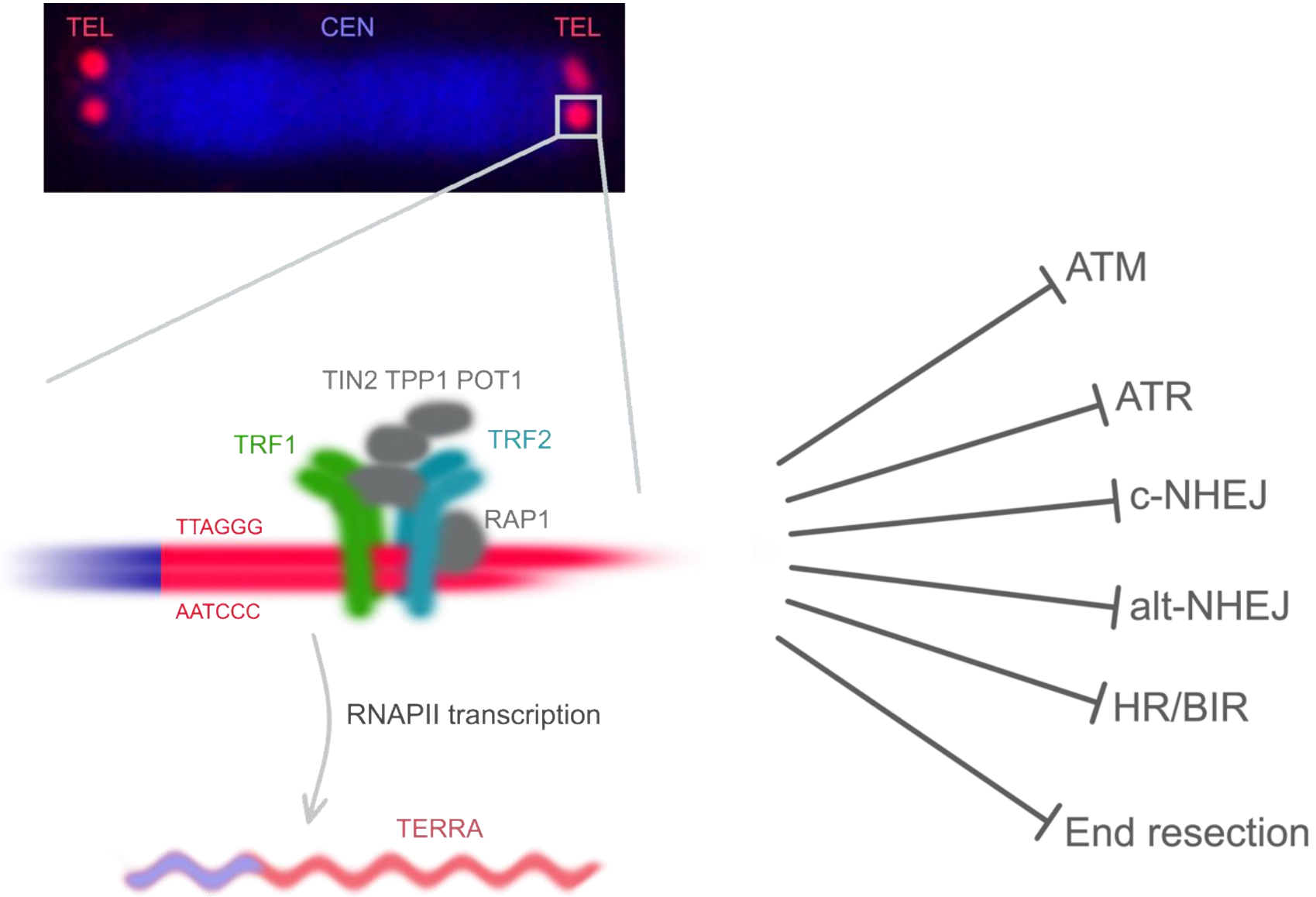
What we do in the lab:
Telomeres and TERRA across evolution

1st TRIAD Meeting
November 2024

GIMM
Gulbenkian Institute *for* Molecular Medicine

Telomeres: HETEROCHROMATIC DNA/PROTEIN COMPLEXES that suppress DNA damage repair at natural chromosome ends



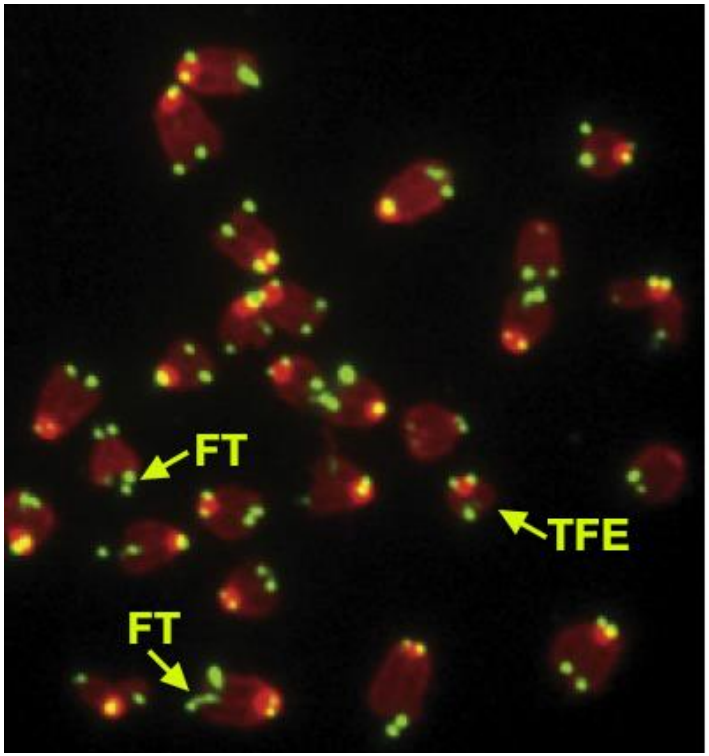
Telomeric repeat-binding factors 1 and 2 (TRF1 & TRF2)

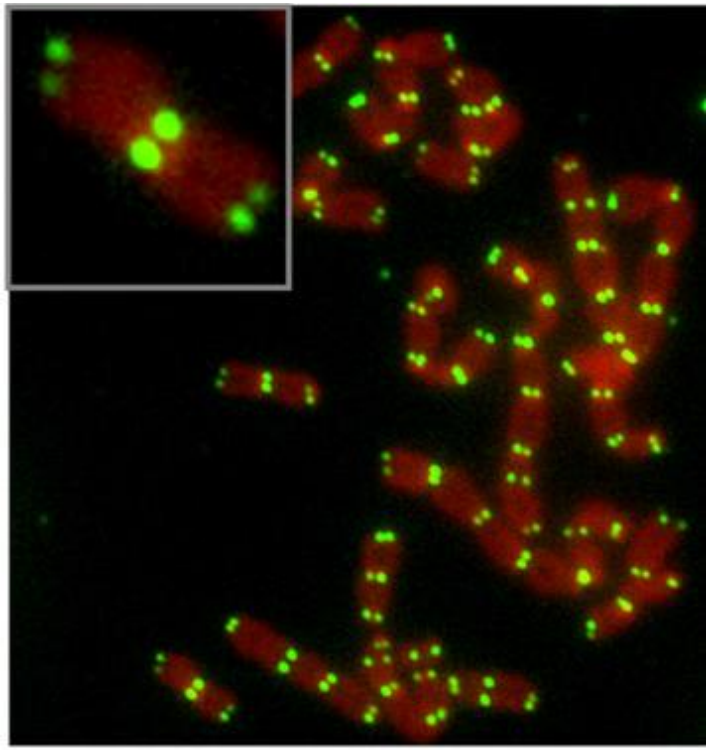
hsTRF1

- Telomeric dsDNA replication
- Suppression of HDR (BIR)

hsTRF2

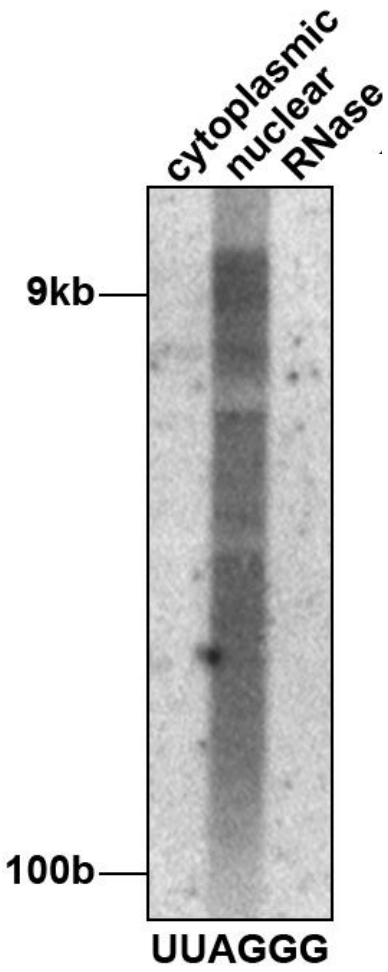
- End protection
- Suppression of NHEJ



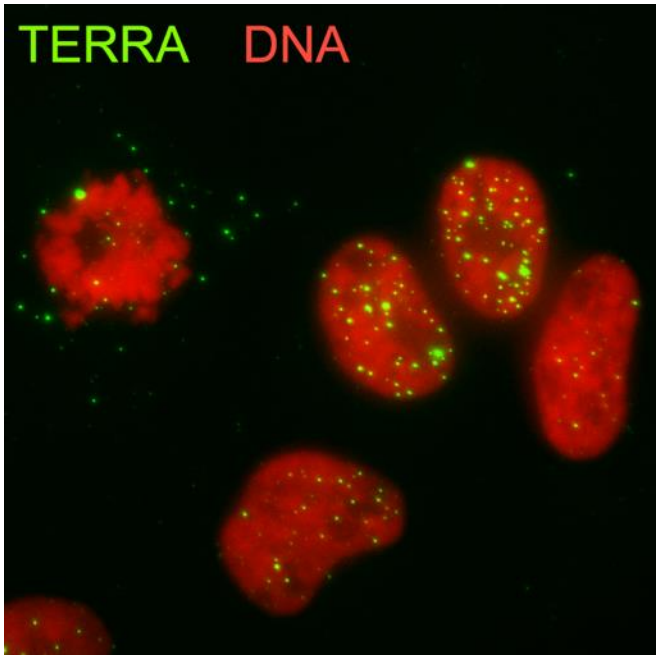


de Lange lab

Telomeric repeat-containing RNA (TERRA)



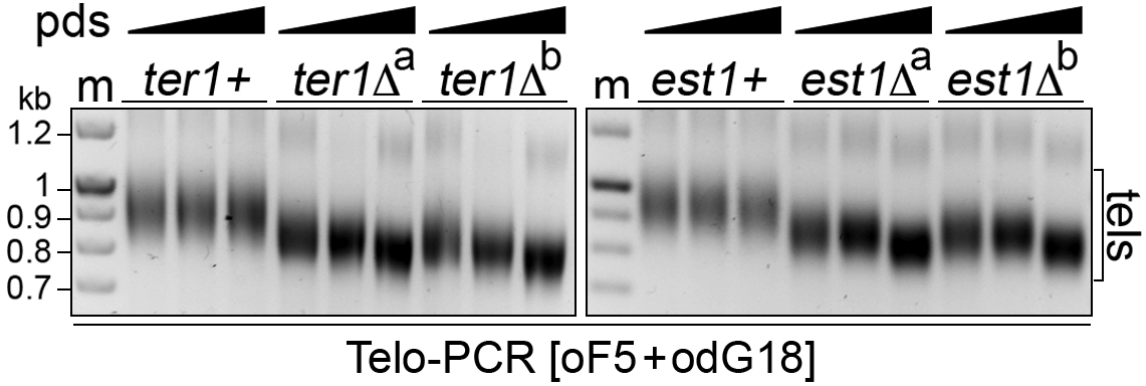
HeLa

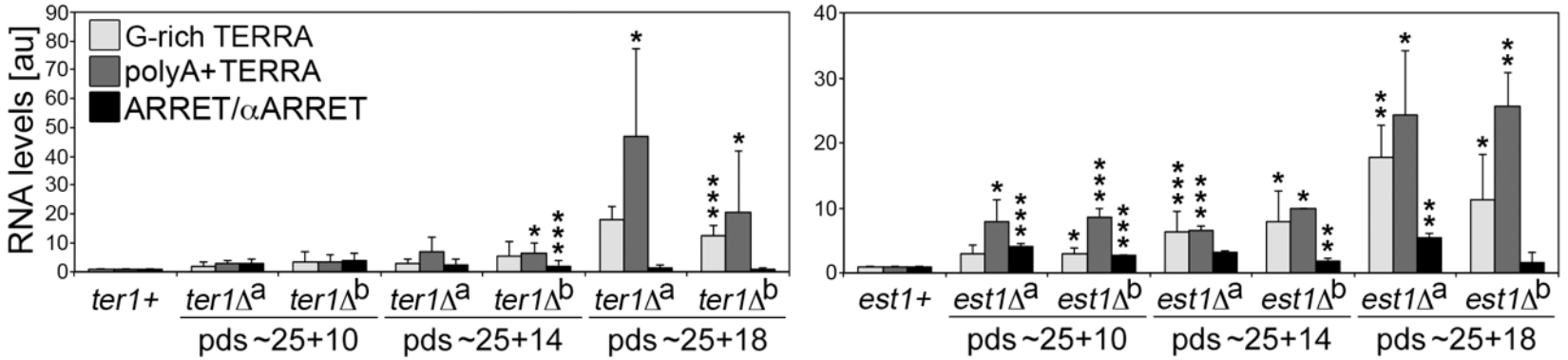


U2OS

Azzalin et al. 2007

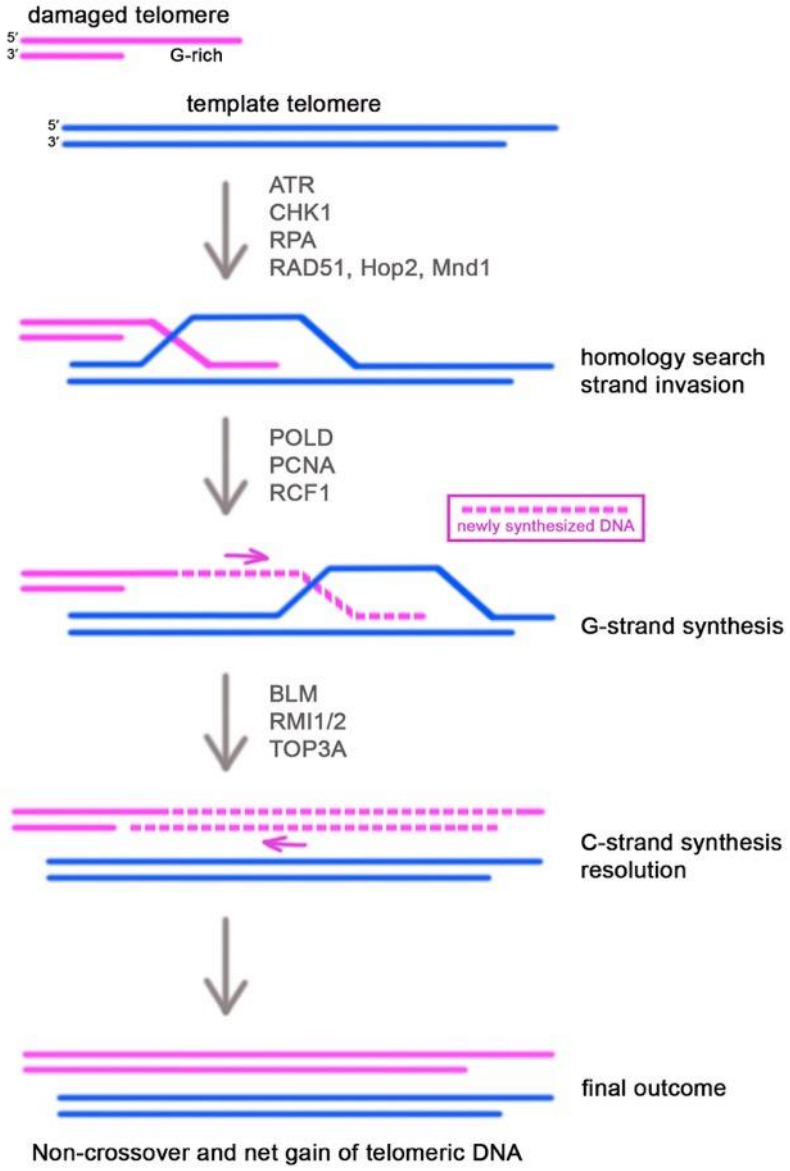
Shorter/damaged telomeres are more avidly transcribed



