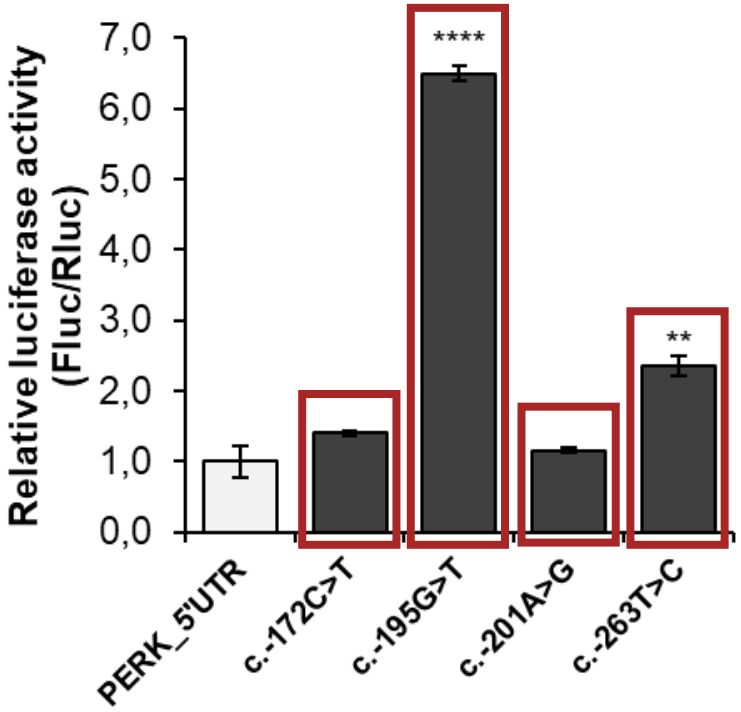
c.-263T>C

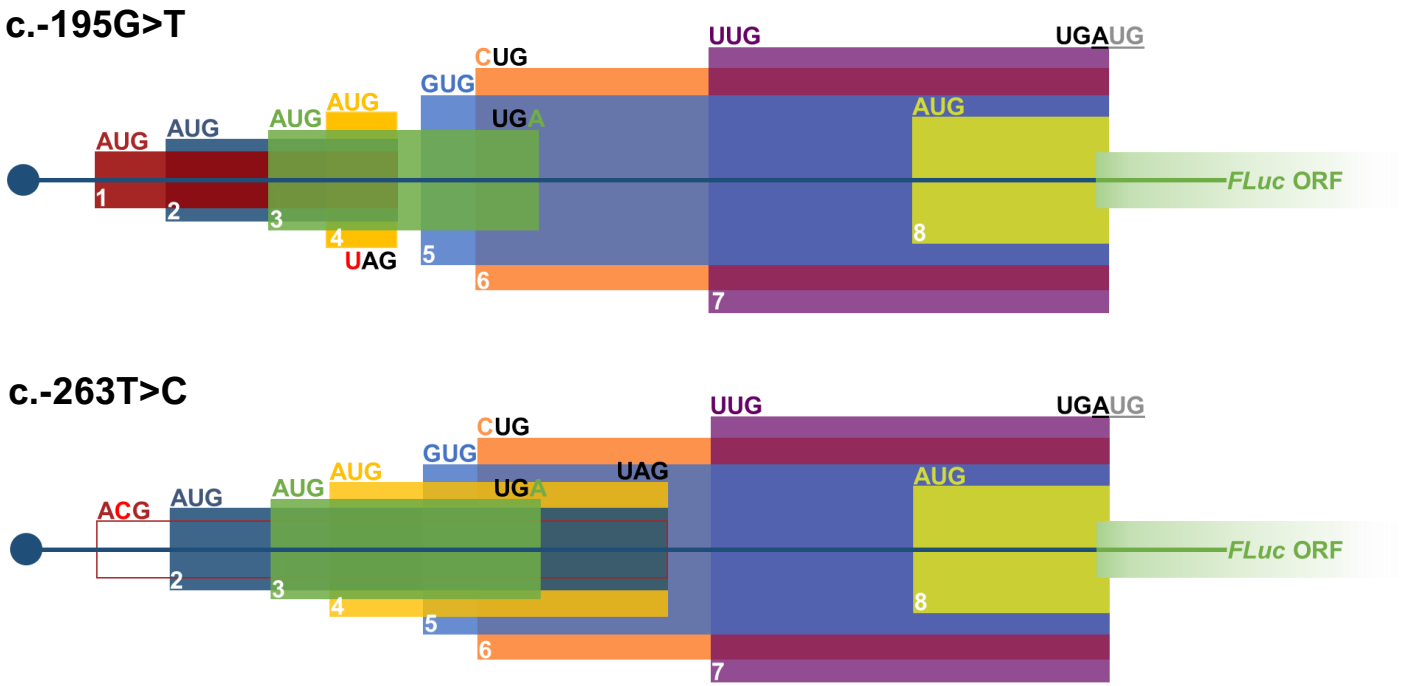


Start codon mutation that eliminates uORF1

Assess the impact of these **5'UTR variants** on the **uORF-mediated regulation** of mORF translation → related to WRS?

