# Studying telomere transcription and its role in senescence

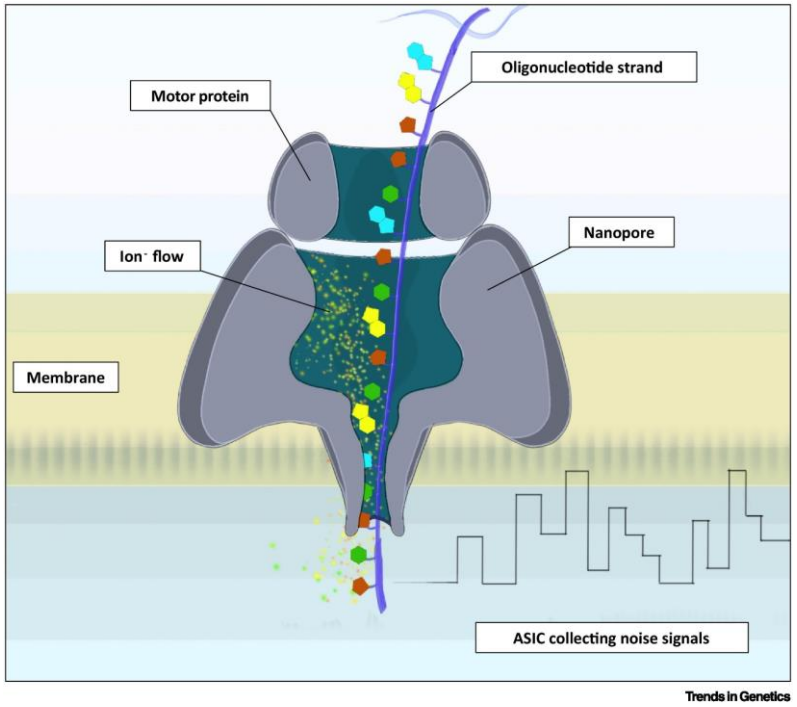
Gulbenkian Institute *for* Molecular Medicine

25 Nov, 2024

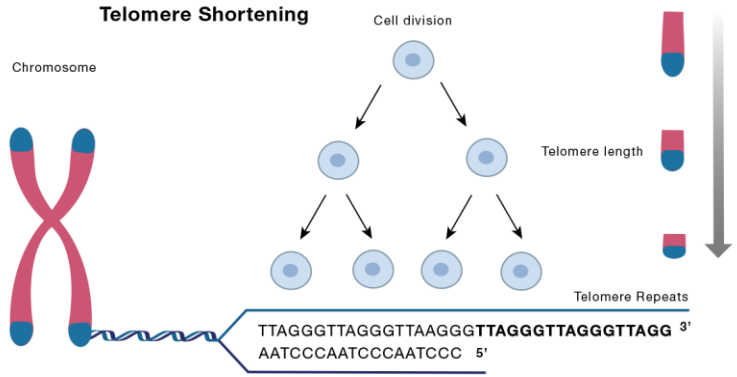
Joana Rodrigues, CMAzzalin Lab

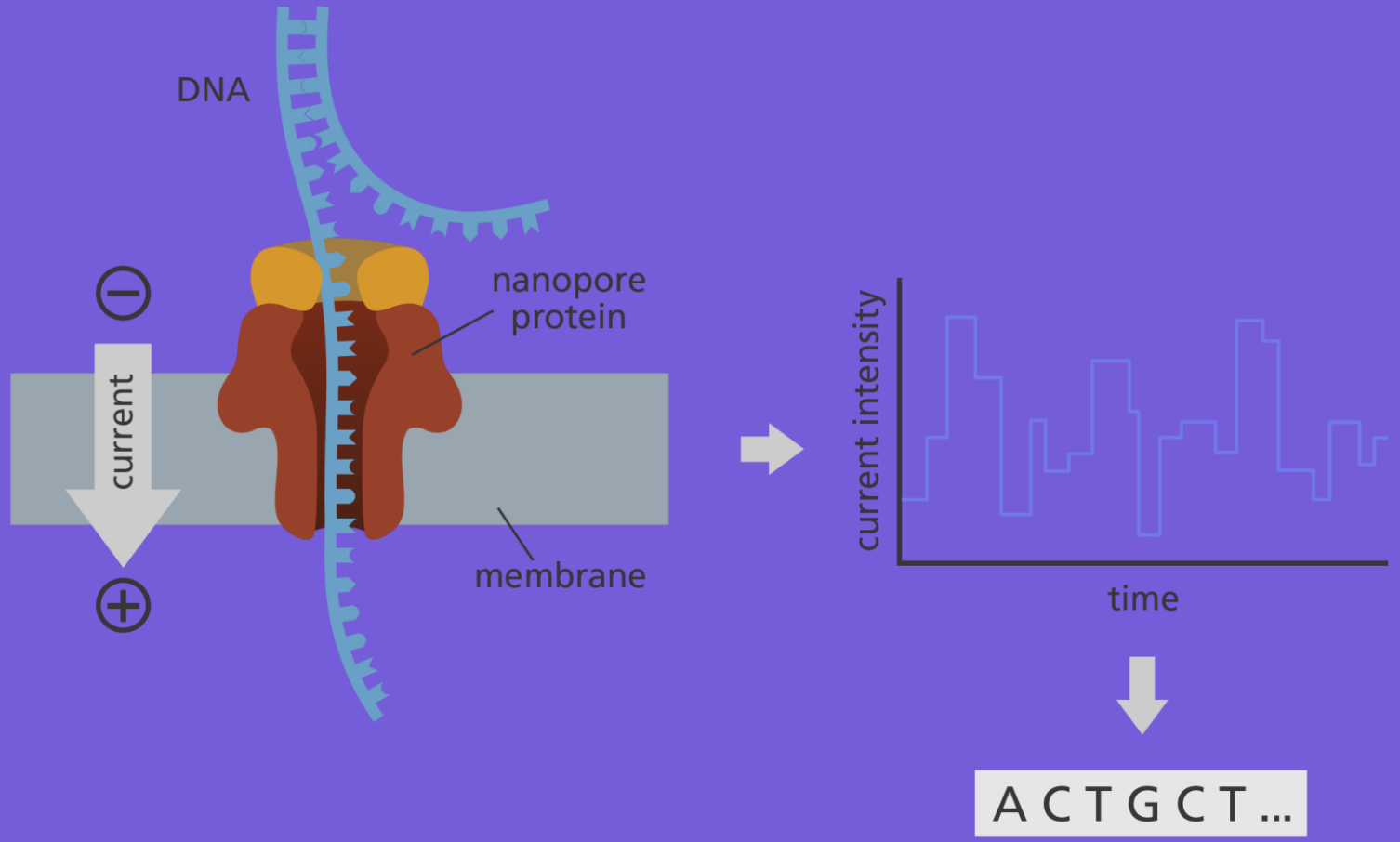
# Studying telomere transcription and its role in senescence

TERRA quantification using nanopore sequencing.