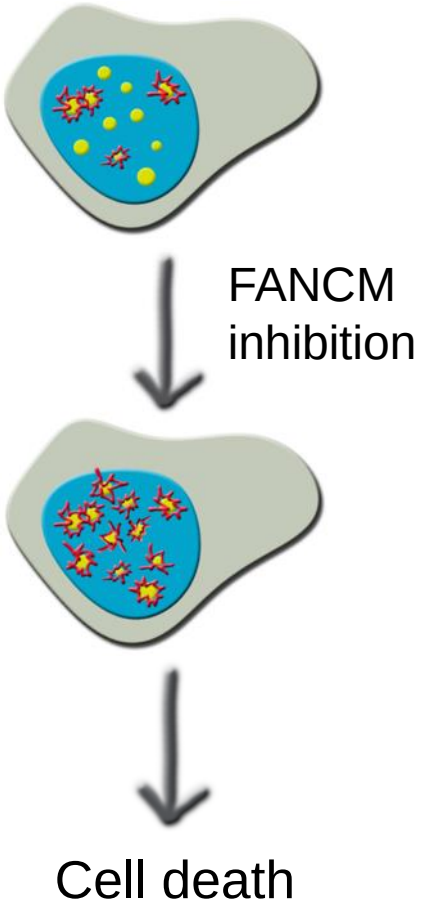


Moravec et al. 2016

Alternative lengthening of telomeres (ALT)



- Tumors of mesenchymal or epithelial origin
- Very aggressive and chemotherapy resistant
- Often develop in children



Silva et al. 2018

TERRA quantification

The ALT mechanism and ALT vulnerabilities

TERRA and shelterin

TERRA far from telomeres

TERRA in an organism

How to quantify TERRA

The ALT mechanism and ALT vulnerabil

TERRA and shelterin

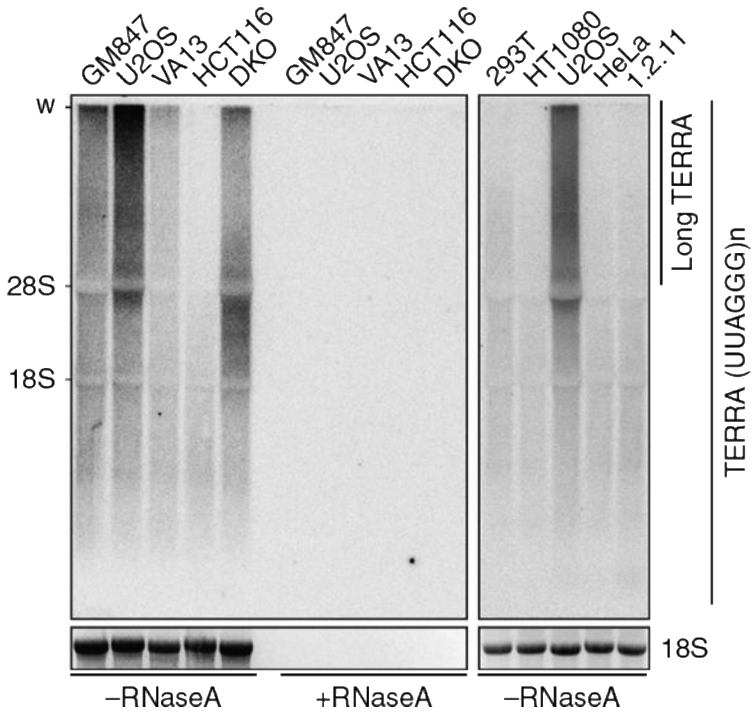
Joana

TERRA far from telomeres

TERRA in an organism

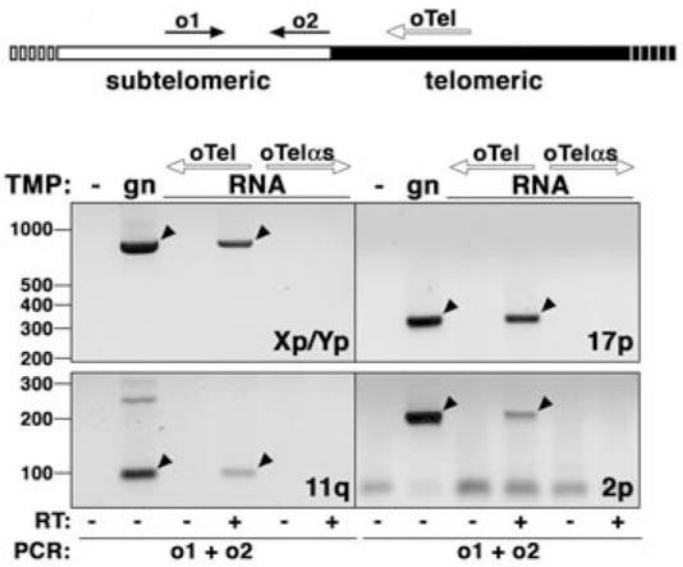
Limitations of canonical ways to quantify human TERRA