PERK uORF-altering mutations affect mORF expression





- Two of the tested *PERK* uORF-altering mutations influence mORF expression
- Related to WRS? → Additional clinical and experimental data is required

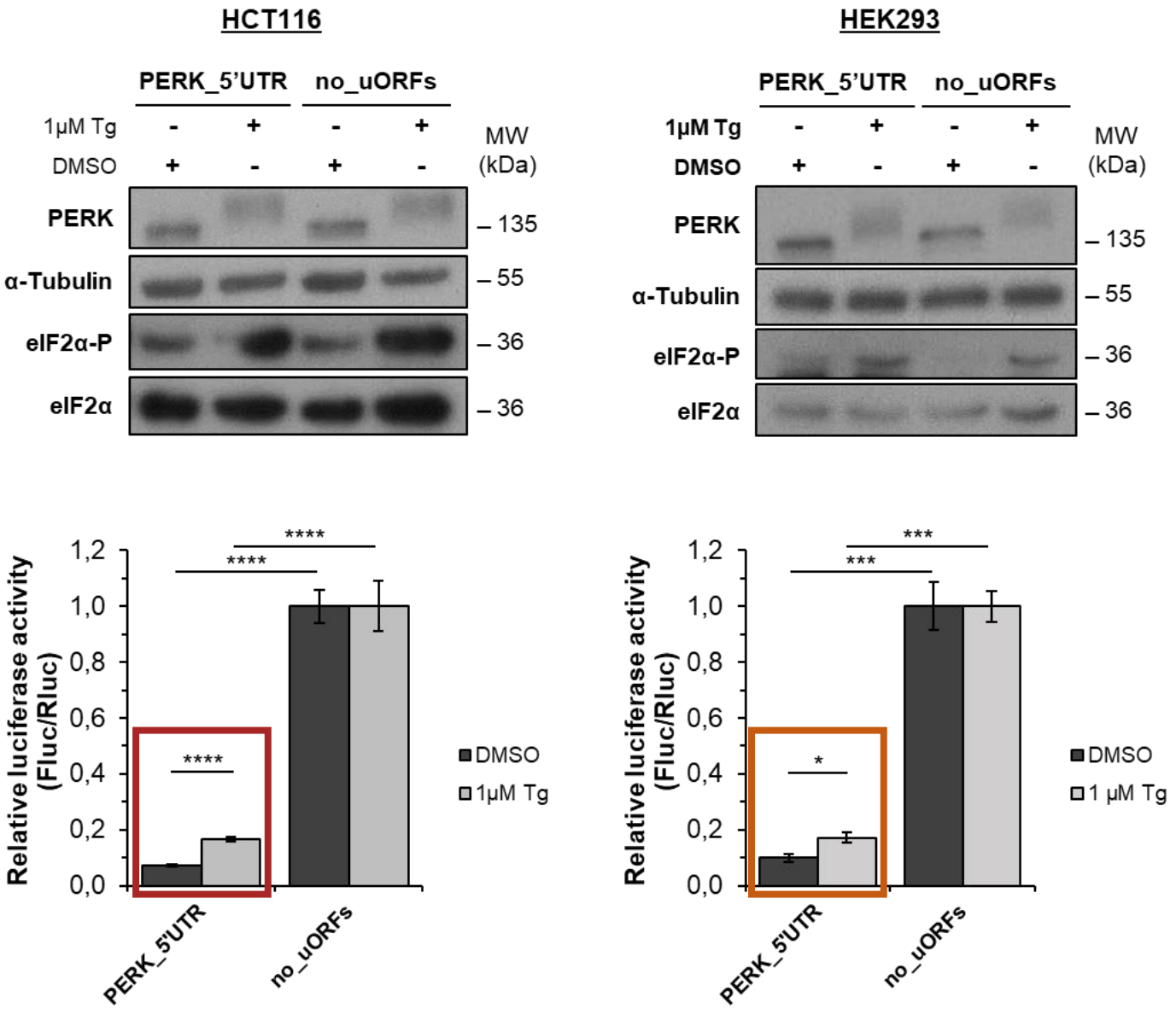
c.-172C>T: Introduces a premature stop codon in uORF3