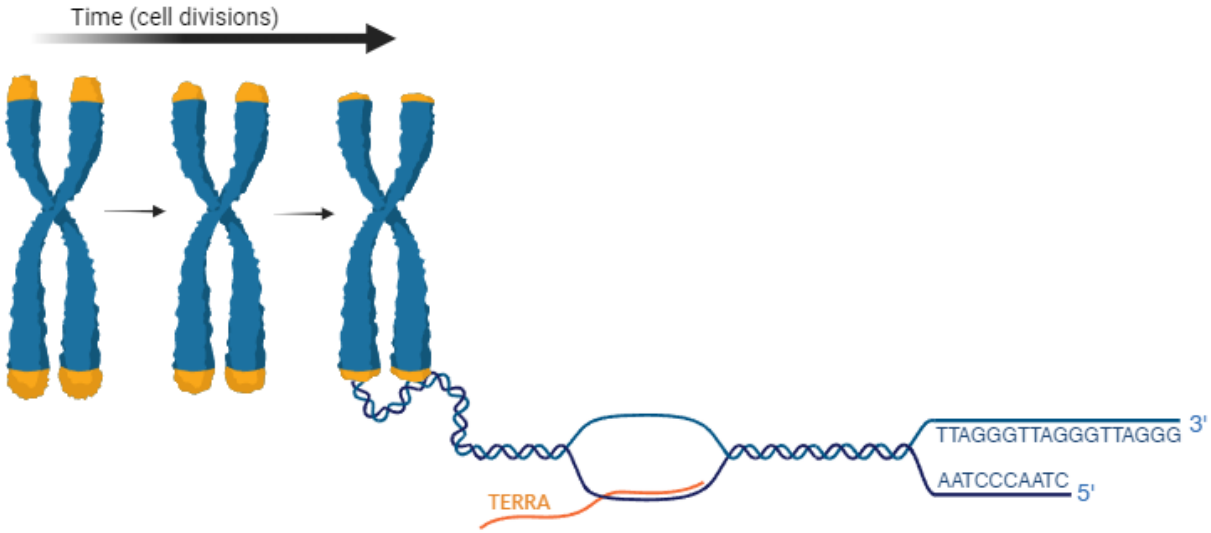

Study the role of TERRA transcription in replicative senescence.






# TERRA quantification using nanopore sequencing.

# Telomere

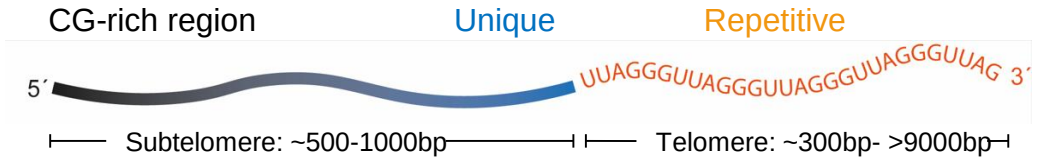



Nucleoprotein structure at the end of linear chromosomes.

Shorten with every cell division.

Transcribed into the telomeric repeat-containing RNA **TERRA.**

# TERRA




- Telomere elongation
- Telomeric heterochromatin establishment
- Replicative senescence
- DNA damage response

Transcribed by RNA pol II, from most telomeres.

29 bp repeat CpG-rich promoters indentified in about half the subtelometeres.

Transcribed at low levels.

- Transcription varies between different chromosome ends and cell types.

Levels increase in response to stress:

- DNA damage
- Telomere shortening
- DNA demethylation

**TABLE 1.** Average number of TERRA molecules per cell

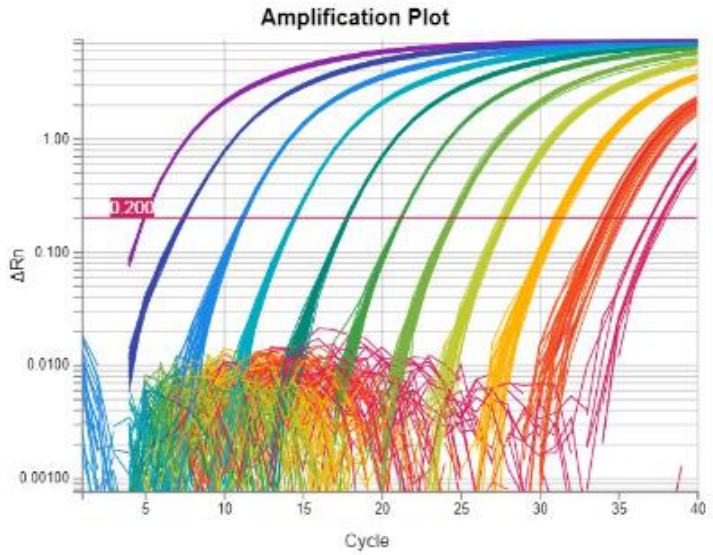
| Cell line | 10q | 15q | 20q |
|---|---|---|---|
| U2OS | 164 ± 41 | 63 ± 9 | 4 ± 1 |
| HLF | 0.8 ± 0.1 | 8 ± 1 | 1 ± 1 |
| HeLa | 2 ± 1 | 5 ± 1 | 1 ± 1 |
| HCT116 WT | 2 ± 1 | 22 ± 4 | 8 ± 2 |
| HCT116 DKO | 954 ± 65 | 298 ± 12 | 4 ± 1 |

Feretzaki M, Renck Nunes P, Lingner J. *RNA*. 2019

# TERRA quantification

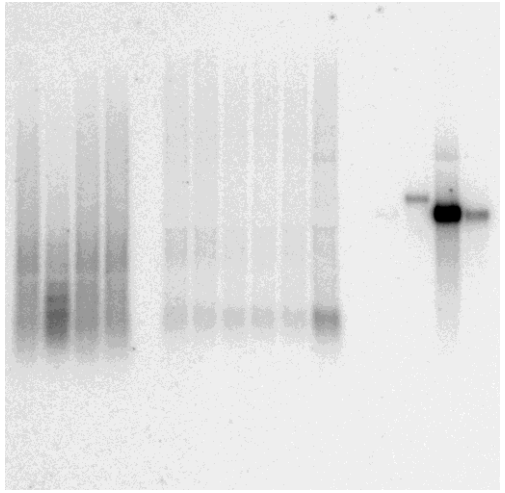

**RT-qPCR**

- Only analyses one telomere at a time.
- Difficult to get specific primers.




**Northern blot**

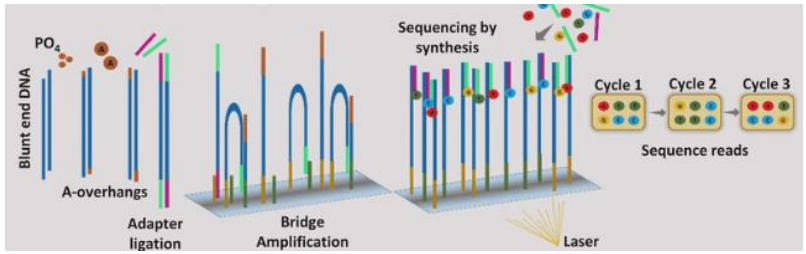
- Analyses all telomeres simultaneously.



# TERRA quantification

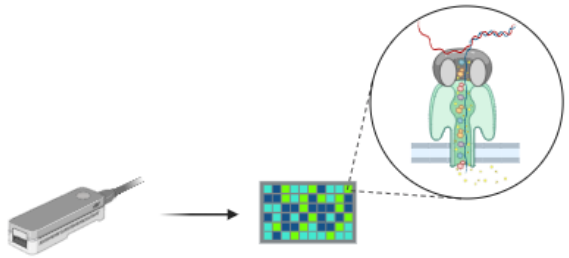

**Illumina sequencing**

- Difficult to unequivocally assign small reads.

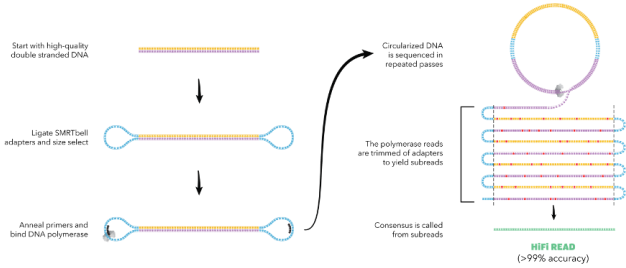



**Long read sequencing**

- Analyses all telomeres simultaneously.
- Most likely to correctly assign reads.