Northern blotting with telomeric probes



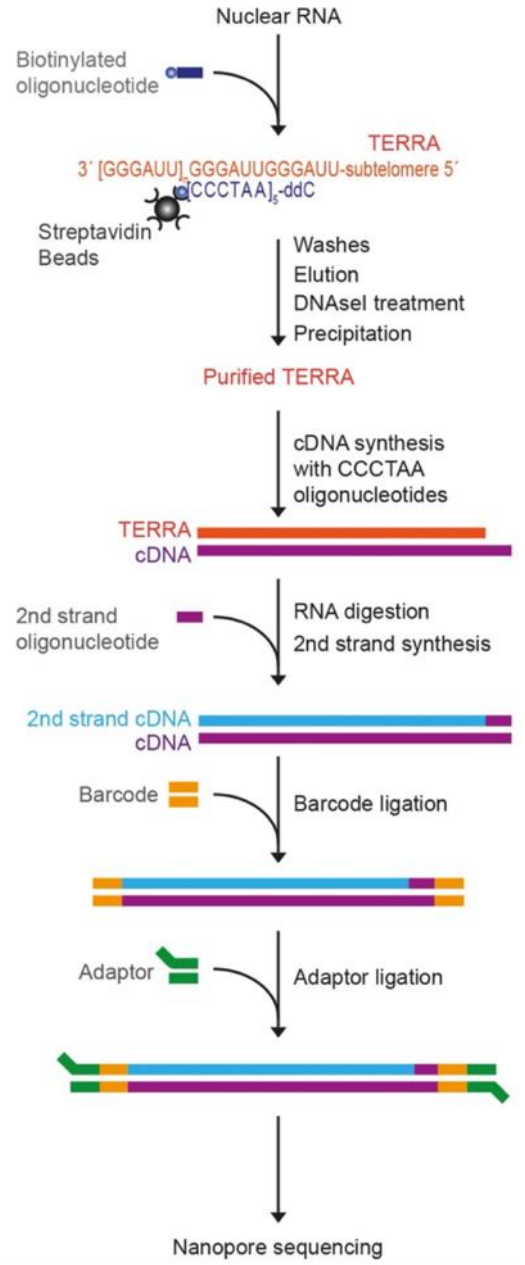
Arora et al. 2014

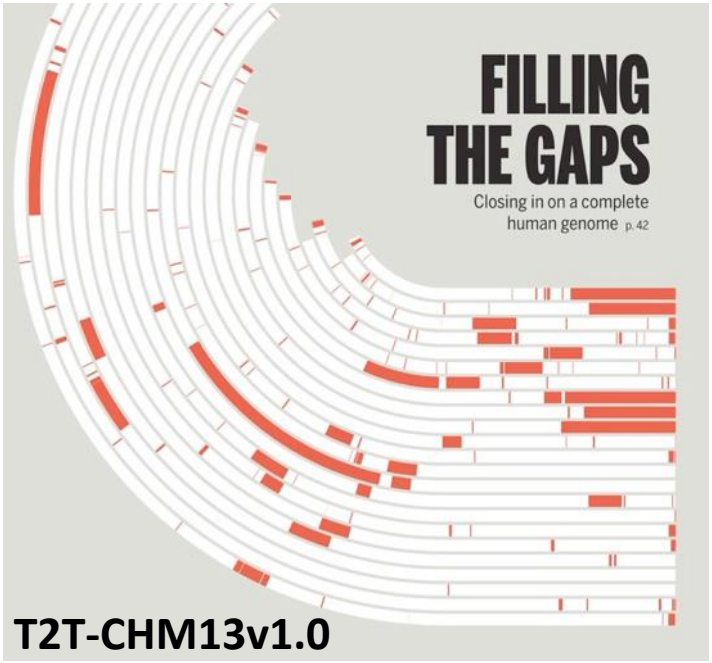
RT-(q)PCR with subtelomeric oligos



Azzalin et al. 2007

TERRA ONTseq – a long read sequencing pipeline to study TERRA transcriptome

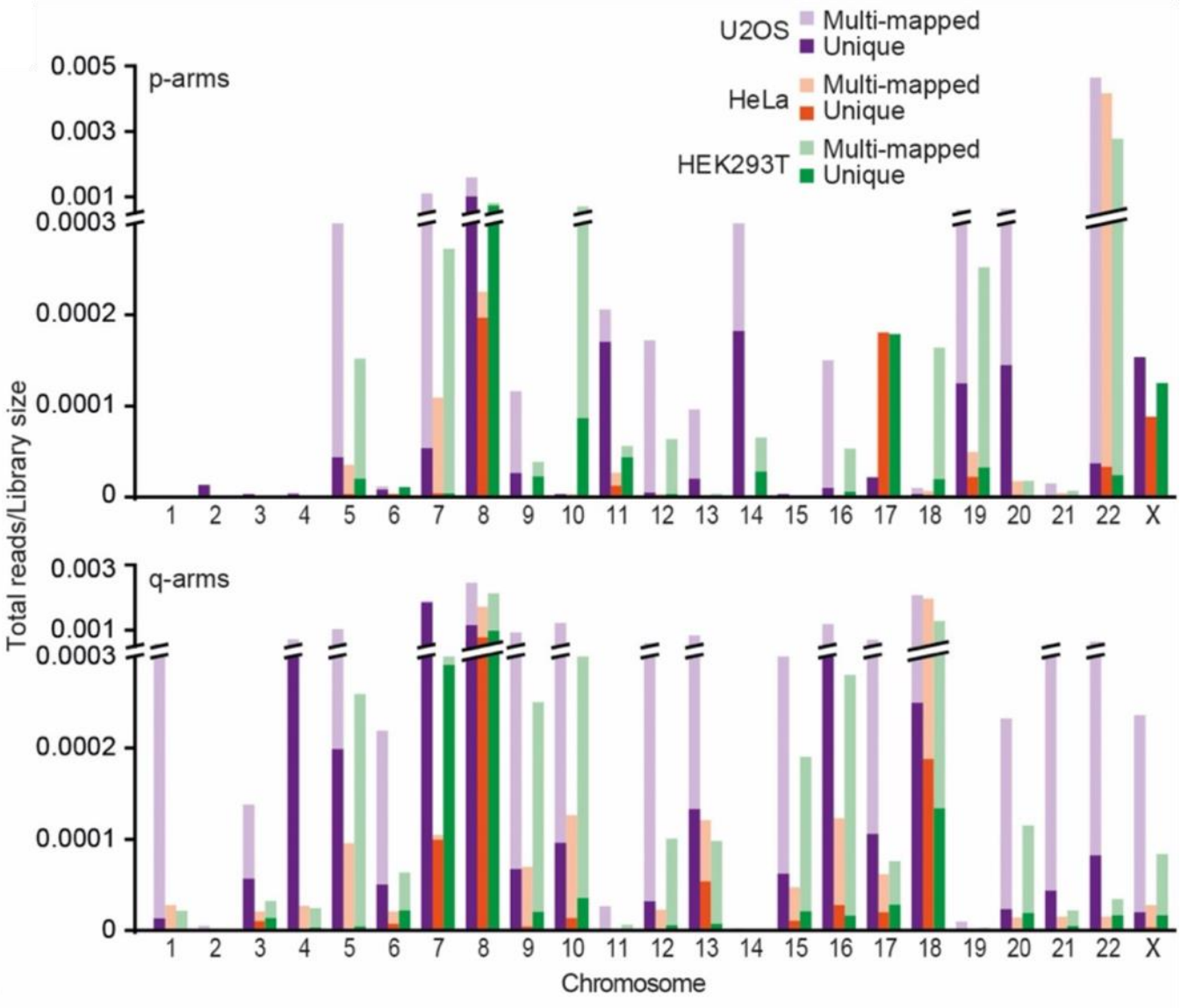




T2T-CHM13v1.0

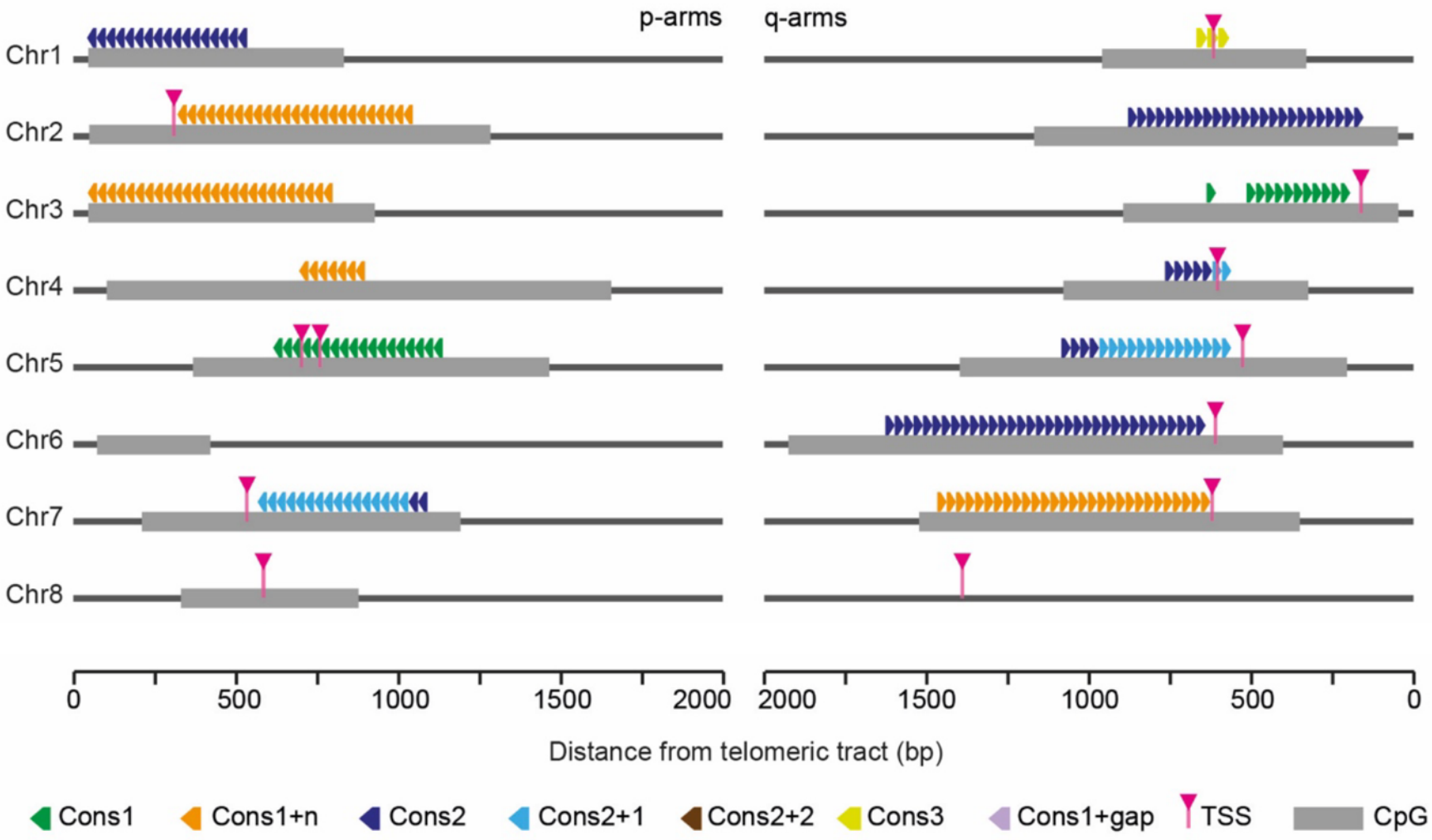
Rodrigues et al. 2024

Virtually all human telomeres produce TERRA



Rodrigues et al. 2024

CpG-rich tandem repeats constitute the majority of TERRA promoters



Rodrigues et al. 2024

How to quantify TERRA

The ALT mechanism and ALT vulnerabilities

TERRA and shelterin


Bruno


Sara


Beatriz

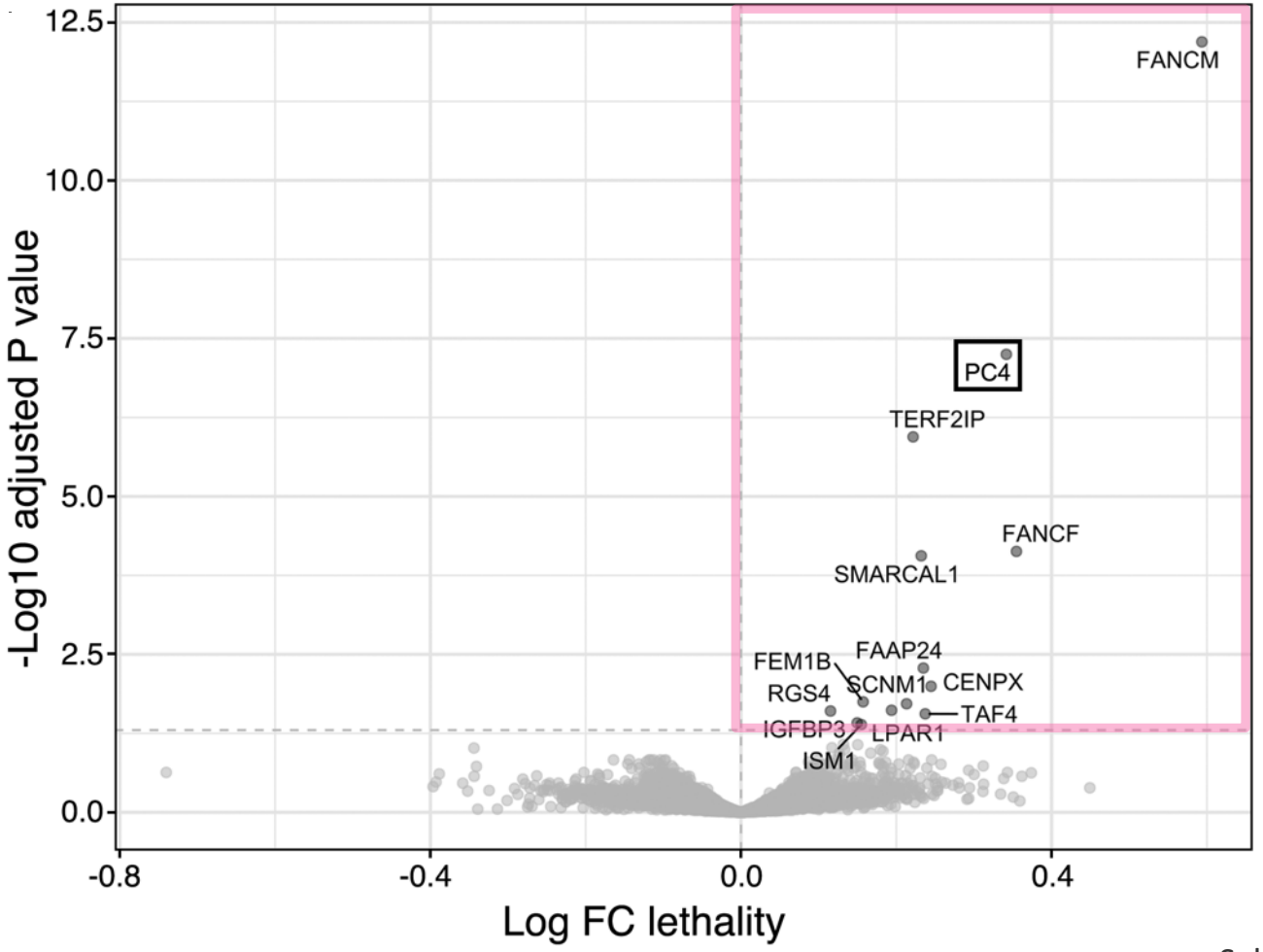

Lee


Patricia


Daniela

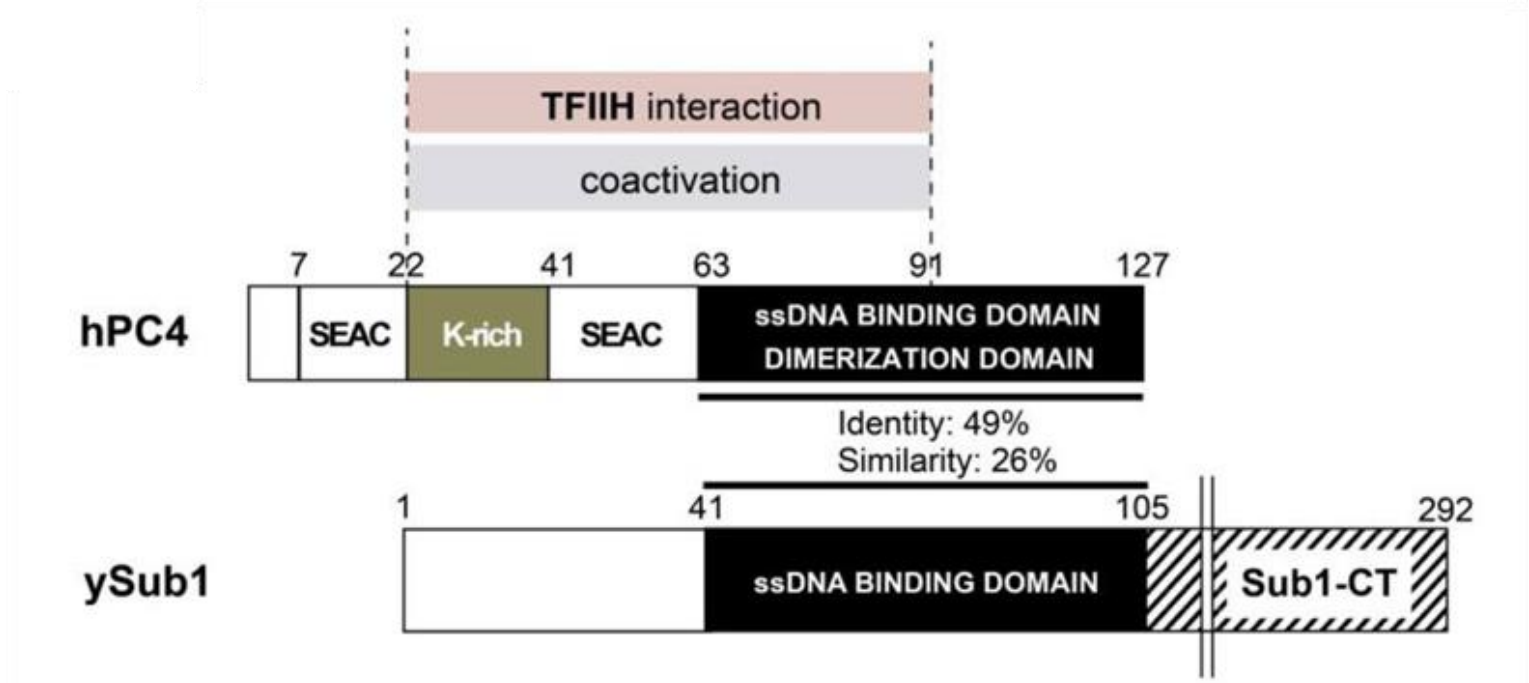
Mining of the Project Achilles cancer dependency database (Cancer Dependency Map Portal, version 23Q2)

- Genome-wide CRISPR/Cas9 screens in 1098 human cancer cell lines (14 ALT)
- Identification of genes with dependency scores significantly higher for the ALT lines



Salgado et al. 2024

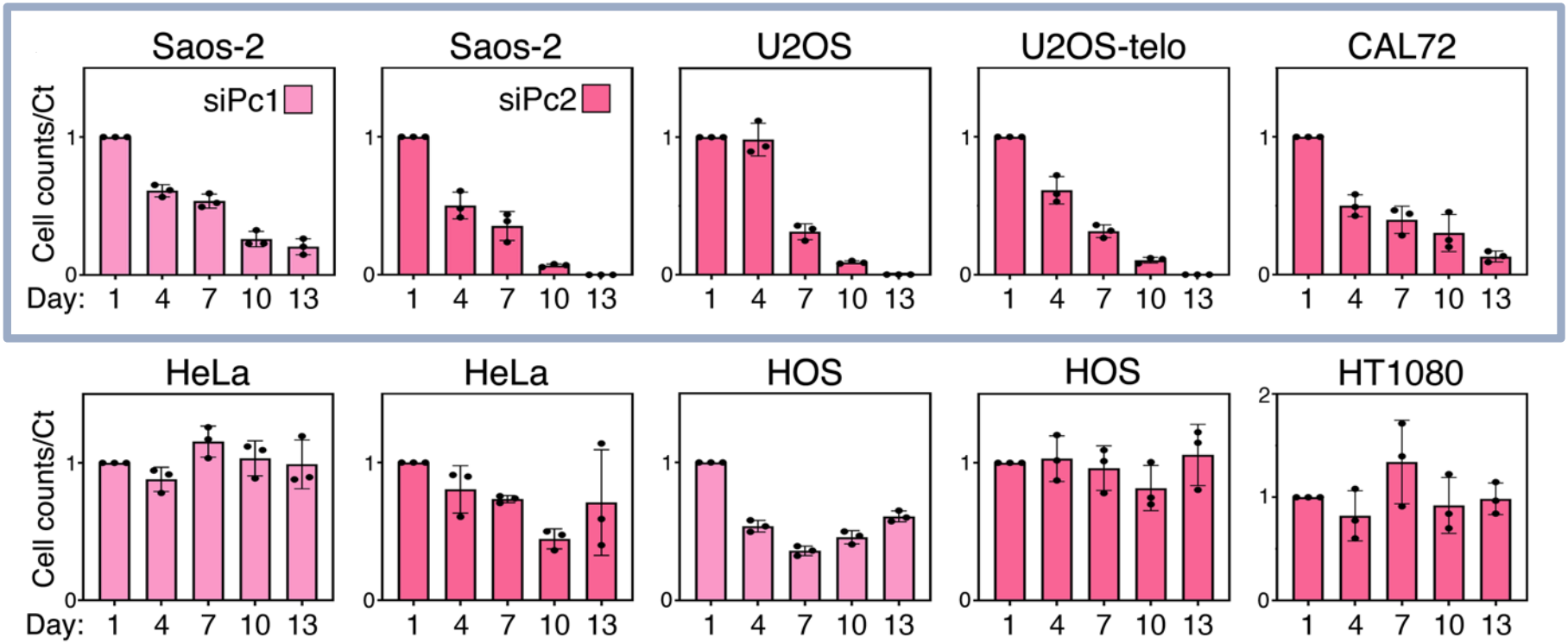
Positive Cofactor 4 (PC4)



Garavís & Calvo, 2017

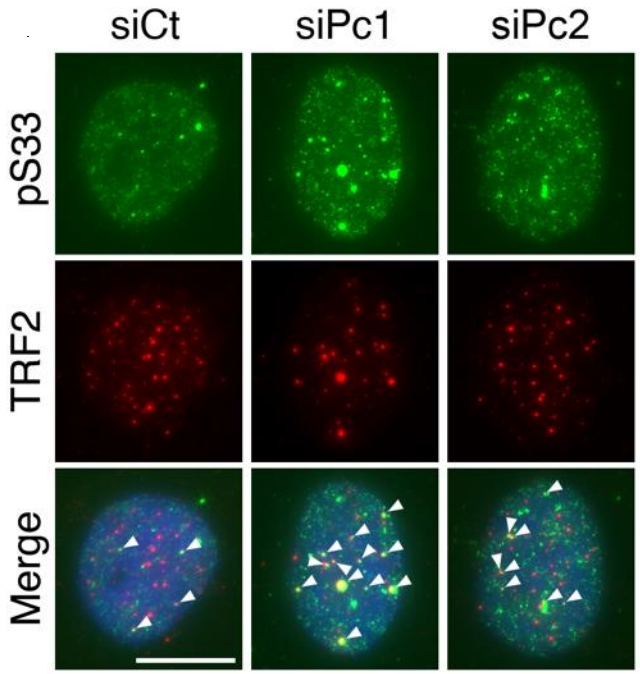
- Protein conserved across eukaryotes and prokaryotes
- Co-activator of RNAPII and RNAPIII
- Supports genome stability upon drug-induced replication stress
- Binds to nuclecic acids including ssDNA, dsDNA and ssRNA

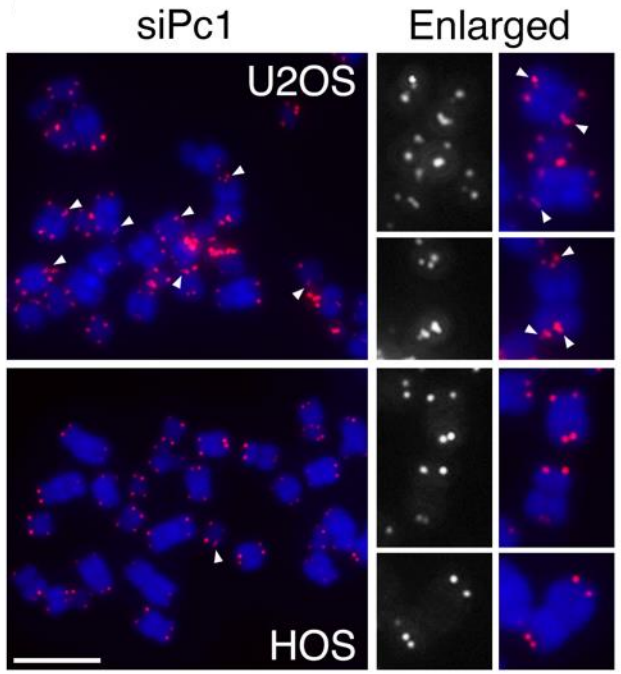
PC4 depletion specifically kills ALT cells

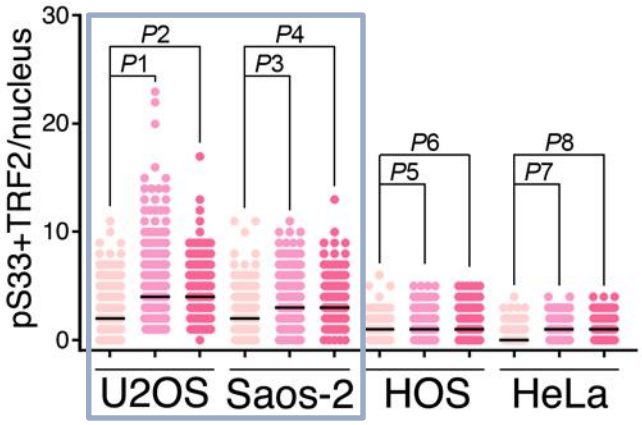


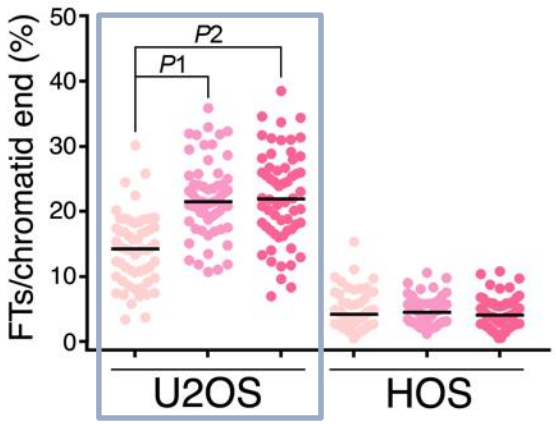
Salgado et al. 2024

PC4 depletion increases replication stress at telomeres in ALT cells





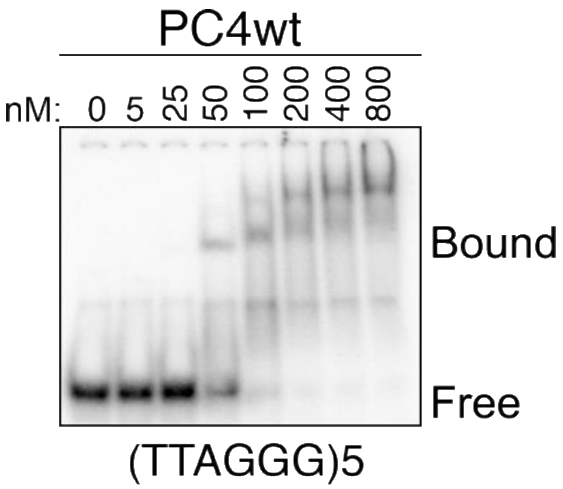


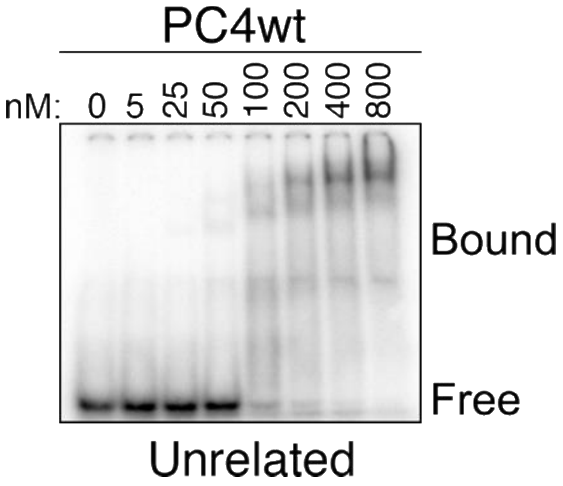


Salgado et al. 2024

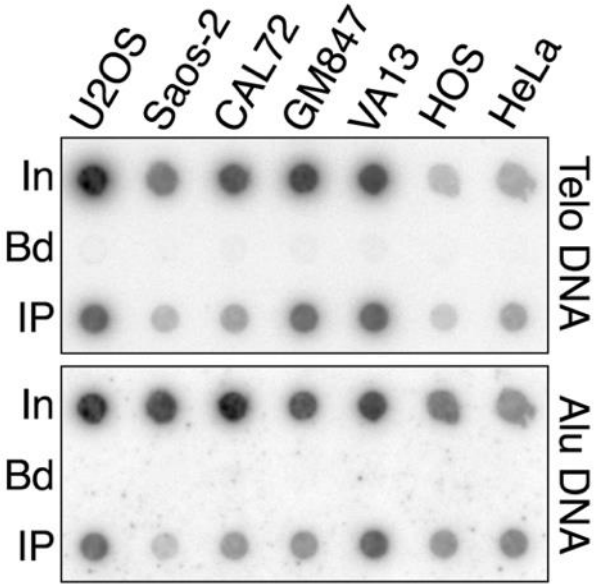
PC4 binds to telomeric ssDNA *in vitro* and to telomeres in cells