c.-195G>T: Introduces a premature stop codon in uORF1, uORF2 and uORF4

c.-201A>G: Start codon mutation that eliminates uORF4

c.-263T>C: Start codon mutation that eliminates uORF1

The *PERK* uORF-mediated translational repression is lessened under Tg-induced stress



- *PERK* uORFs **maintain a notable inhibitory activity** over mORF translation during conditions of increased eIF2α-P
- uORFs **lose part** of their repressive activity in these conditions

Micropeptides have functions beyond their regulatory role

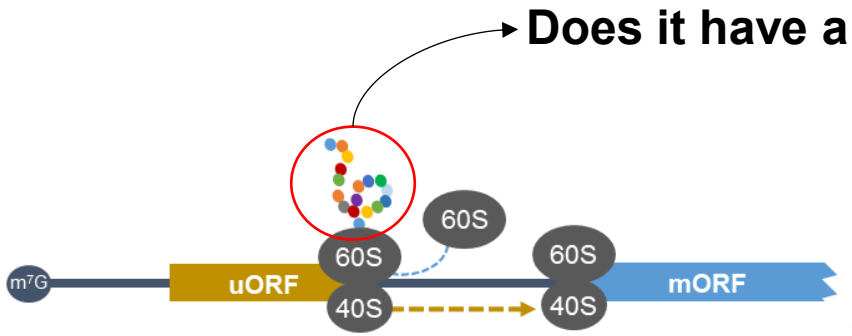
uPEP2 (*PKCη*)

MP31 (*PTEN*)

ASDURF (*ASNSD1*)

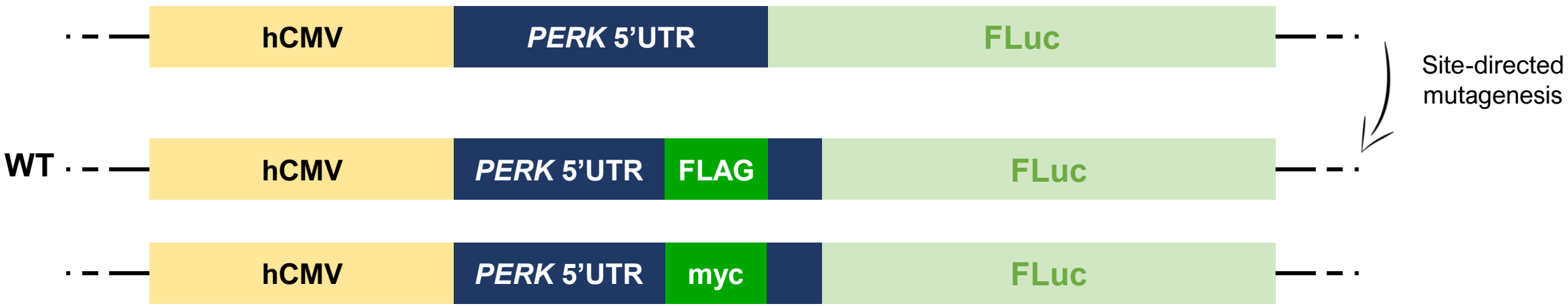
Micropeptides encoded by uORFs that have a function beyond their regulatory role in main open reading translation