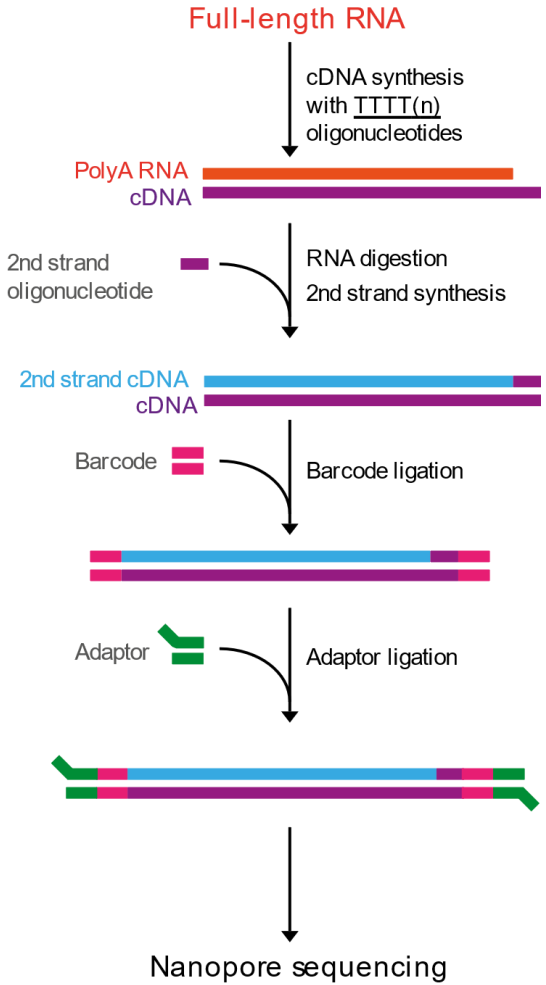


Ex. Nanopore



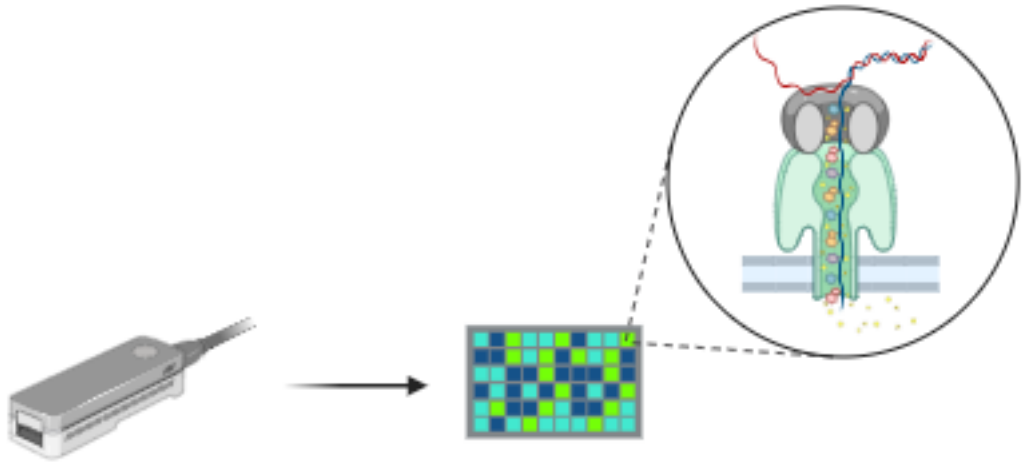

Ex. PacBio

# Nanopore sequencing: direct cDNA sequencing

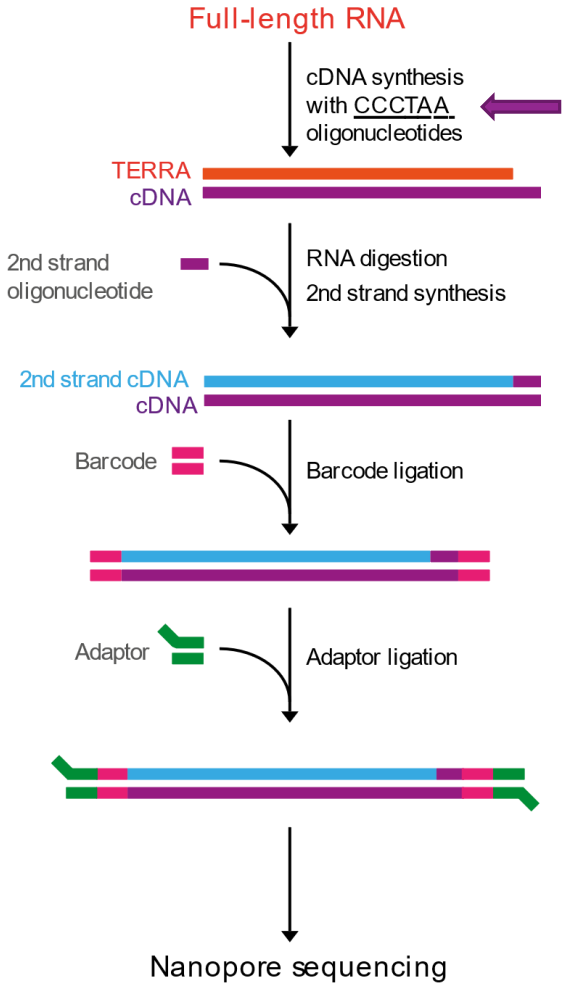




TERRA is mostly non-polyadenylated.



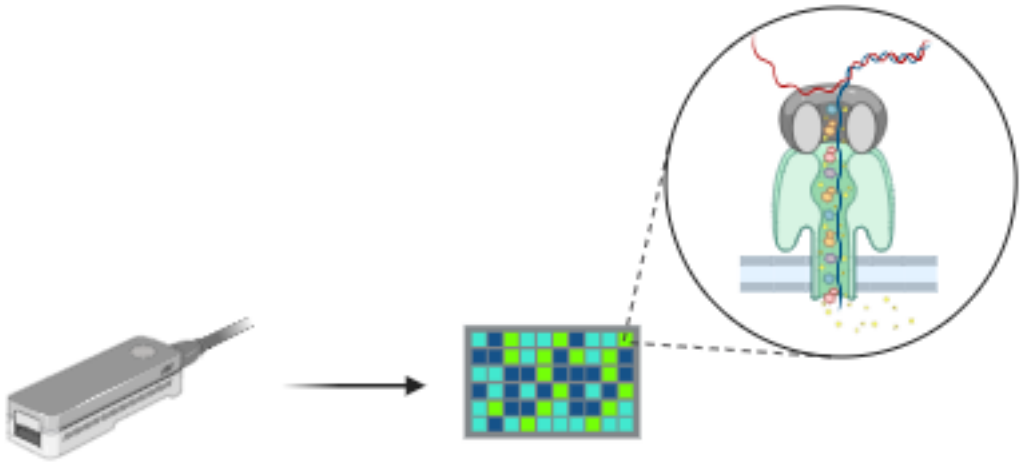
# Nanopore sequencing: direct cDNA sequencing