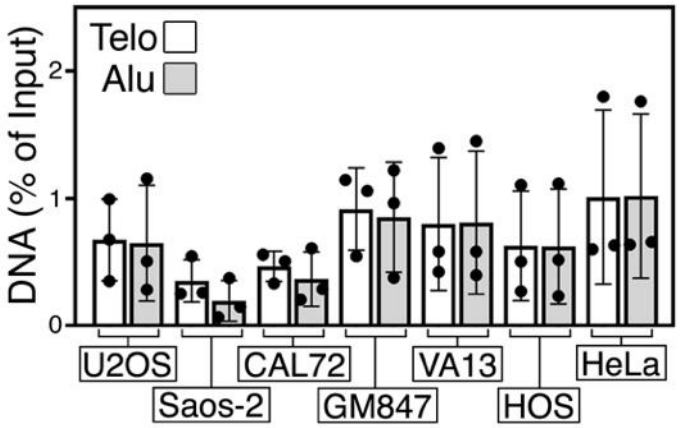
EMSAs (*in vitro*)





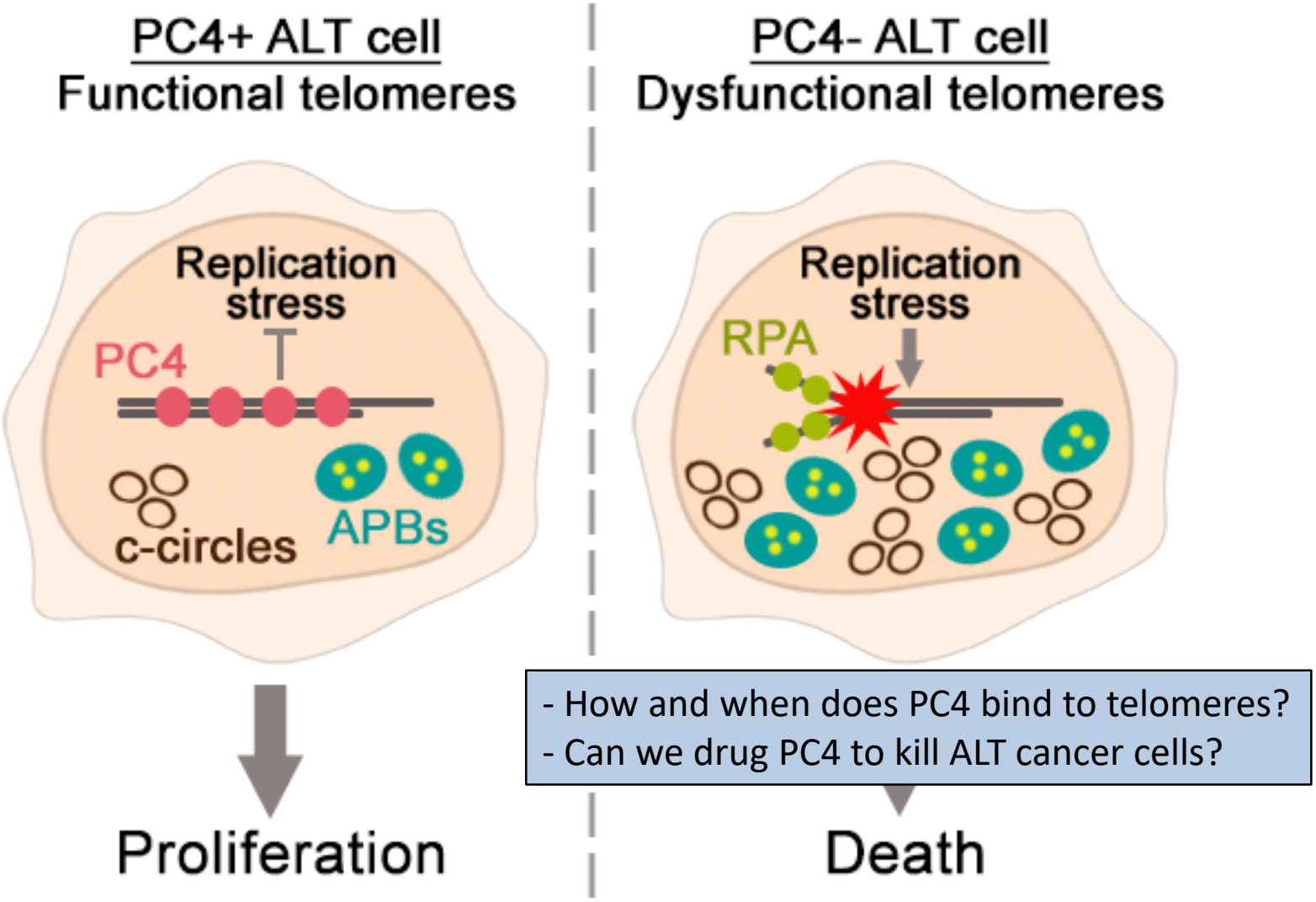
ChIPs (*in cellulo*)





Salgado et al. 2024

PC4 and ALT



- How and when does PC4 bind to telomeres?
- Can we drug PC4 to kill ALT cancer cells?

Salgado et al. 2024

How to quantify TERRA

The ALT mechanism and ALT vulnerabilities

TERRA and shelterin

TERRA functions at telomeres

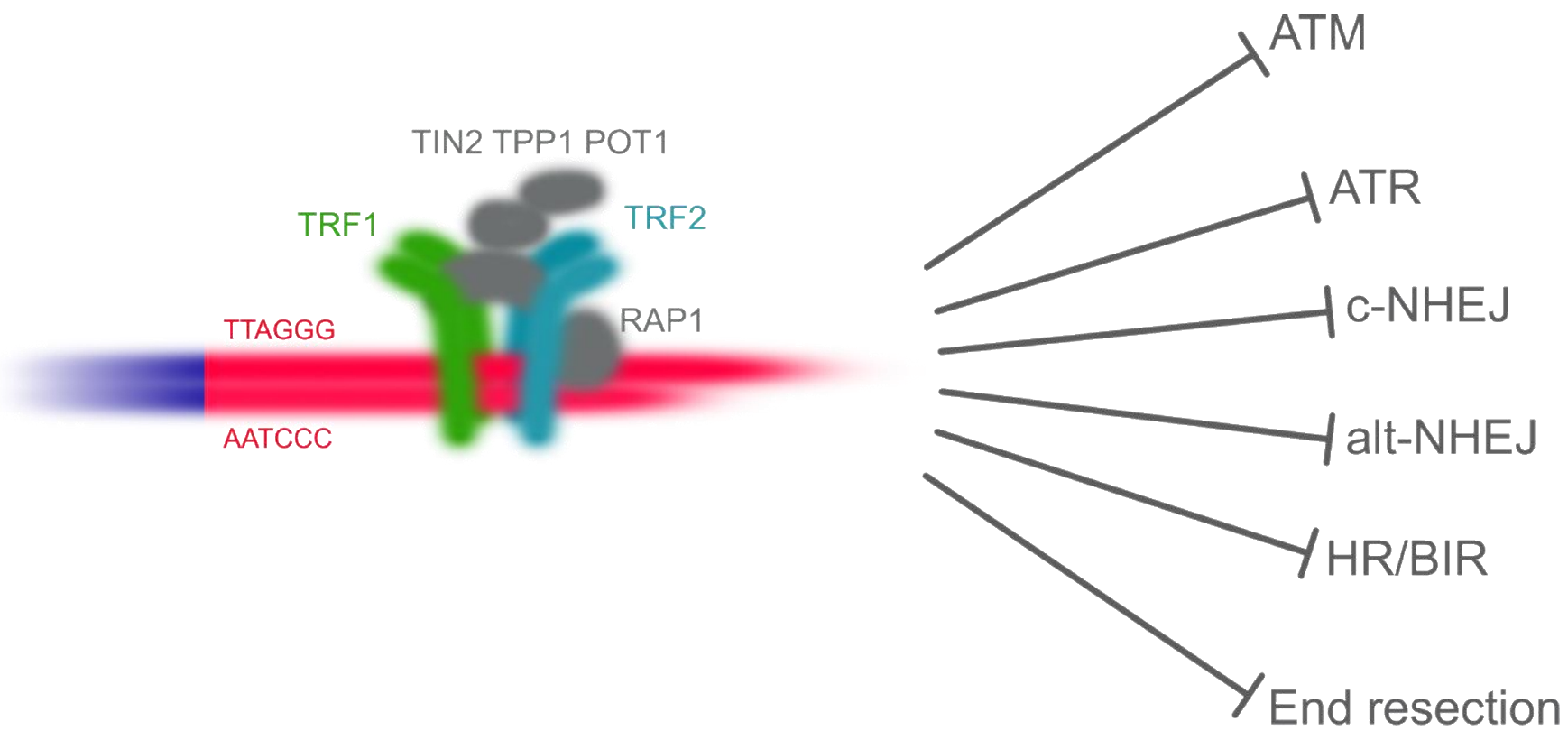
TERRA in other organism

Yong Woo

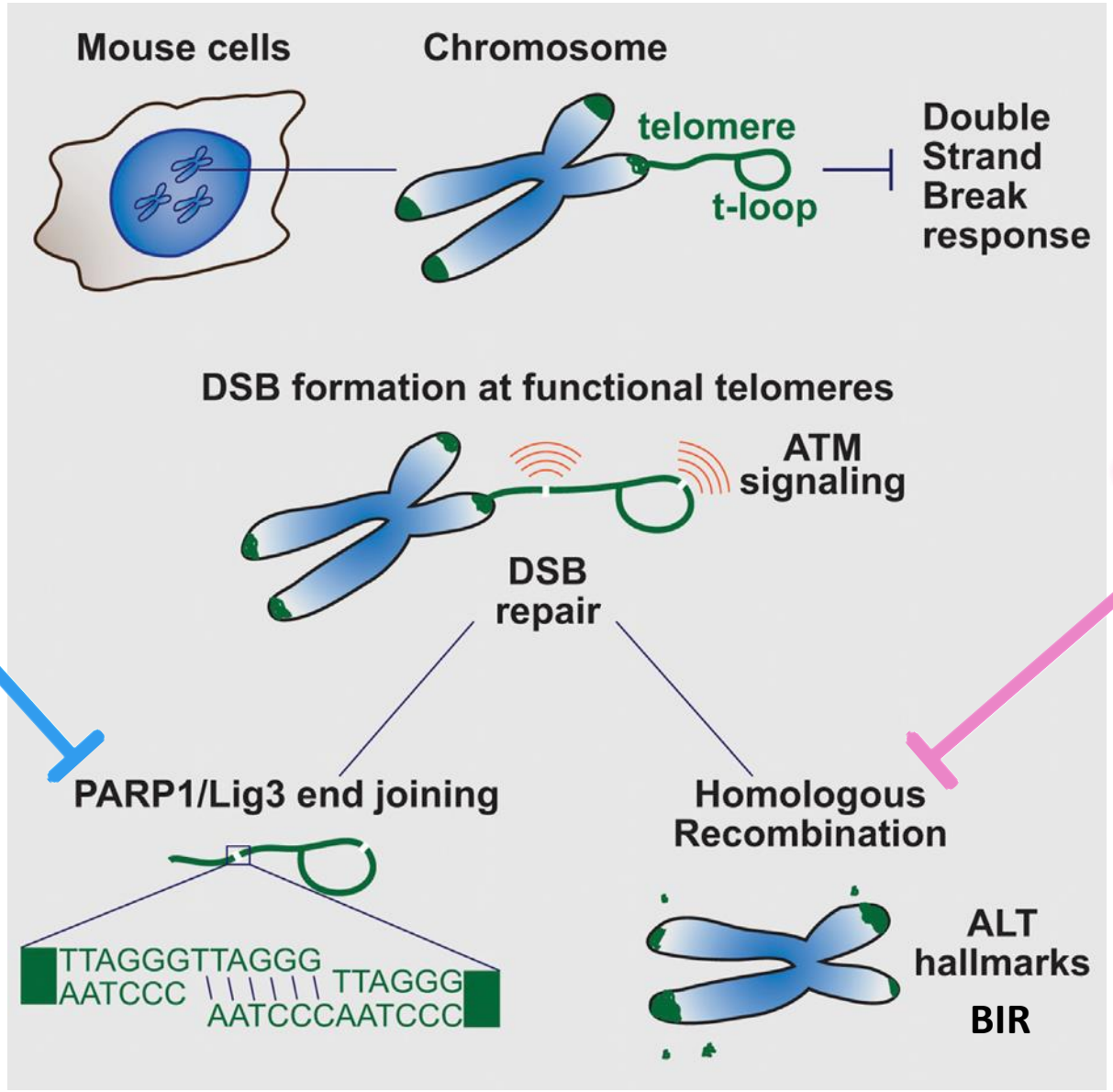
Patricia

Valentina

Telomeres: HETEROCHROMATIC DNA/PROTEIN COMPLEXES that suppress inappropriate DNA damage repair at chromosome ends