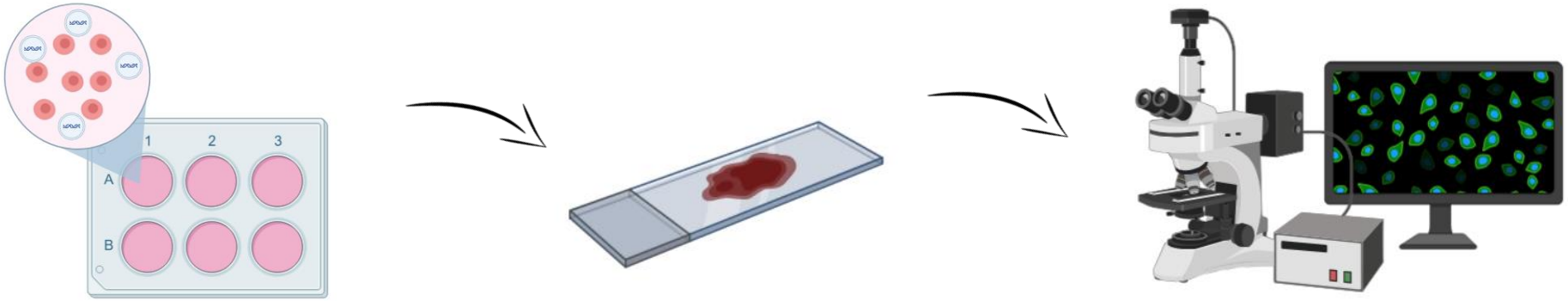
- **uPEP2**: Acts as a kinase inhibitor reducing cancer cell survival and migration
- **MP31**: Inhibits tumorigenesis and sensitizes glioblastoma cells to chemotherapy
- **ASDURF**: Completes the prefoldin-like module of the PAQosome