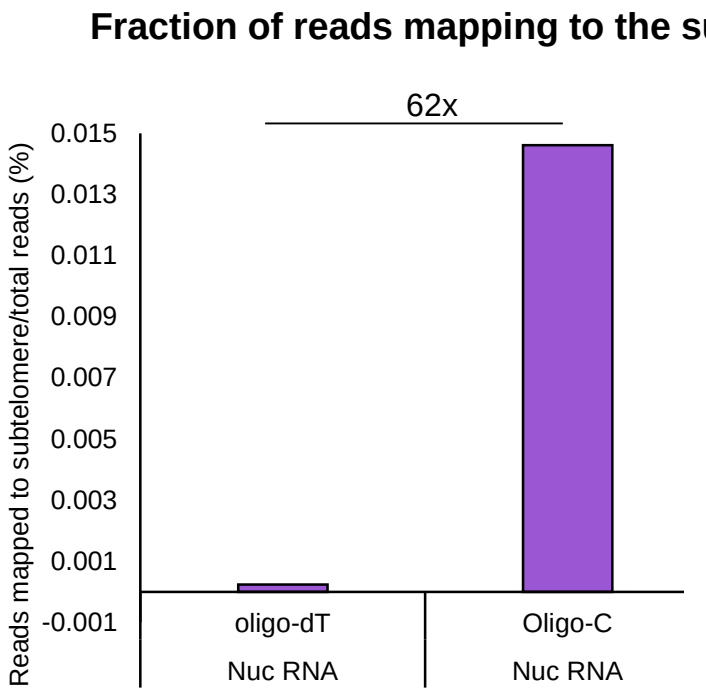


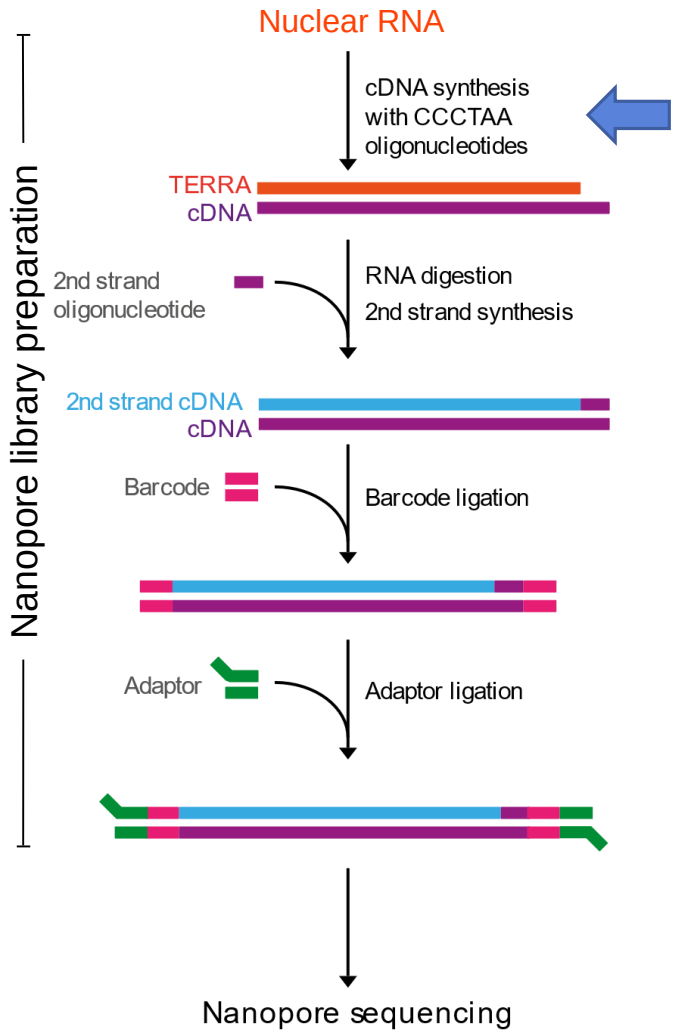
TERRA is mostly non-polyadenylated.

Replace the oligo dT by and oligo complementary to the telomeric repeats (CCCTAA(5)).



# Nanopore sequencing: direct cDNA sequencing



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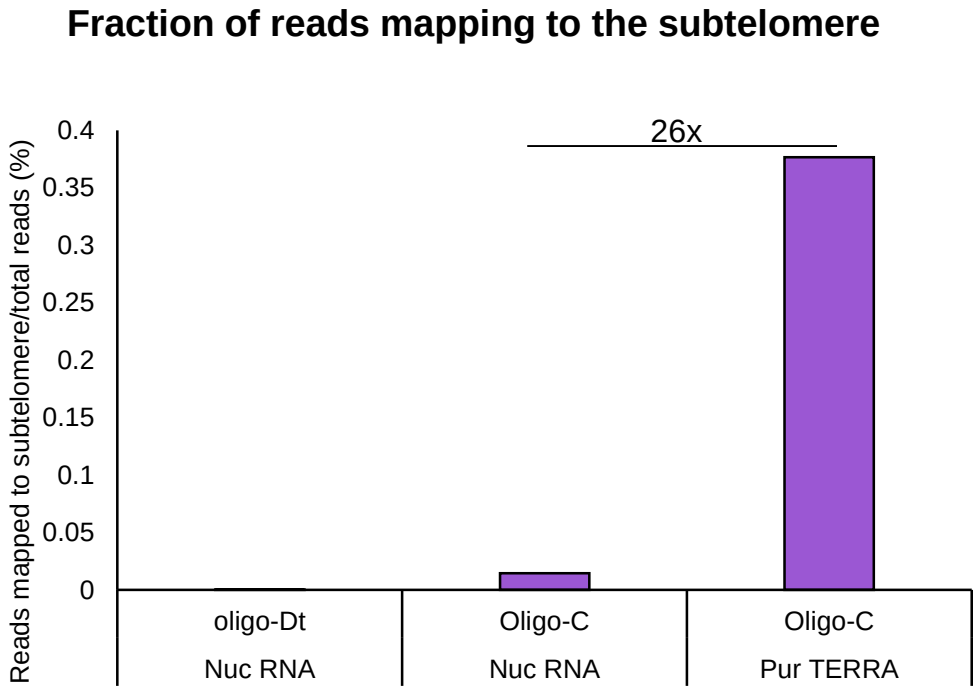
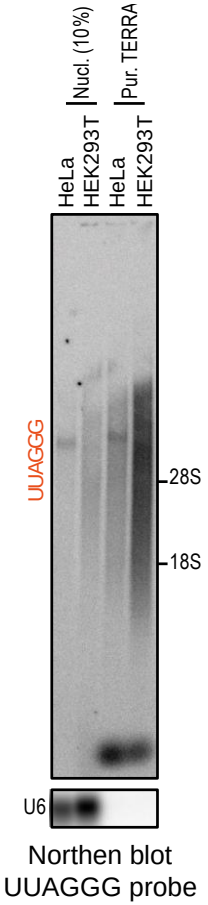
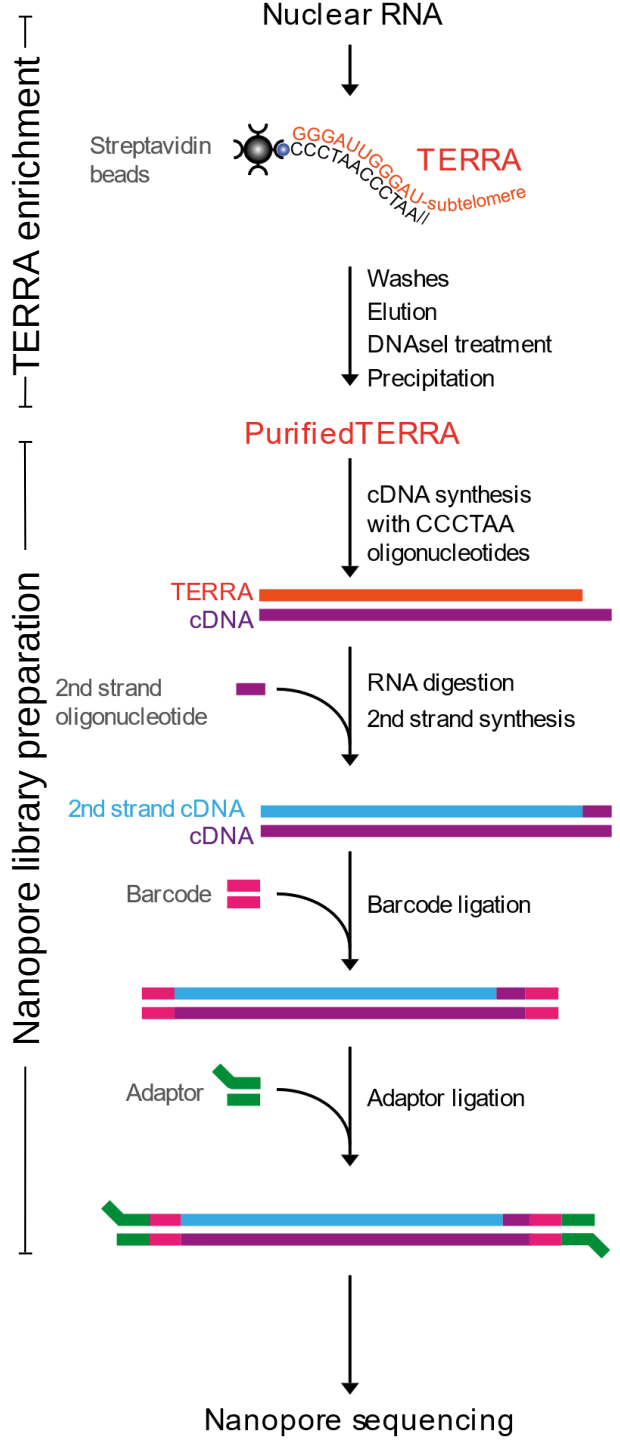
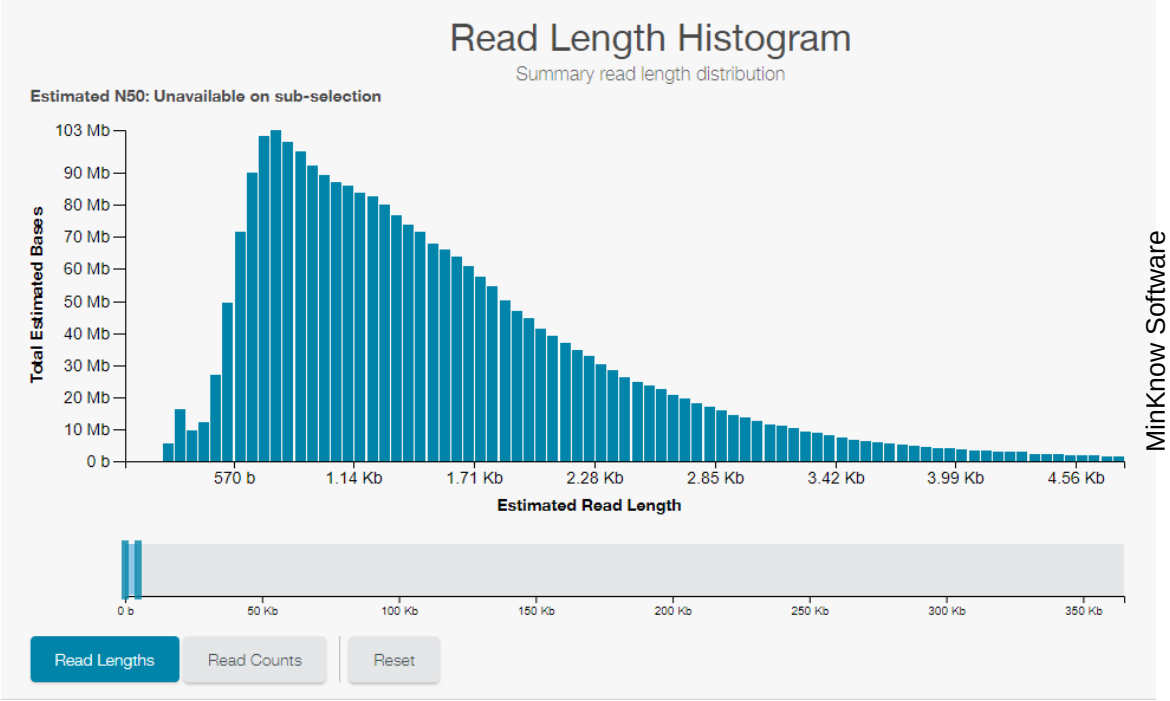