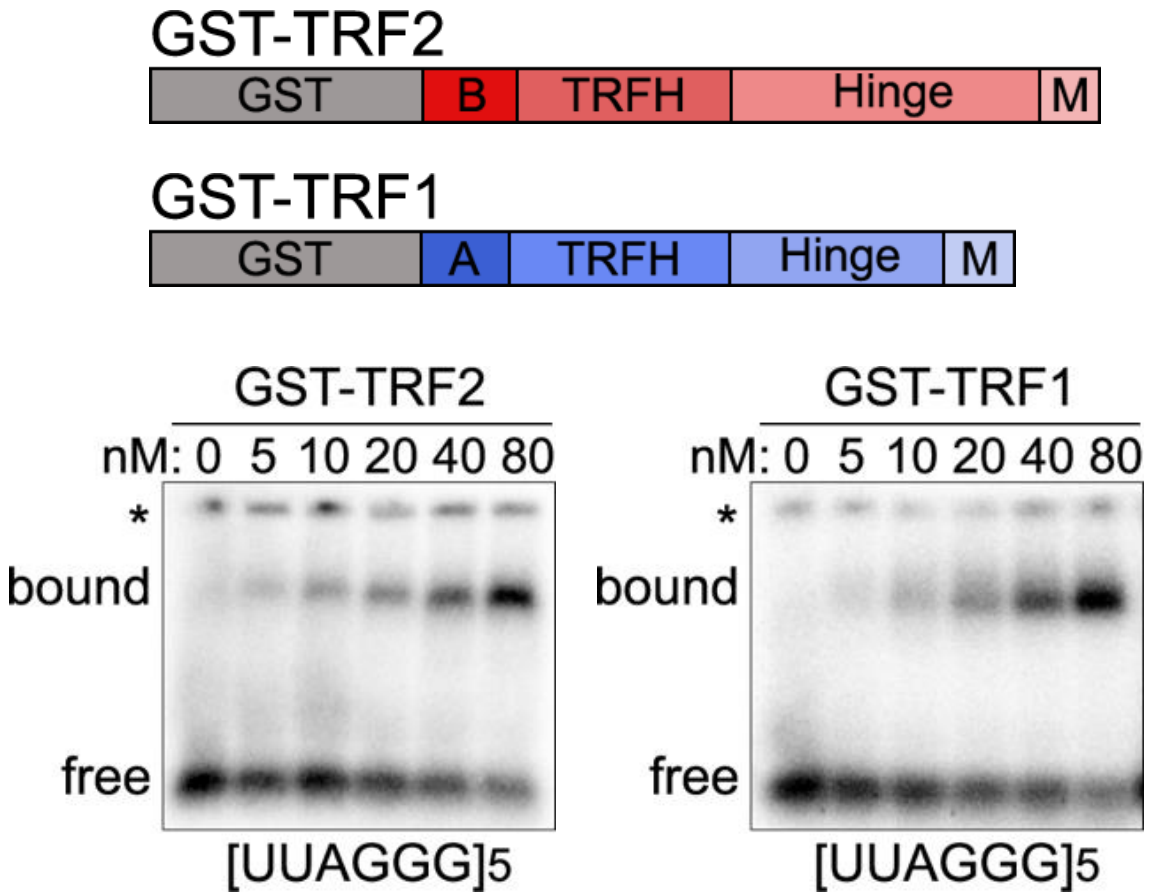


Damaged telomeric DNA can be repaired



Doksani & de Lange, 2016

TERRA and TRF proteins

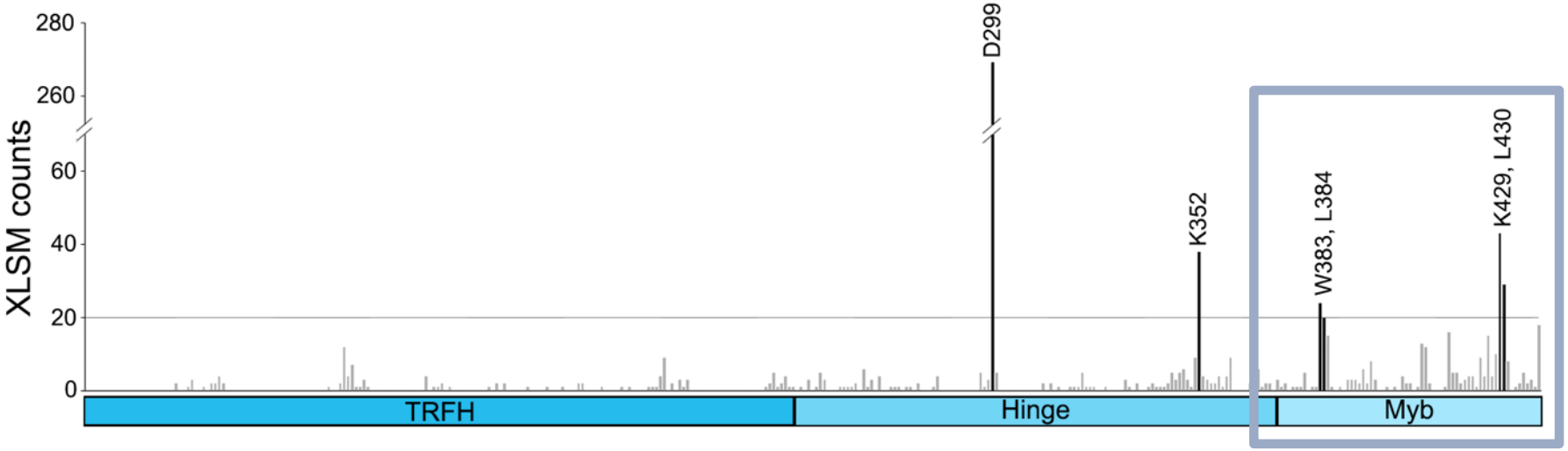


Lee et al. 2018; Abreu et al. 2022

CLIR-MS/MS of recombinant TRF1ΔA

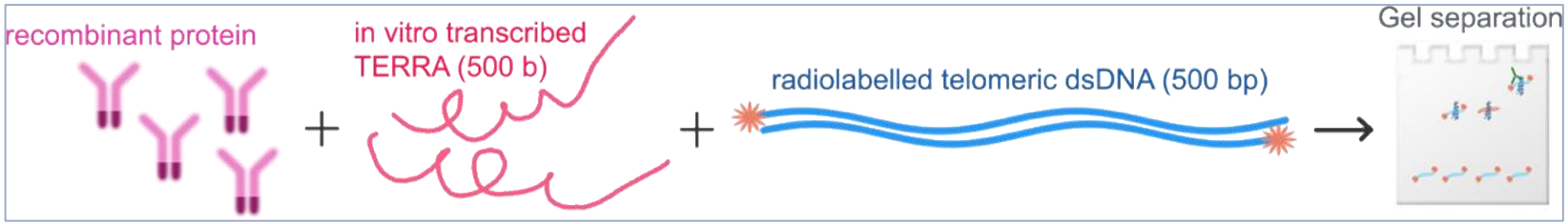
GST-TRF1ΔA

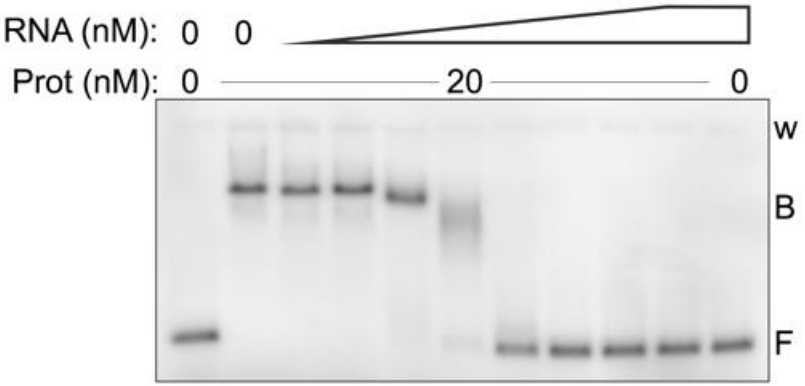
+ TERRA



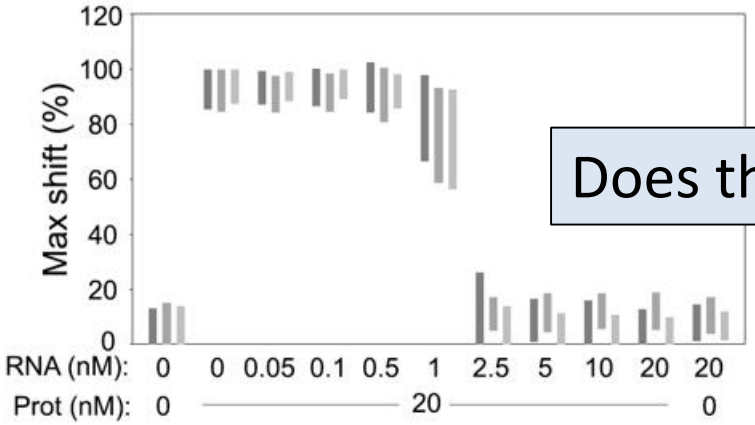
Collaboration with Alex Leitner and Chris Sarnowski (ETH Zürich)

TERRA inhibits TRF1 binding to dsDNA



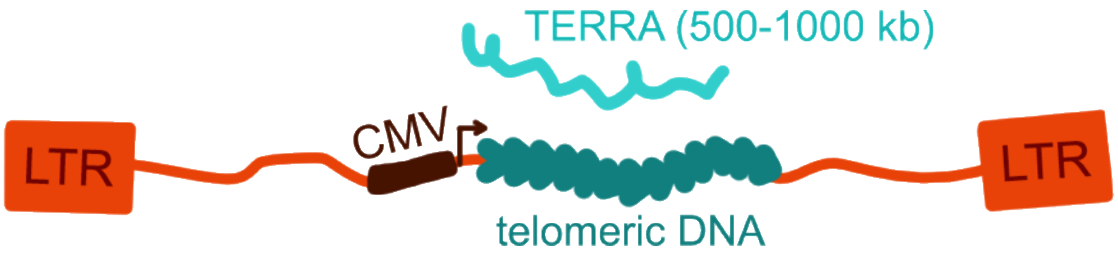


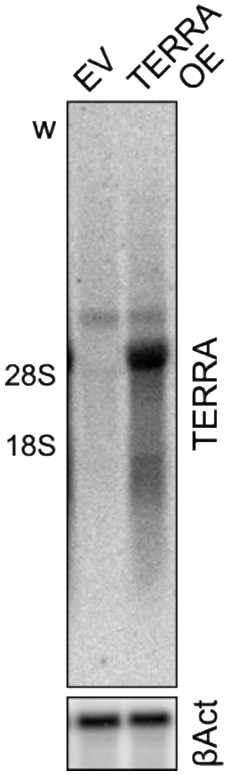
dsDNA + TRF1 > TERRA

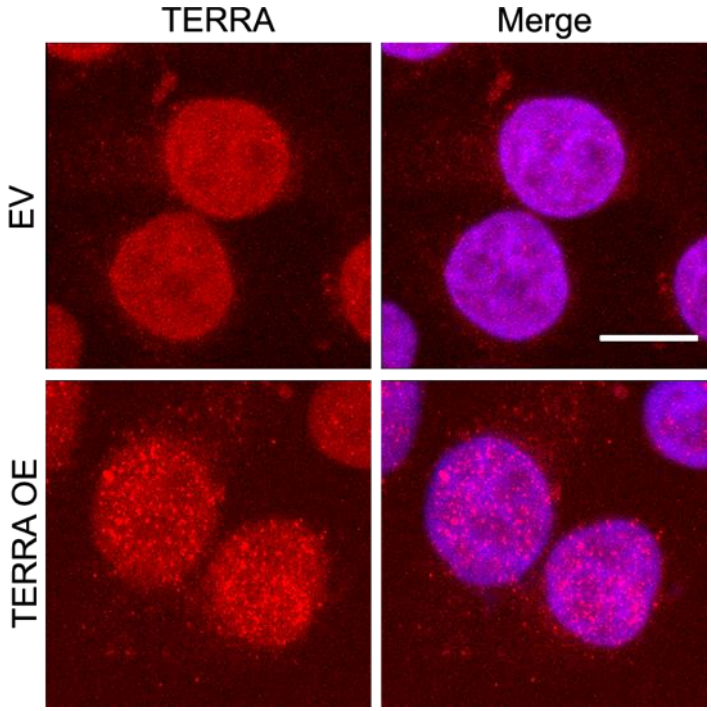


Does this happen in cells?

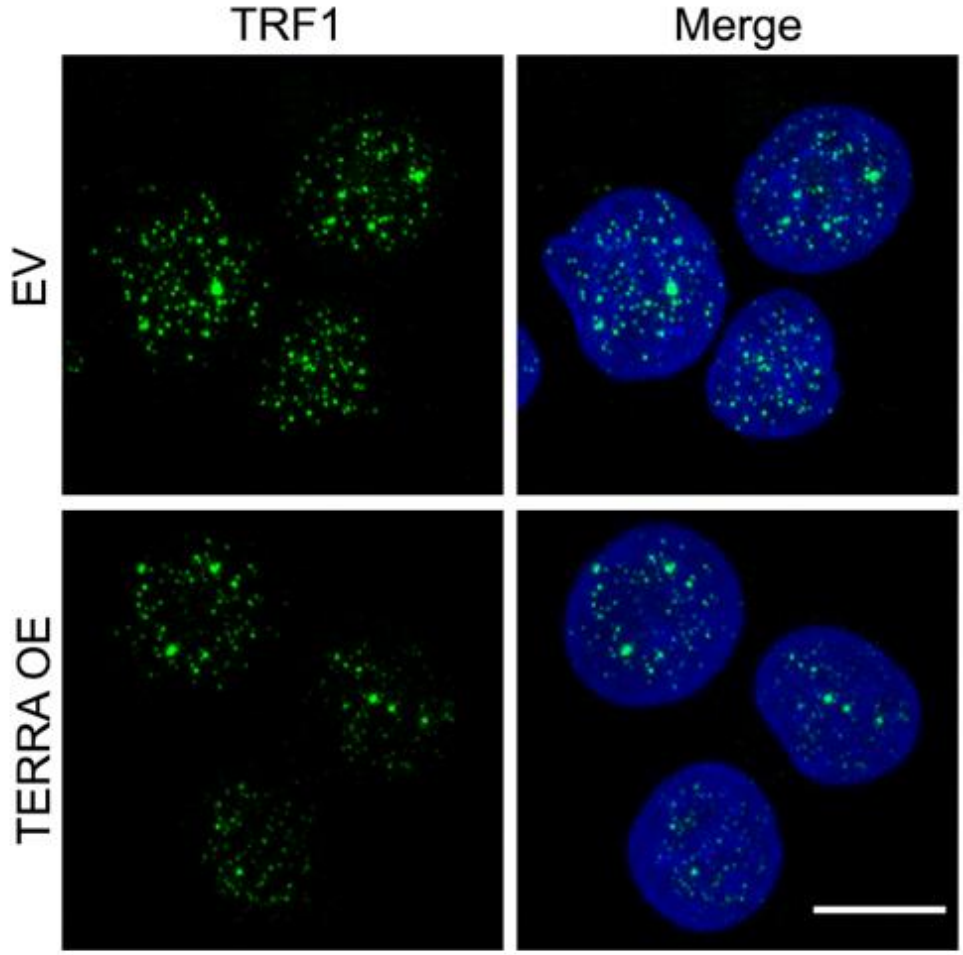
TERRA over-expression in HeLa cells

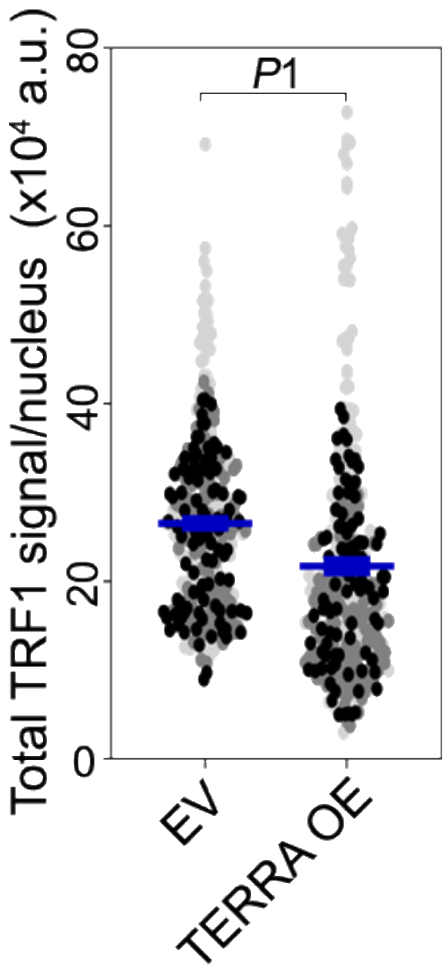




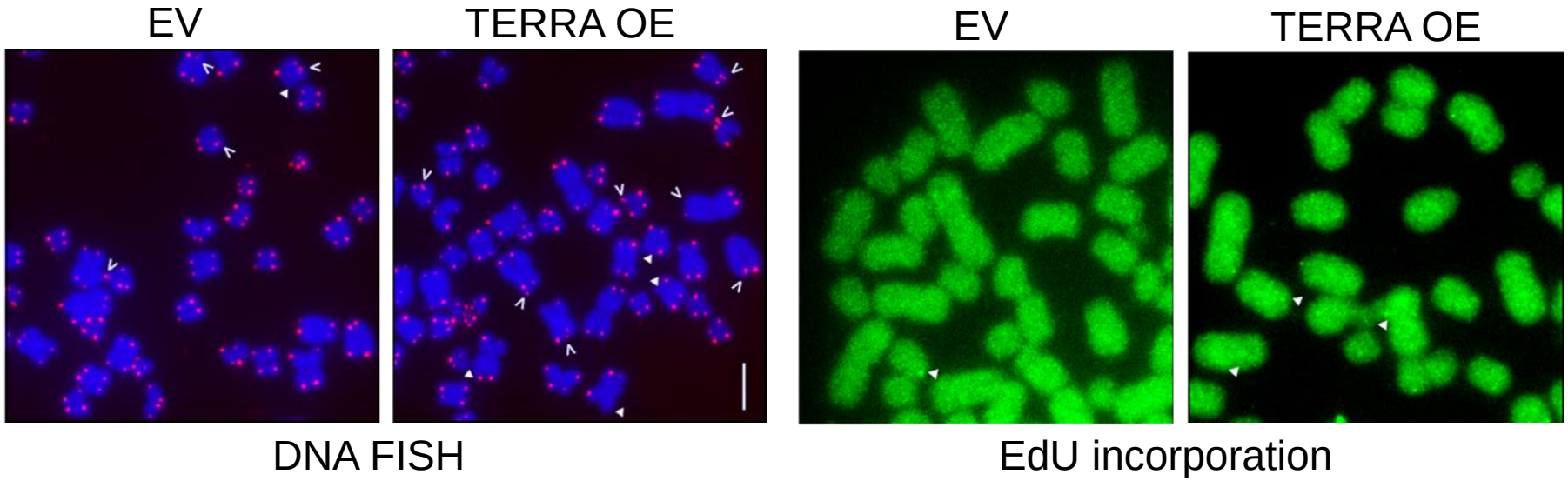


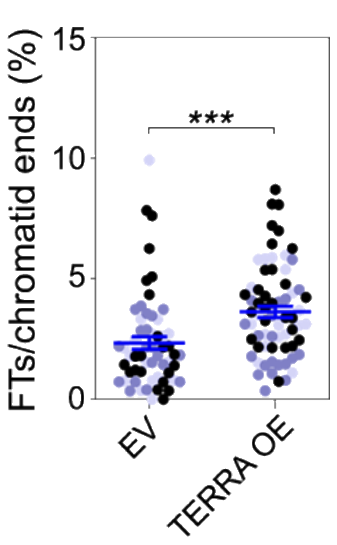
TERRA over-expression diminishes the levels of telomere-bound TRF1