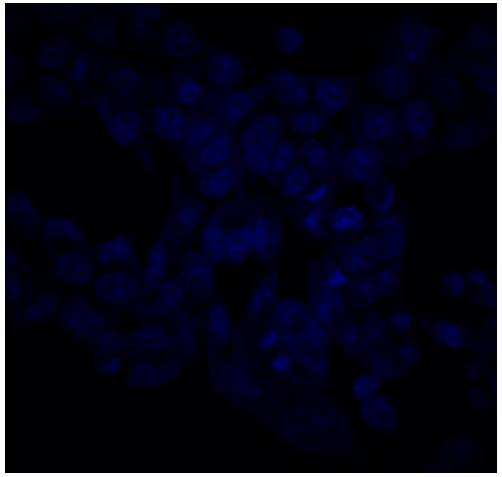
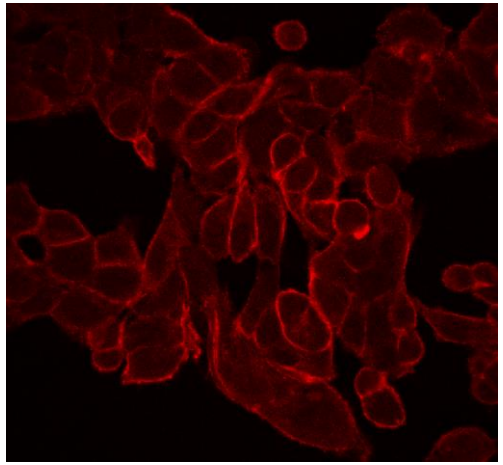
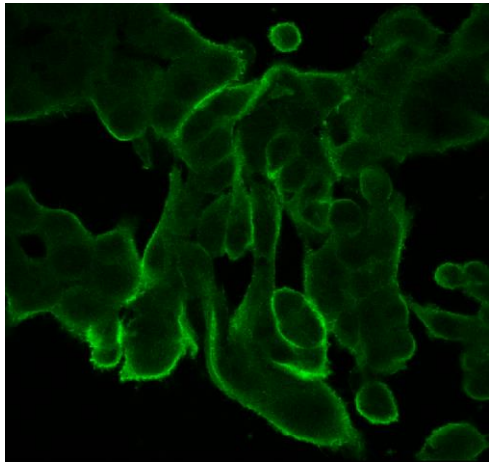
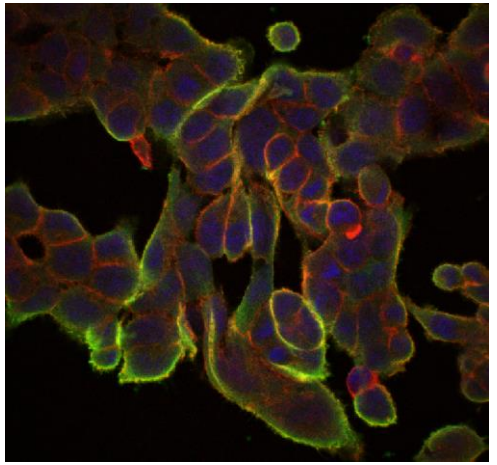
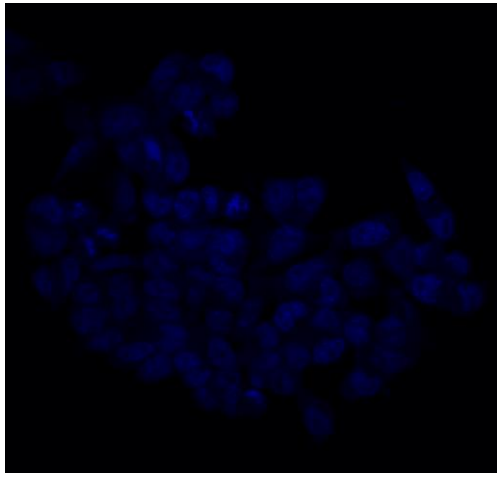
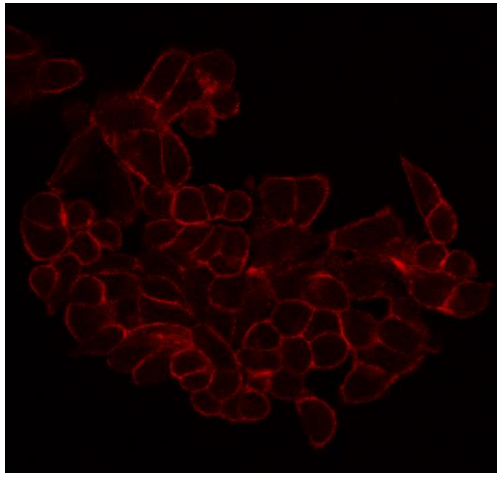
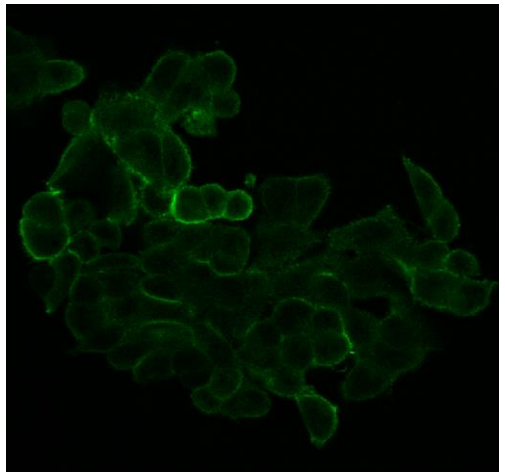
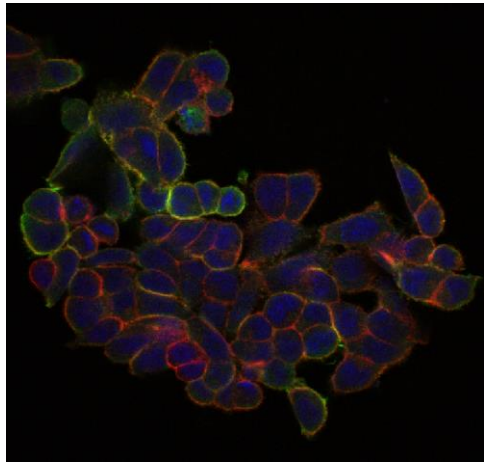
(Li et al., 2024; Belsamma et al., 2023; Poitras et al., 2020)