Northen blot
UUAGGG probe



# TERRA ONTseq

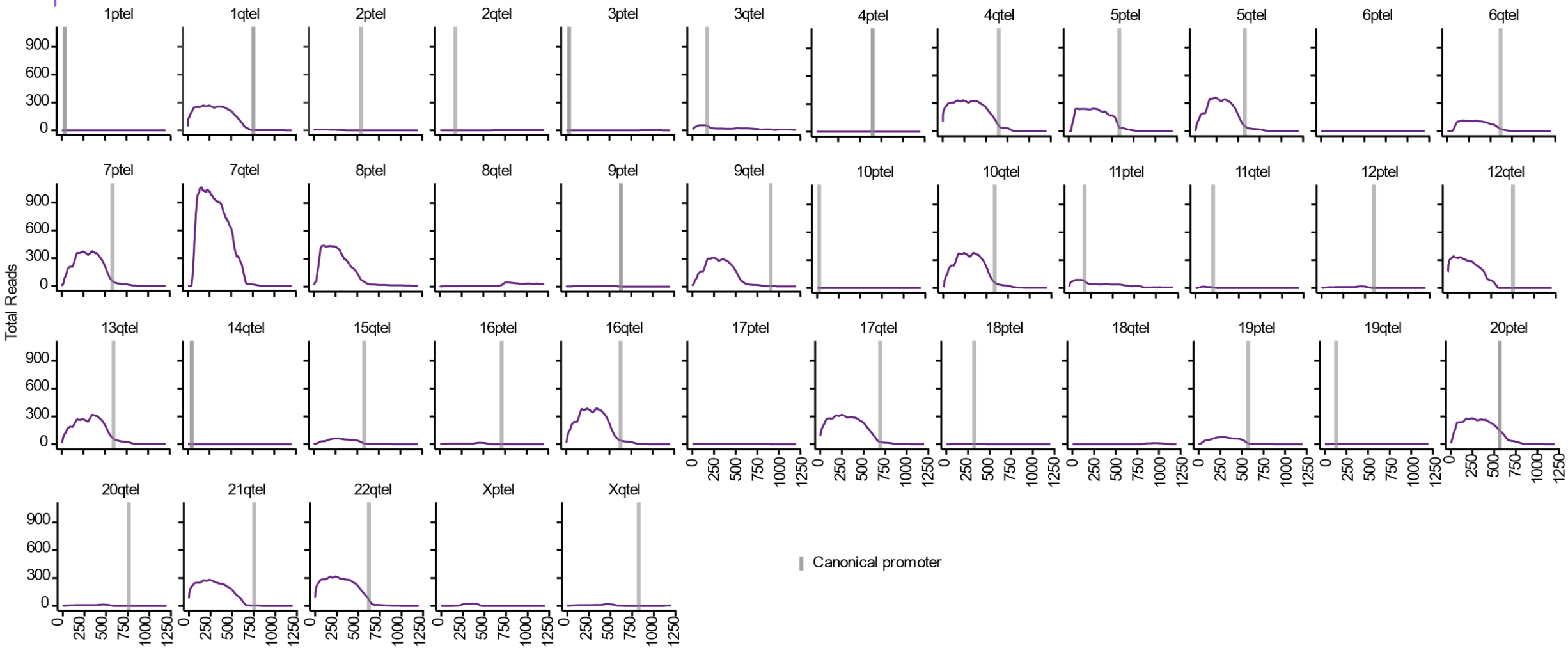



Selected reads that had a minimum of 3xTTAGGG repeats at the 3´.

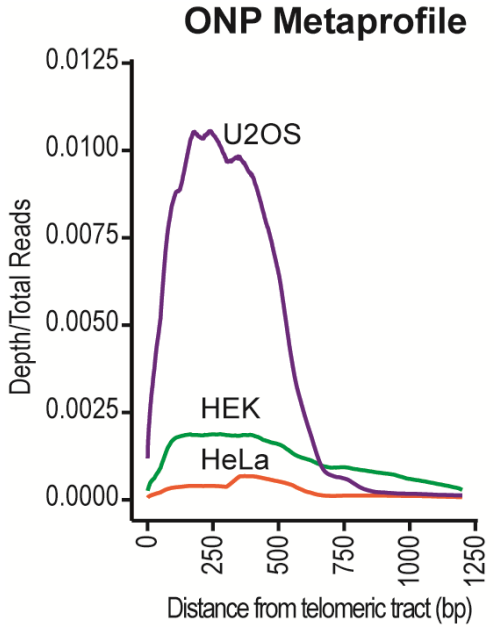
- Subtelomeric read lengths varied from 167 and 1394 bp with an average length of 842 bases.