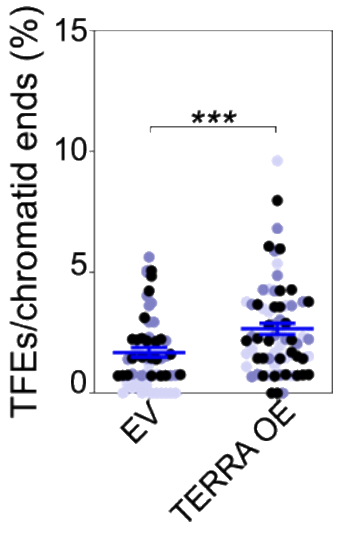


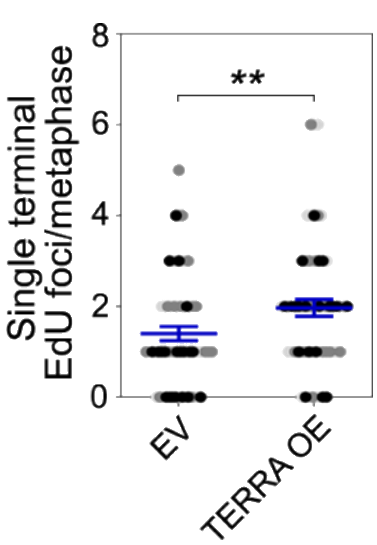


TERRA over-expression causes FTs and TFEs

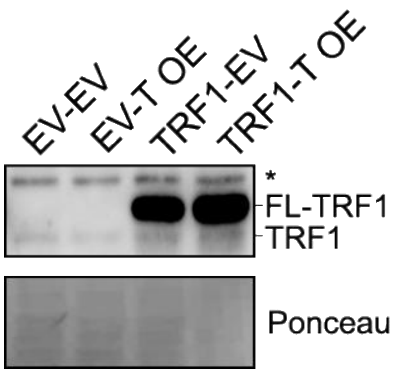


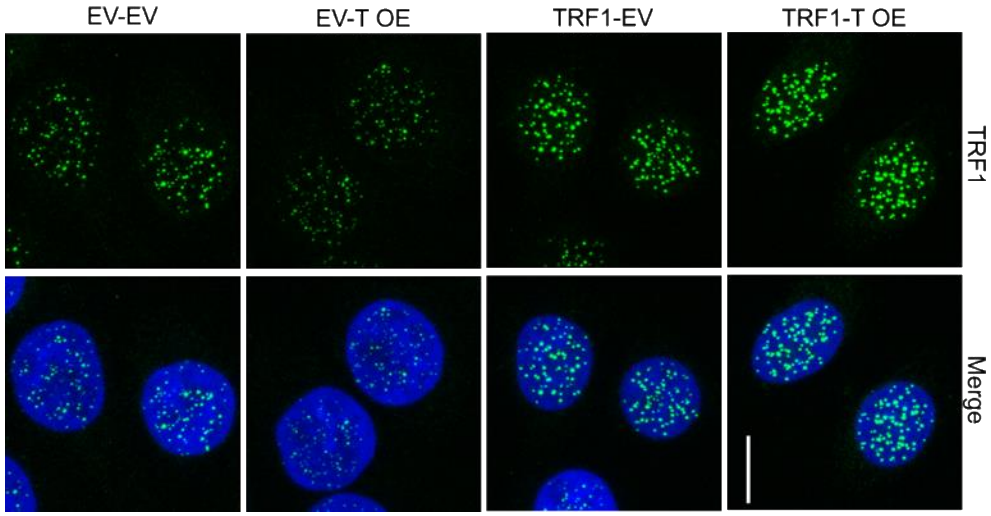


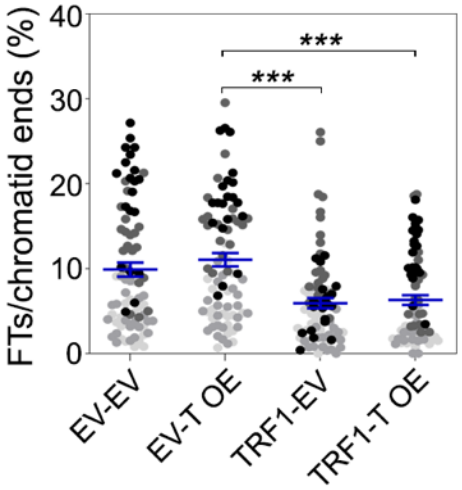


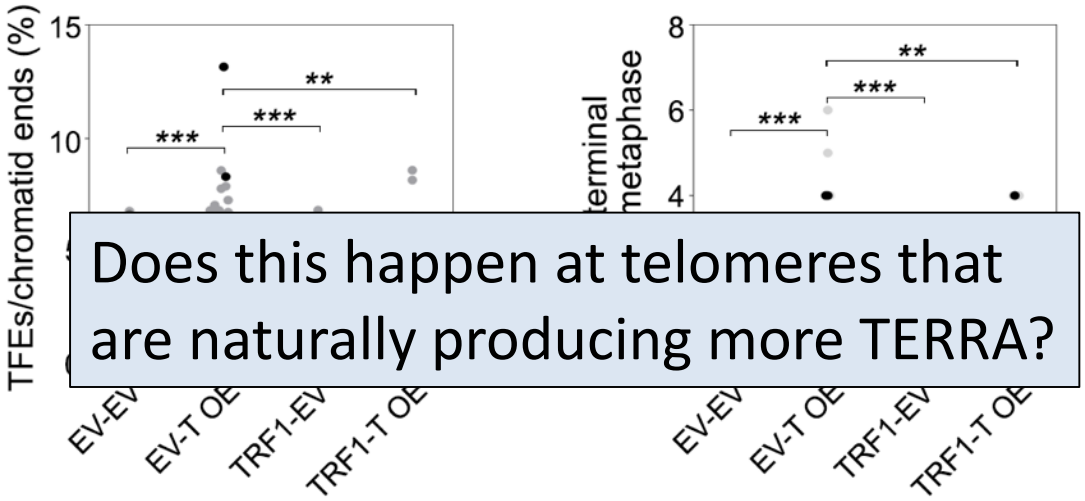


TRF1 over-expression suppresses the defects caused by TERRA over-expression



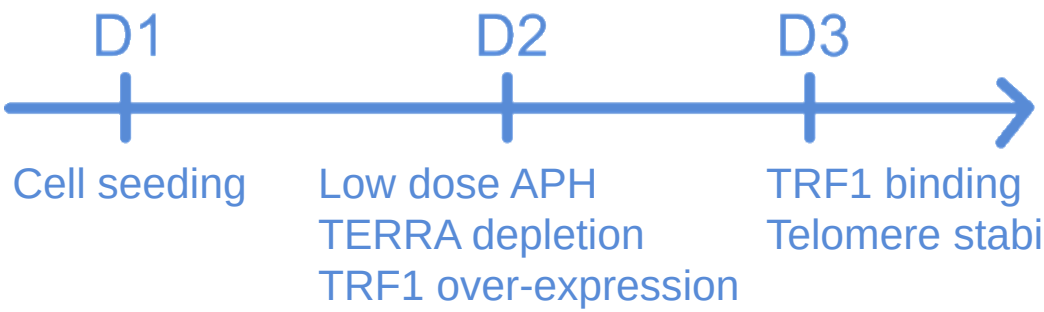


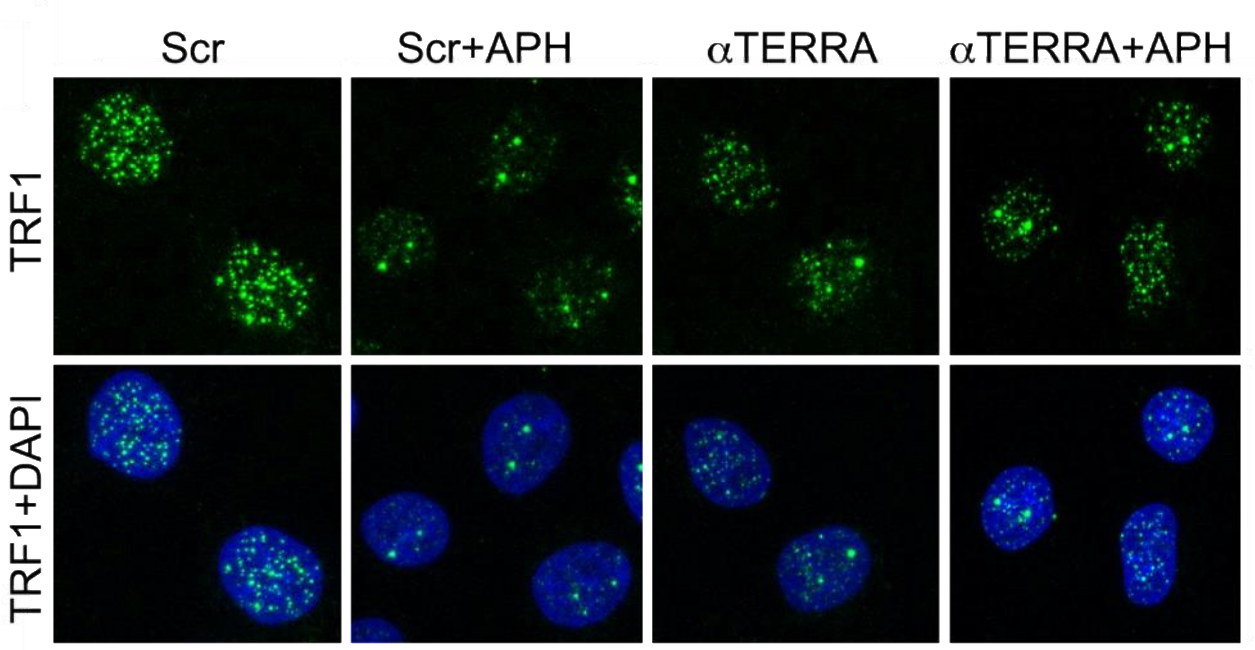


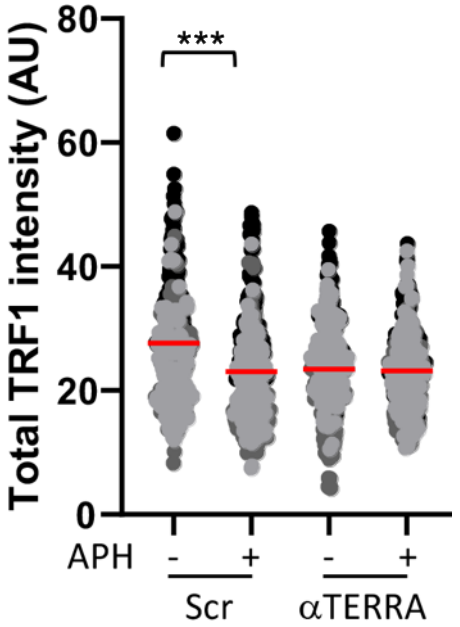


Does this happen at telomeres that are naturally producing more TERRA?

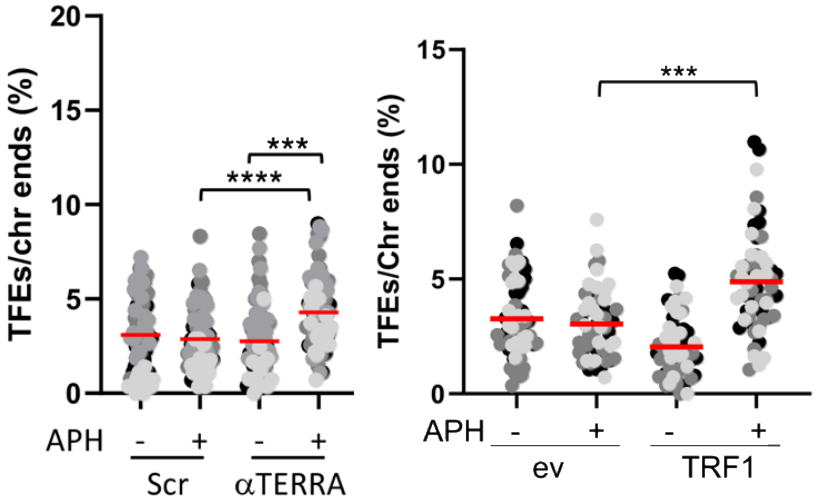
TRF1 is depleted from telomeres in APH-treated cells in a TERRA-dependent manner