Studying the localization of the *PERK* uORFs

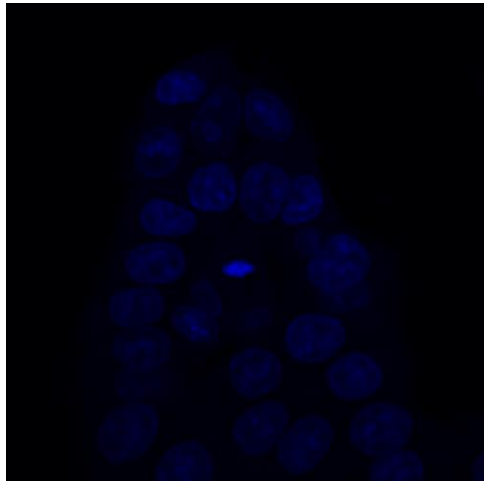
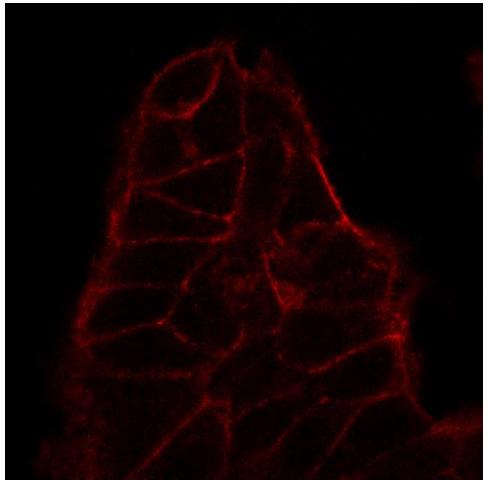
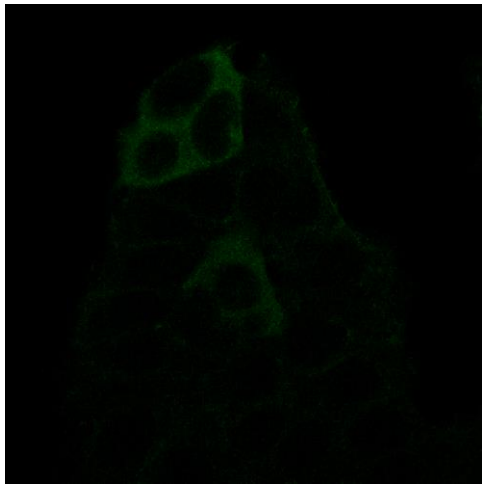
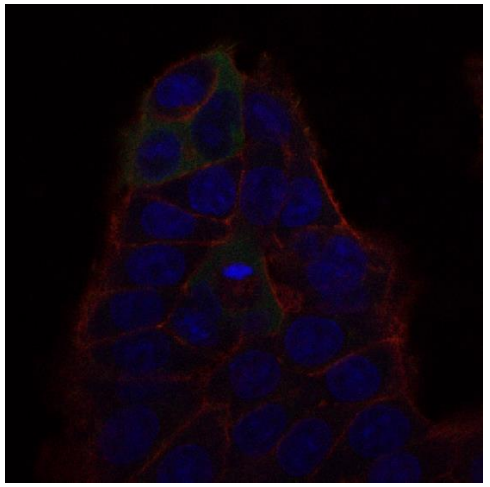
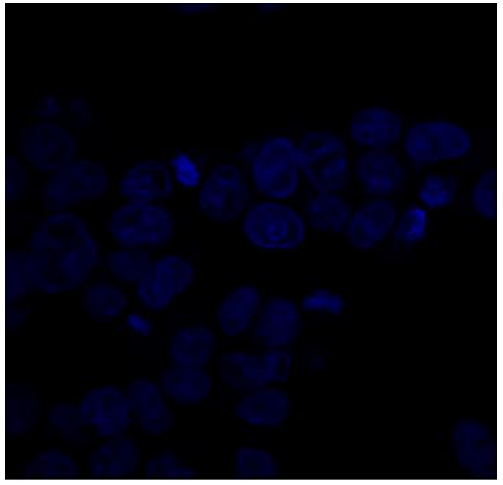
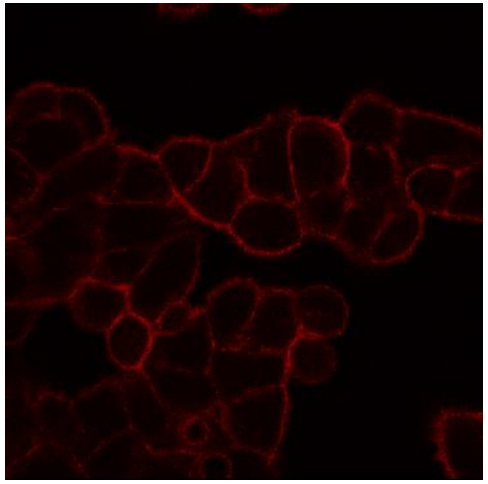
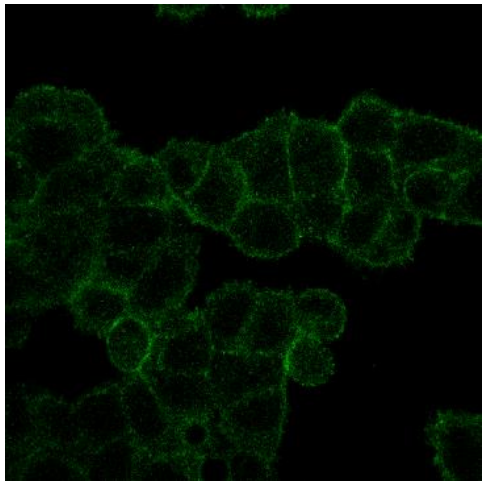
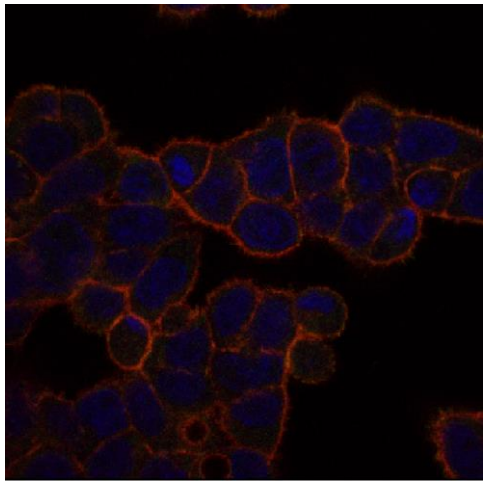