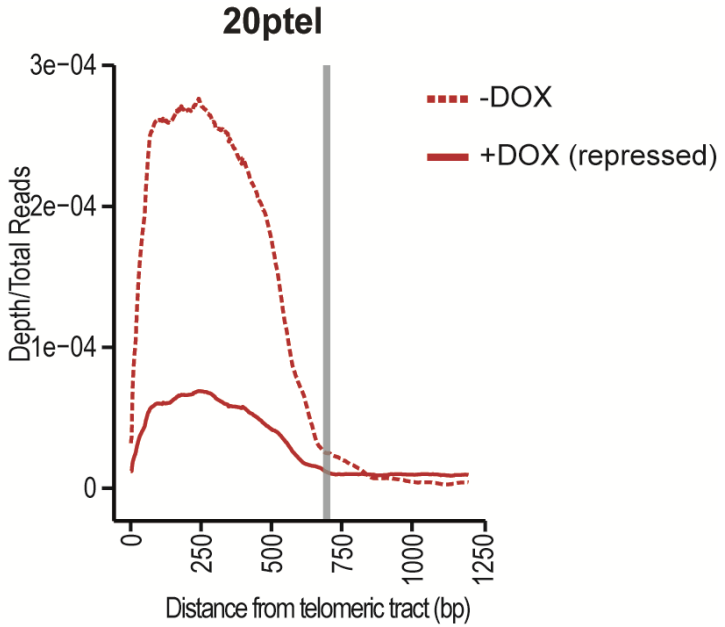
Mapped those reads to the most telomere-proximal 1250 bp (T2T genome assembly).

# TERRA ONTseq



# TERRA ONTseq is quantitative

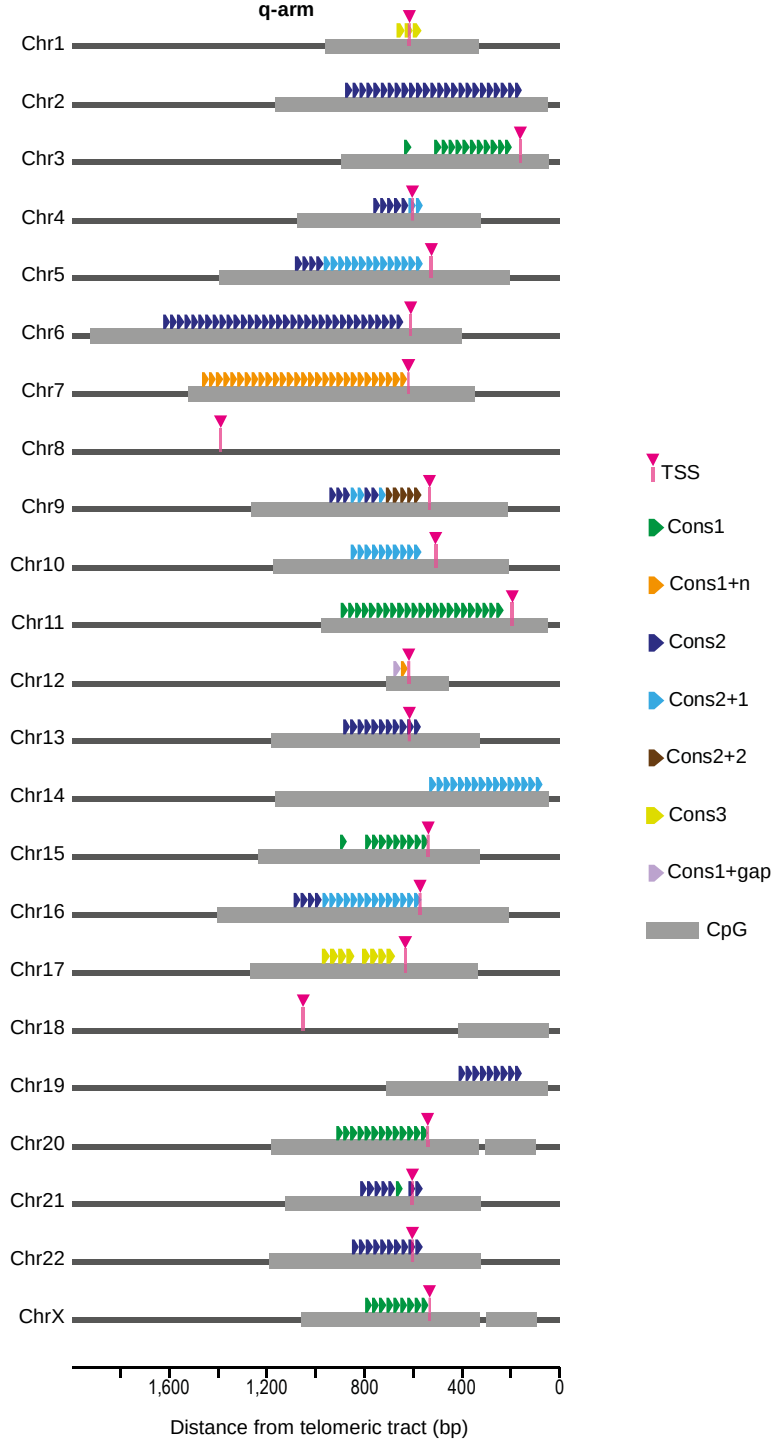


# TERRA ONTseq identifies TERRA TSS

TERRA ONTseq allowed the mapping of TERRA transcription start sites:

- Within CpG islands.
- Mostly dowstream of tandem repeats of the 29bp-repeat promoters.