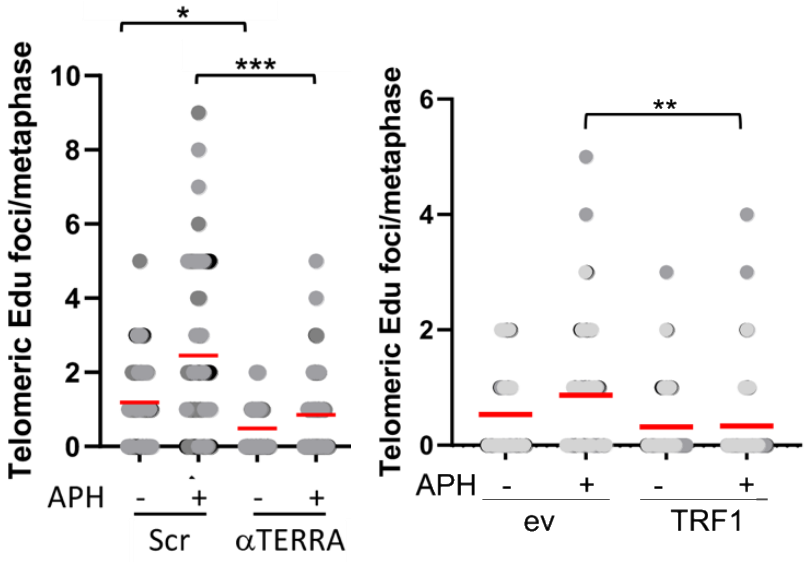




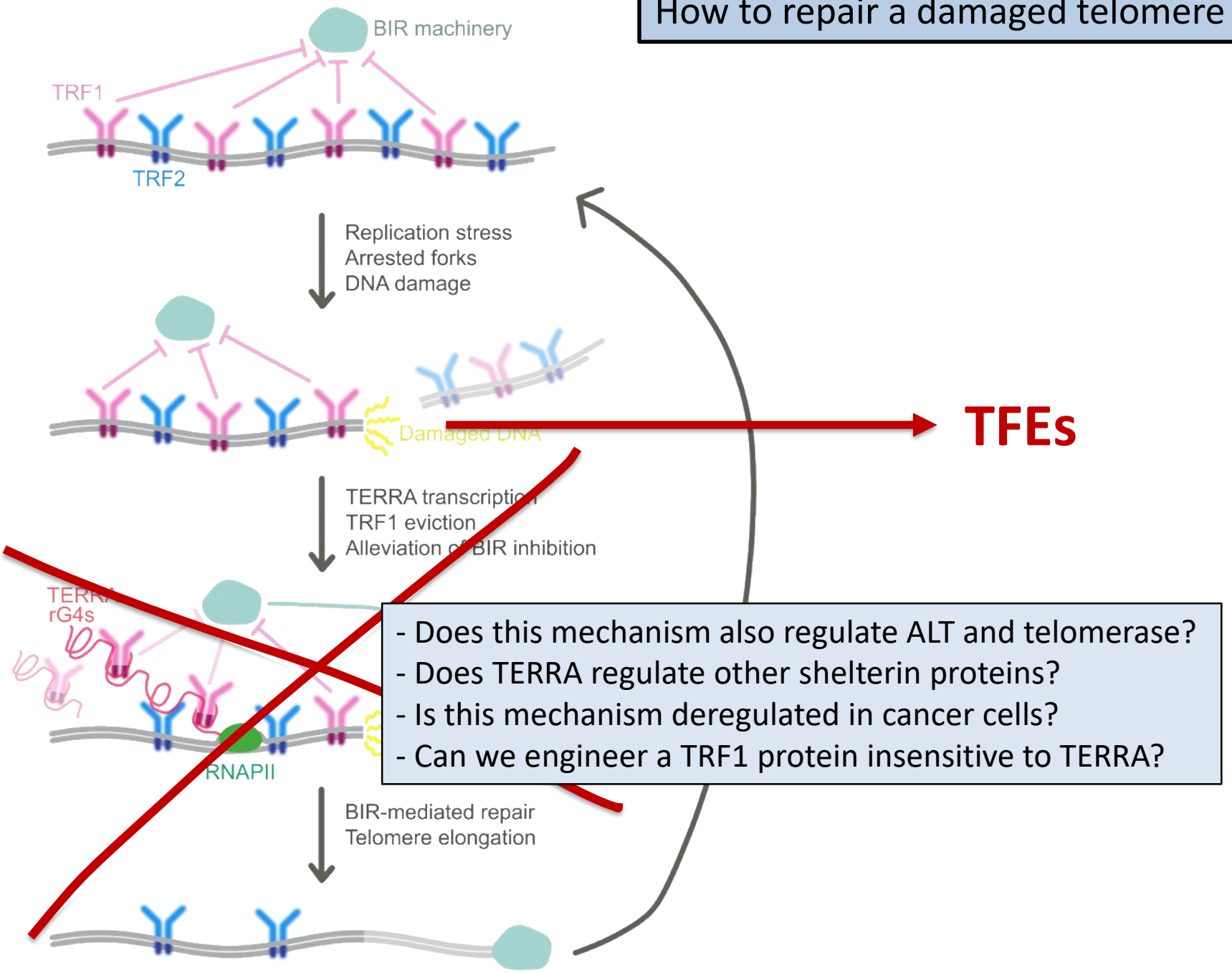


TERRA and TRF1 suppress TFE accumulation and BIR activation in APH-treated cells





How to repair a damaged telomere



- Does this mechanism also regulate ALT and telomerase?
- Does TERRA regulate other shelterin proteins?
- Is this mechanism deregulated in cancer cells?
- Can we engineer a TRF1 protein insensitive to TERRA?

How to quantify TERRA

The ALT mechanism and ALT vulner

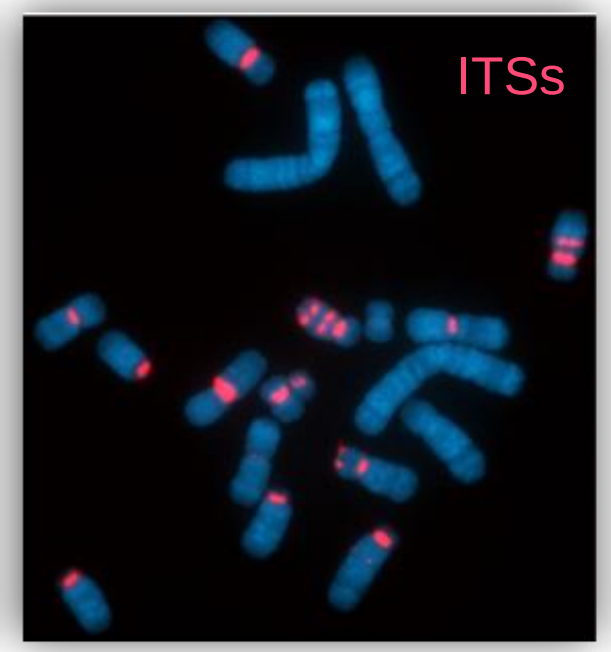
TERRA and shelterin

Beatriz

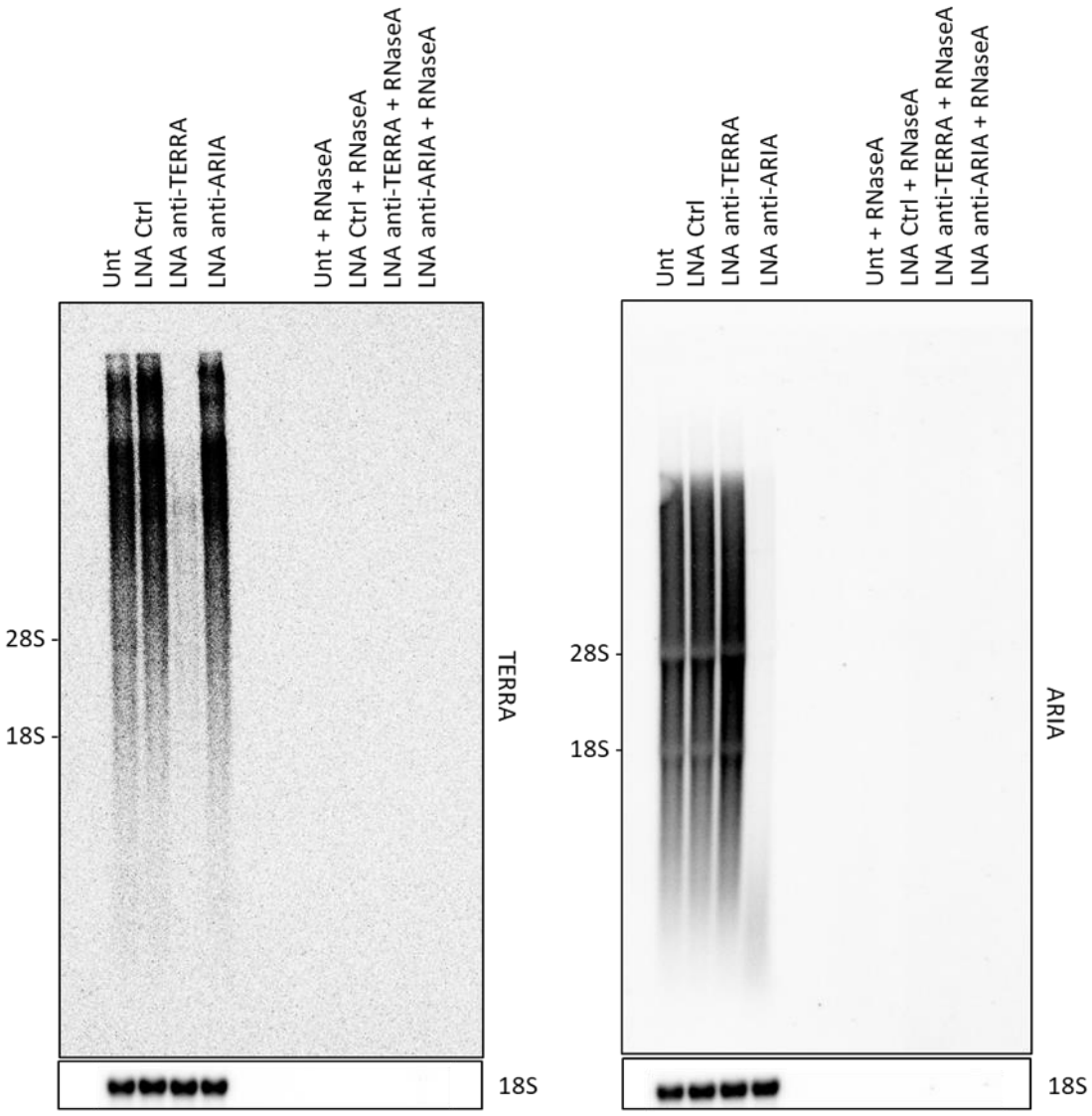
TERRA far from telomeres

TERRA in an organism

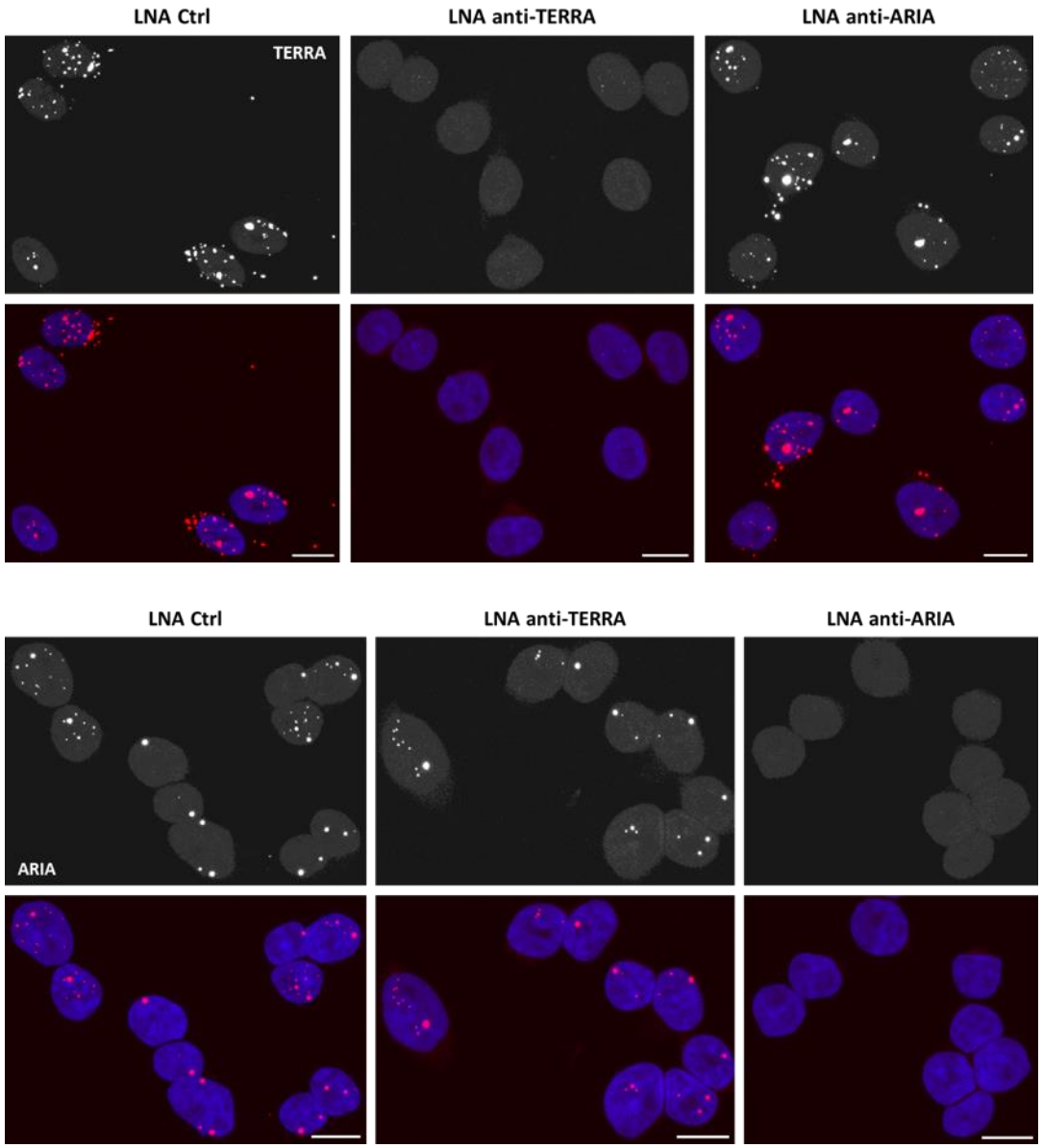
Chinese hamster - Intrachromosomal Telomeric Sequences



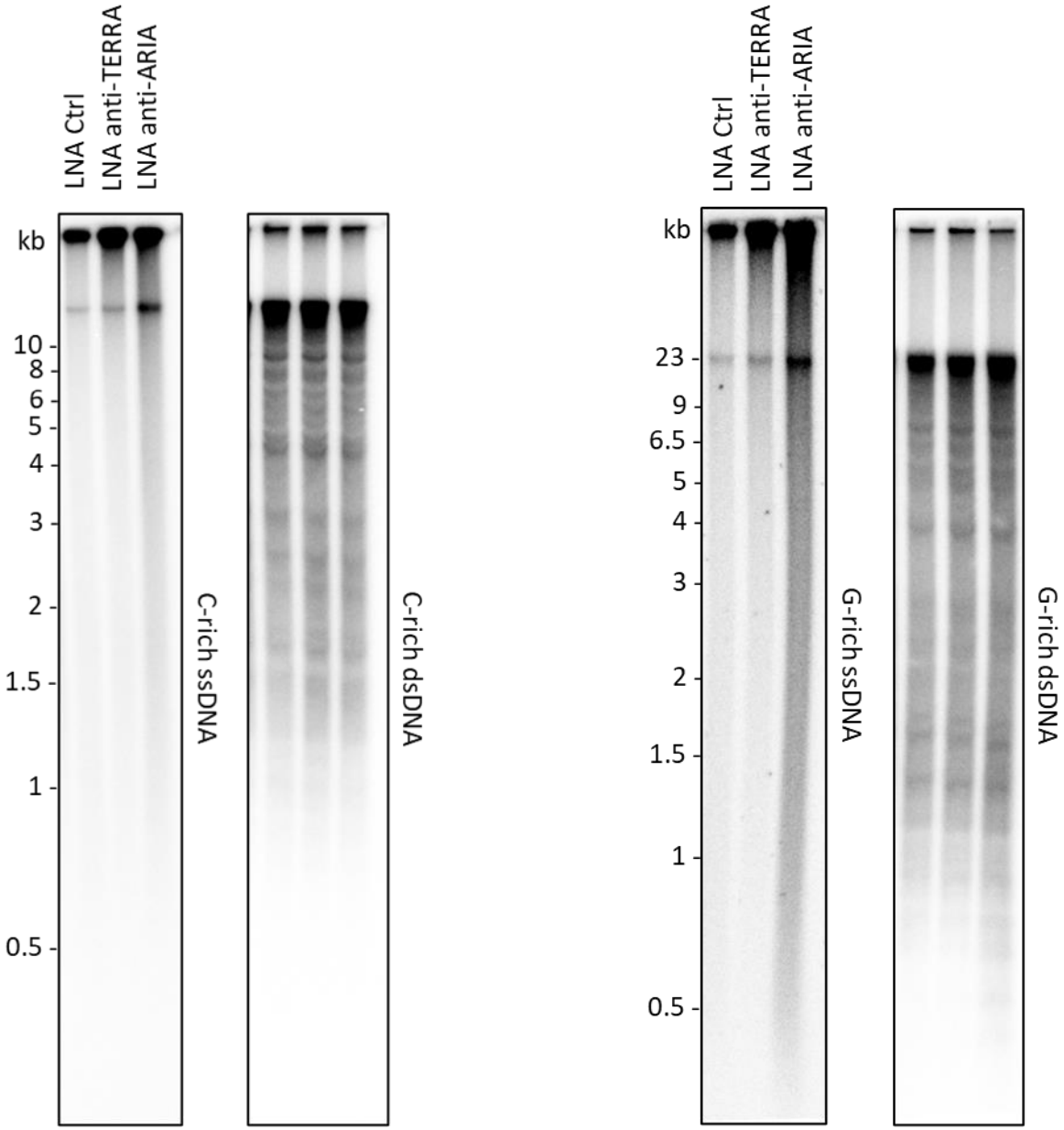
CHO cells contain elevated levels of TERRA and its complementary RNA (ARIA)