Myc Tag does not allow to conclude where the peptide is located

	DAPI	Phalloidin	myc	MERGE
CONTROL				
ORF1				

FLAG Tag indicates that the peptide is located in the cytoplasmic membrane

	DAPI	Phalloidin	FLAG	MERGE
CONTROL				
WT				

Conclusions

- uORF2 and the non-AUG-uORFs 5, 6 and 7 do not seem to have a significant regulatory role
- uORF1, uORF3, uORF4 and uORF8 together present a strong repressive effect over mORF translation in basal conditions, without affecting the PERK mRNA levels
- The combined repressive activity of uORF1, uORF3, uORF4 and uORF8 is likely involved in maintaining PERK in an inactive state, by promoting its expression at low levels
- During stress conditions, uORF1 allows some translational de-repression, which may help to gradually counterbalance PERK increased degradation
- It is possible that when these uORF-mediated regulatory mechanisms fail or are altered, PERK expression becomes unbalanced, which may drive harmful biological processes and ultimately lead to disease.
- Peptides encoded by uORFs tend to move to the cytoplasmic membrane.

Together, our results illustrate how disturbed uORF-mediated translational control can be involved in the etiology of human genetic disorders.

Is the peptide secreted outside the cell?

Does it have any exocrine function?

Does the peptide interact with PERK?

Does the micropeptide act via *trans* (physical/regulatory interaction) or is the known repressive effect mainly via *cis* (via control of translation initiation)?

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