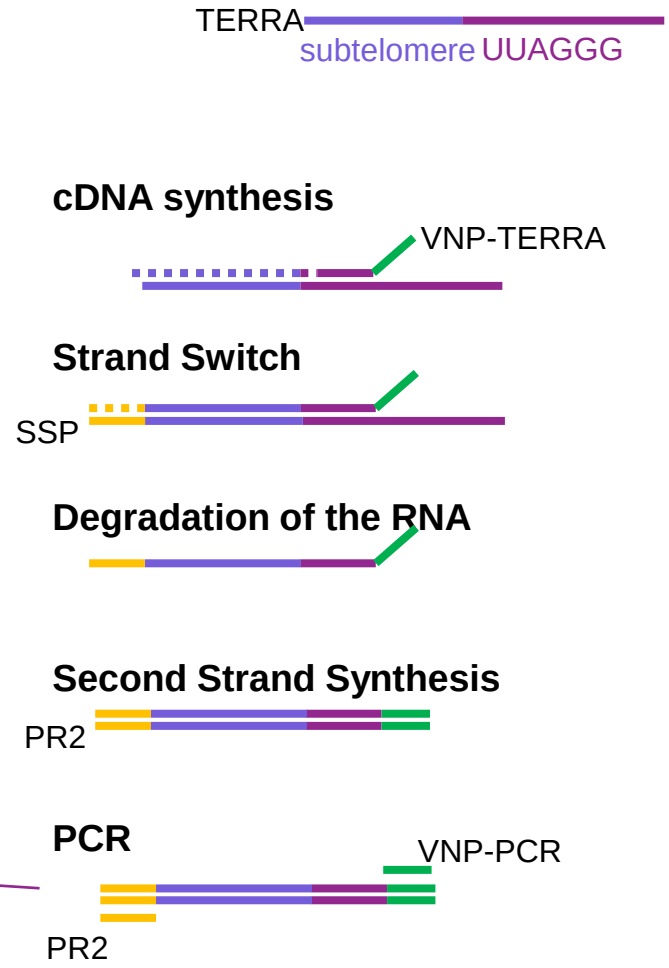


# Introducing PCR

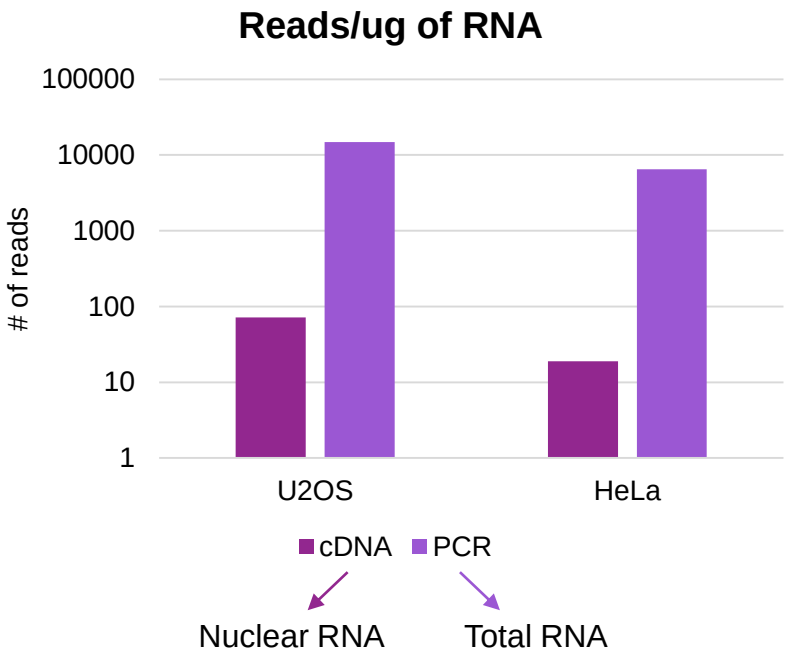
*For U2OS, a total of 225 µgs of nuclear RNA was used for 2 sequencing runs; for HeLa, 727 µgs of nuclear RNA was used for 6 sequencing runs (3 of which barcoded); for HEK293T 1 mg of nuclear RNA was used for 7 sequencing runs (4 of which barcoded).*

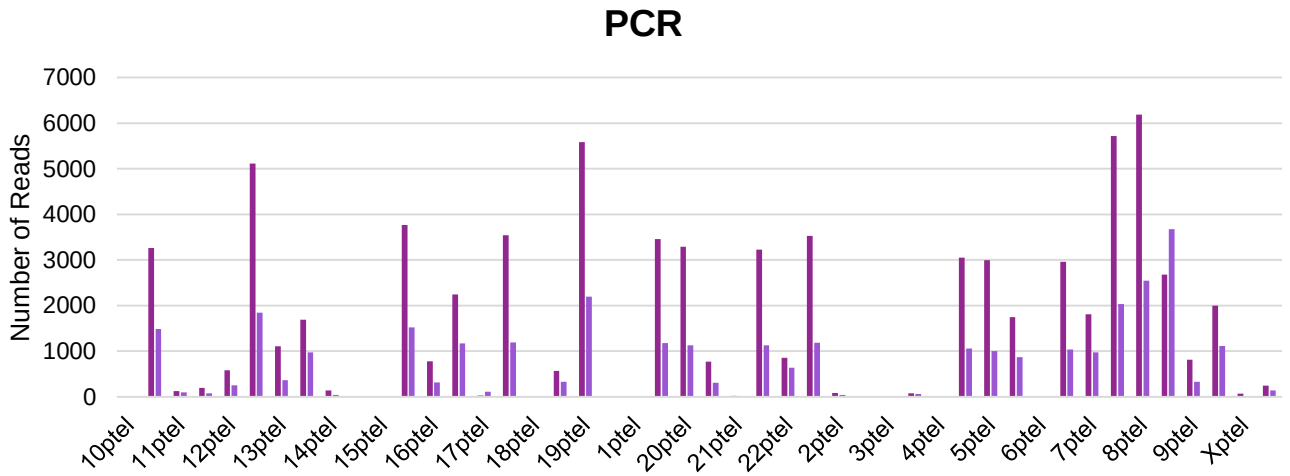
**The subtelomeric sequences of TERRA have very comparable sizes.**

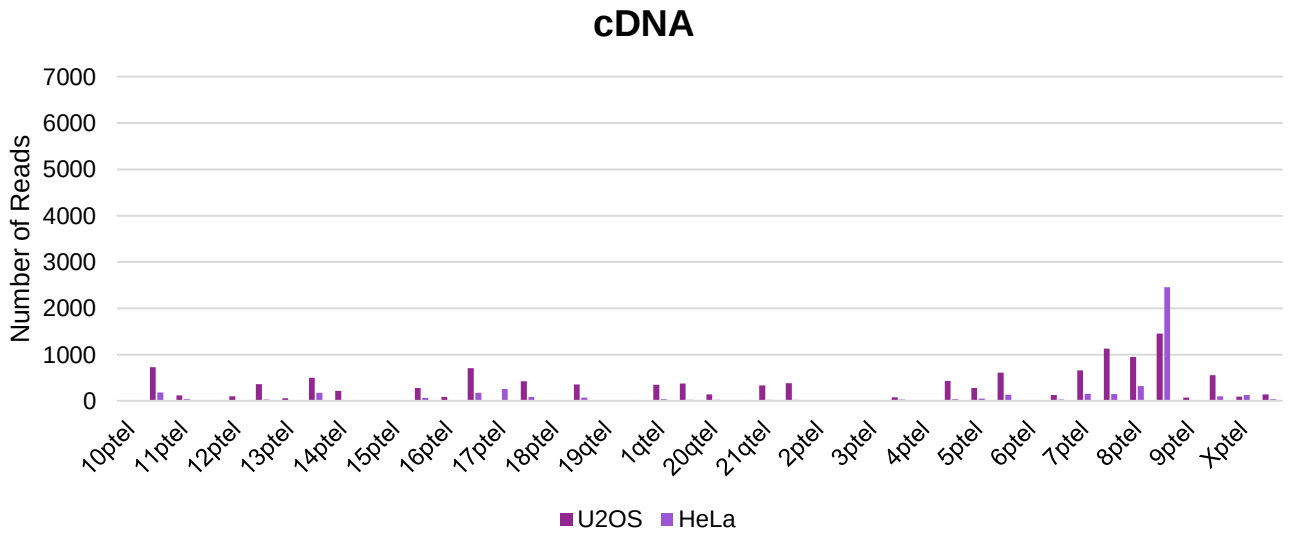
- Repair,
- dA tailing,
- Ligation to barcodes,
- Ligation to adaptors,
- Sequencing.



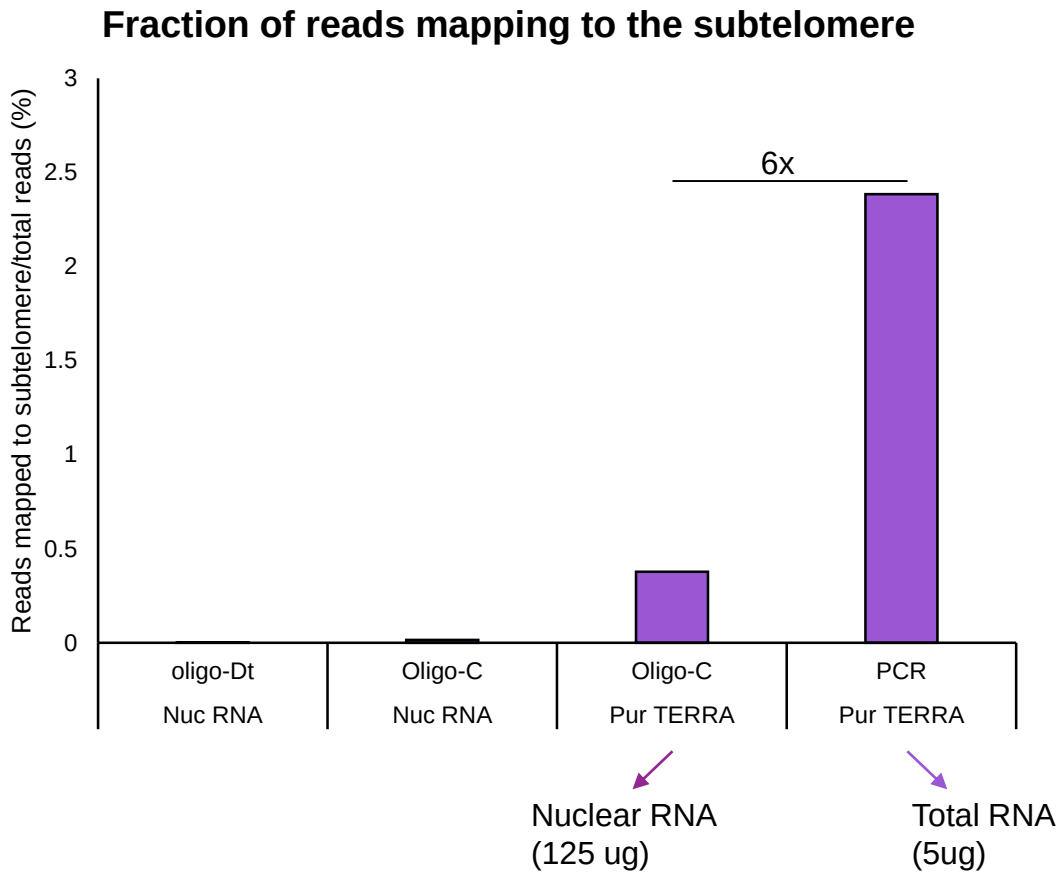
# PCR greatly increases read yield, while maintaining quantitativeness