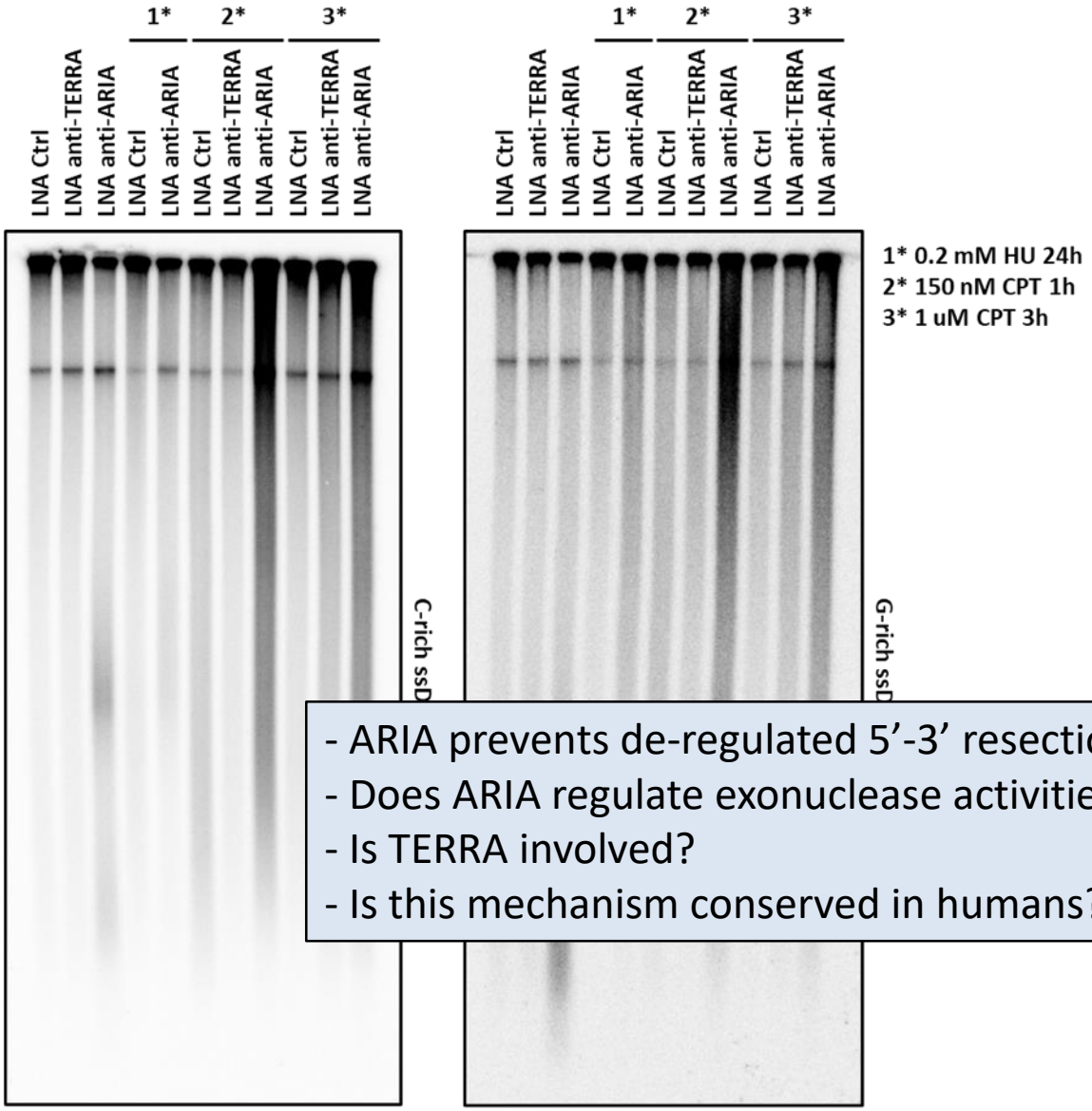
TERRA is nuclear and cytoplasmic while ARIA is mostly nuclear



ARIA suppresses the accumulation of telomeric ssDNA



ARIA suppresses telomeric ssDNA accumulation upon DSB induction



- ARIA prevents de-regulated 5’-3’ resection at DSBs and telomeres
- Does ARIA regulate exonuclease activities?
- Is TERRA involved?
- Is this mechanism conserved in humans?

How to quantify TERRA

The ALT mechanism and ALT vulnerabilities

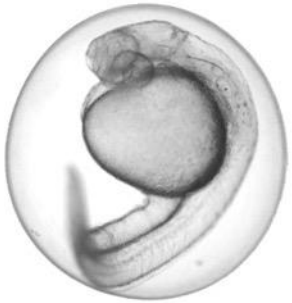
TERRA and shelterin

TERRA far from telomeres

Marta

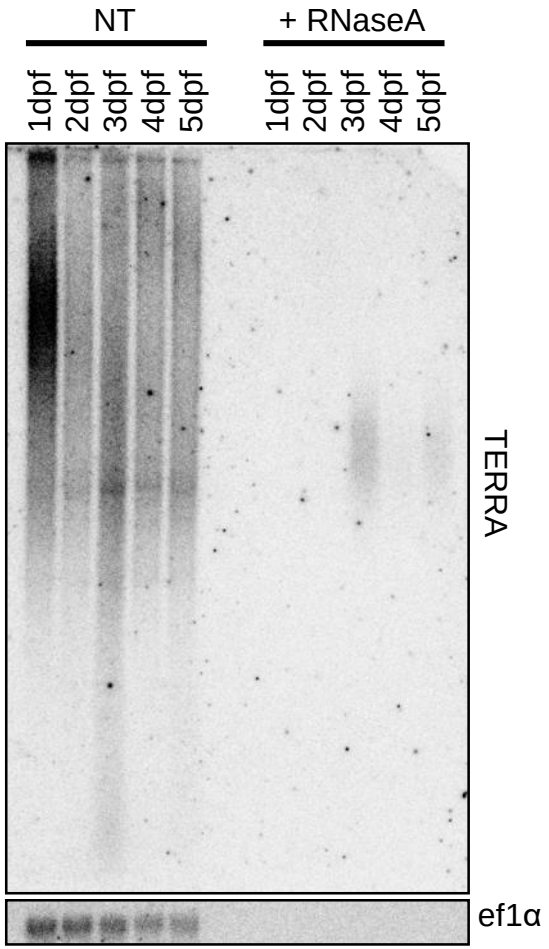
TERRA in an organism

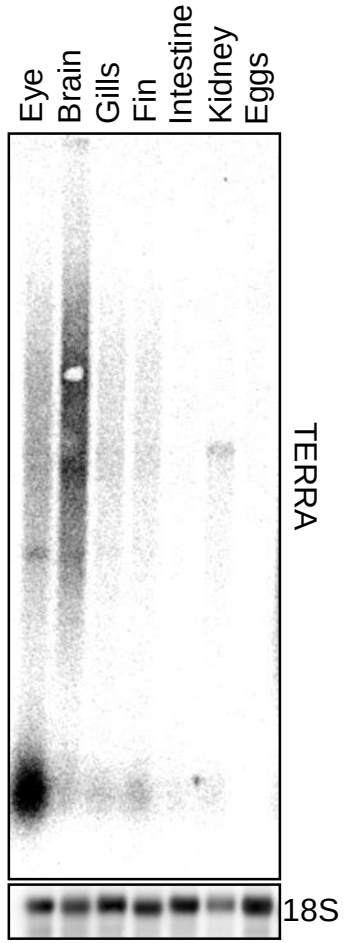
Zebrafish – a vertebrate with human-like telomeres



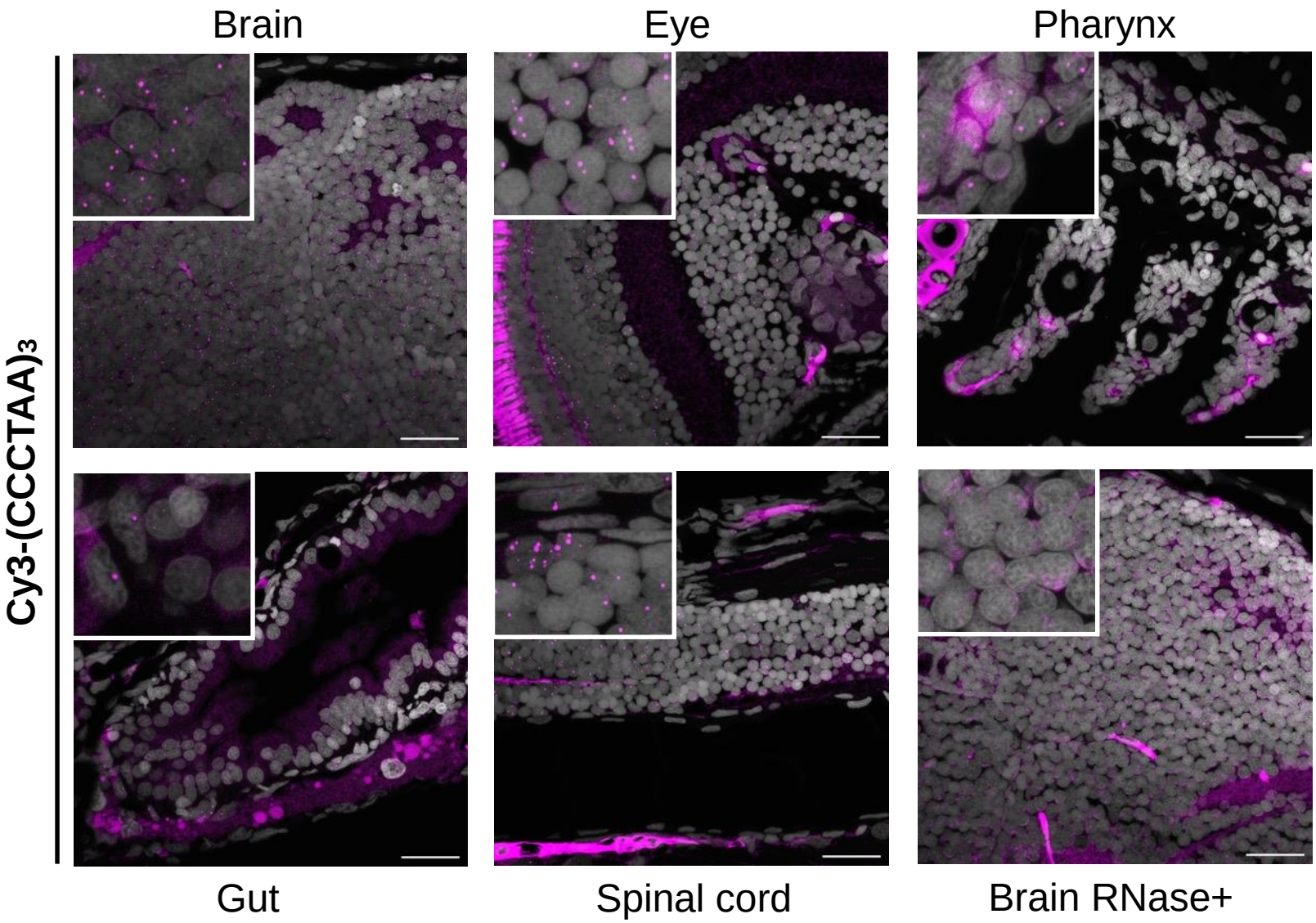
- Characterized development
- Sequenced genome
- Heterogeneous telomeres of human-like length
- Telomeres shorten with age
- Telomerase activity decreases with age
- Telomerase mutants show premature aging already in G1

Zf TERRA is developmentally and ubiquitously expressed

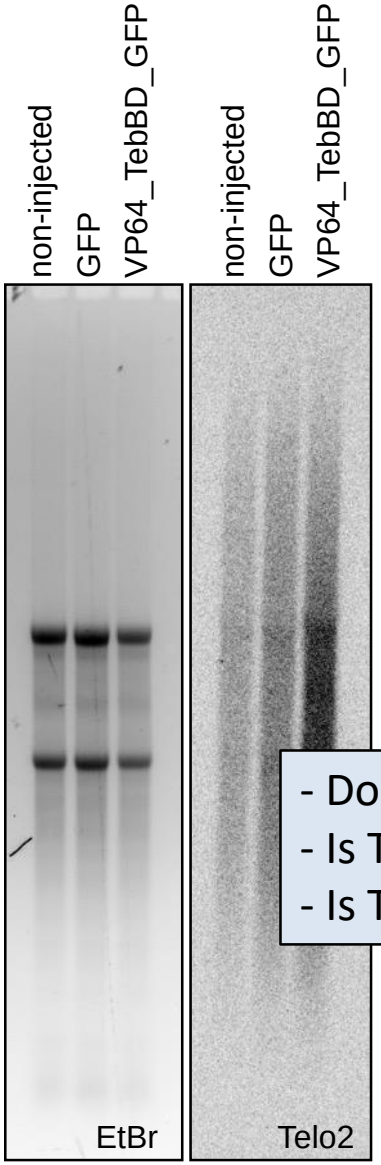


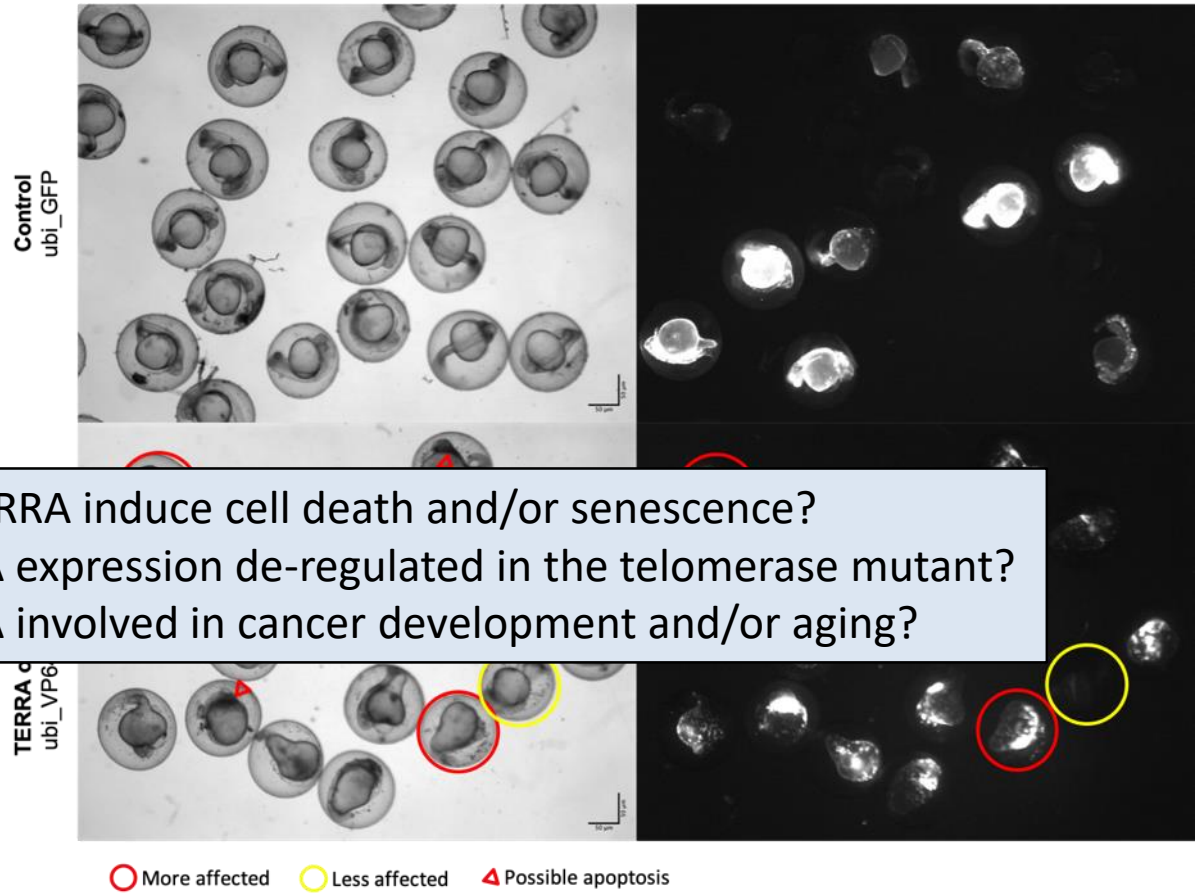


TERRA foci are elevated in the nervous system



TERRA manipulation in Zf larvae





- Does TERRA induce cell death and/or senescence?
- Is TERRA expression de-regulated in the telomerase mutant?
- Is TERRA involved in cancer development and/or aging?

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FCT Fundação para a Ciência e a Tecnologia

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Search for novel regulators of ALT activity and cell viability


Bruno


Sara


Beatriz


Lee


Patricia


Daniela

TERRA and shelterin functional interactions


Yong Woo


Patricia


Valentina