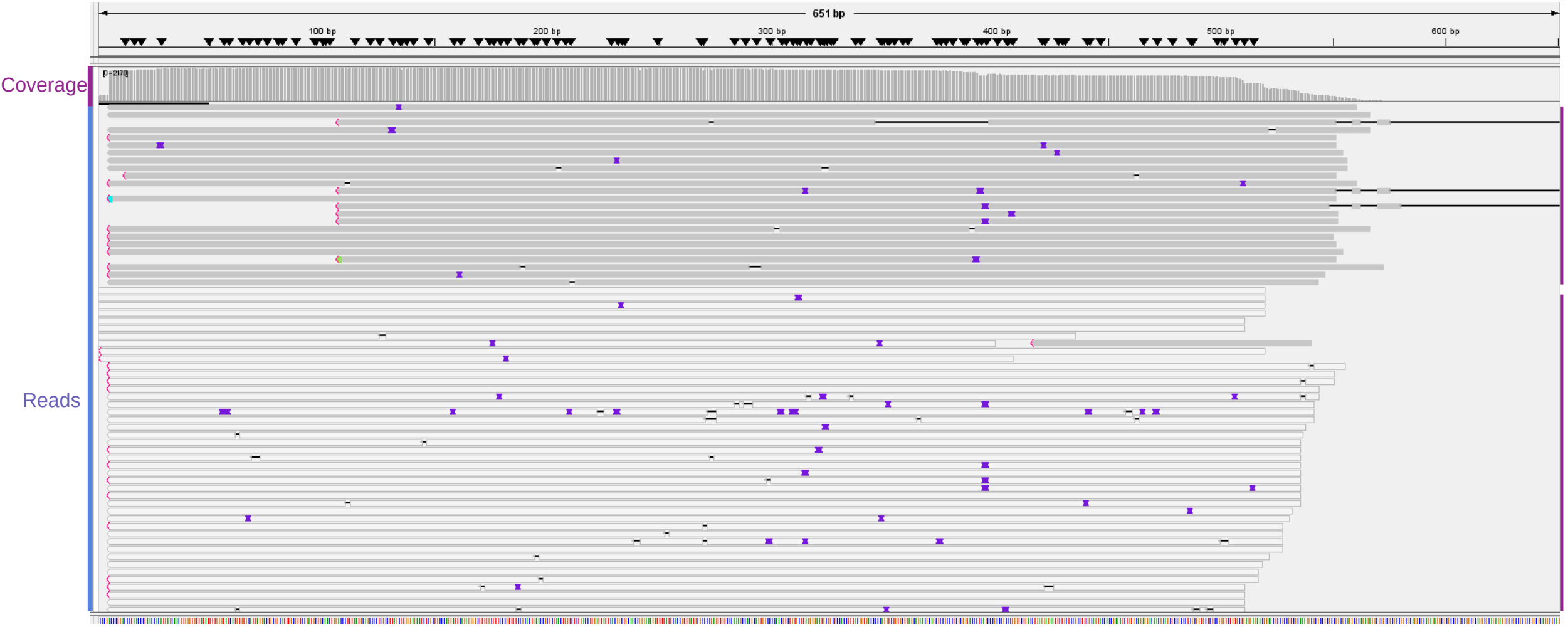



# PCR yields more reads from less material



DKO: 7q

# Reads map across the entire subelomere




← Telomere

Centromere →

# Conclusions

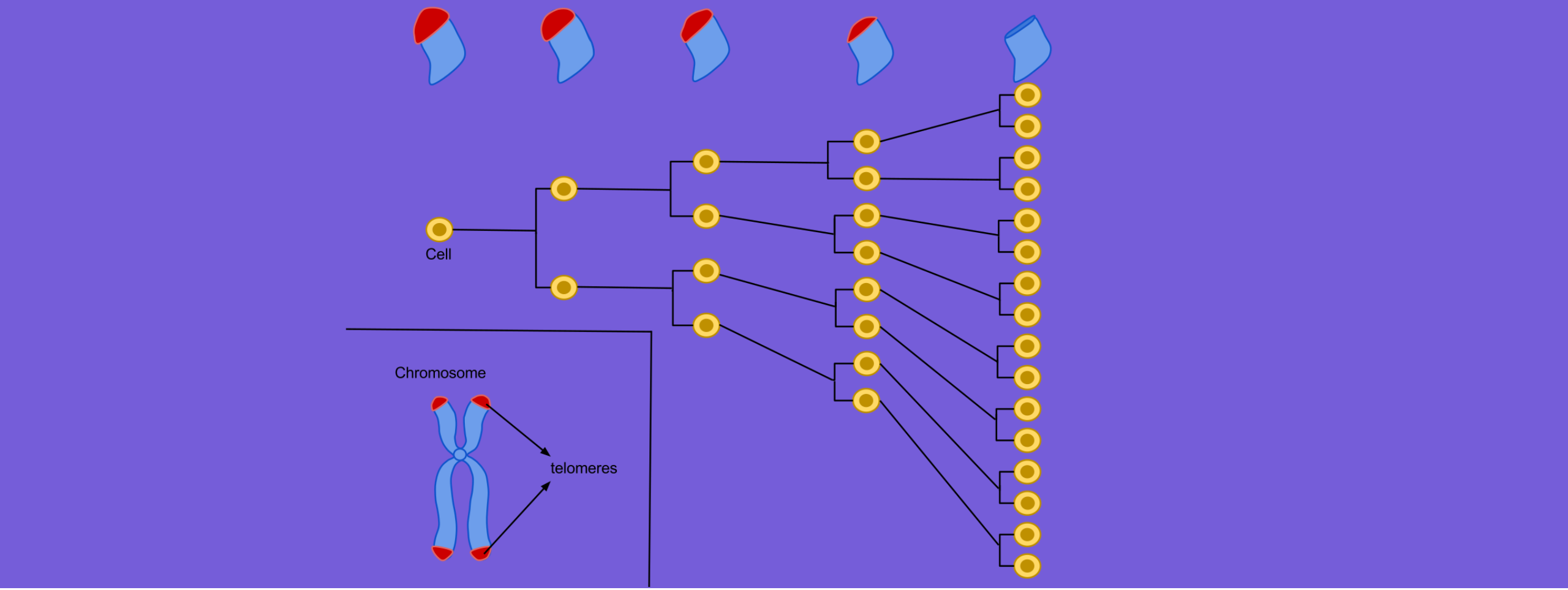
TERRA ONTseq is a reliable method to qualitatively and quantitatively analyse the telomeric transcriptome.

- Potential to become a tool for the rapid and precise analysis of TERRA in different laboratorial and clinical settings.

TERRA ONTseq allowed the mapping of most TERRA TSS.

The most transcribed chromosome ends are consistent between cell lines, suggesting the existence of conserved regulatory elements in different cell types.

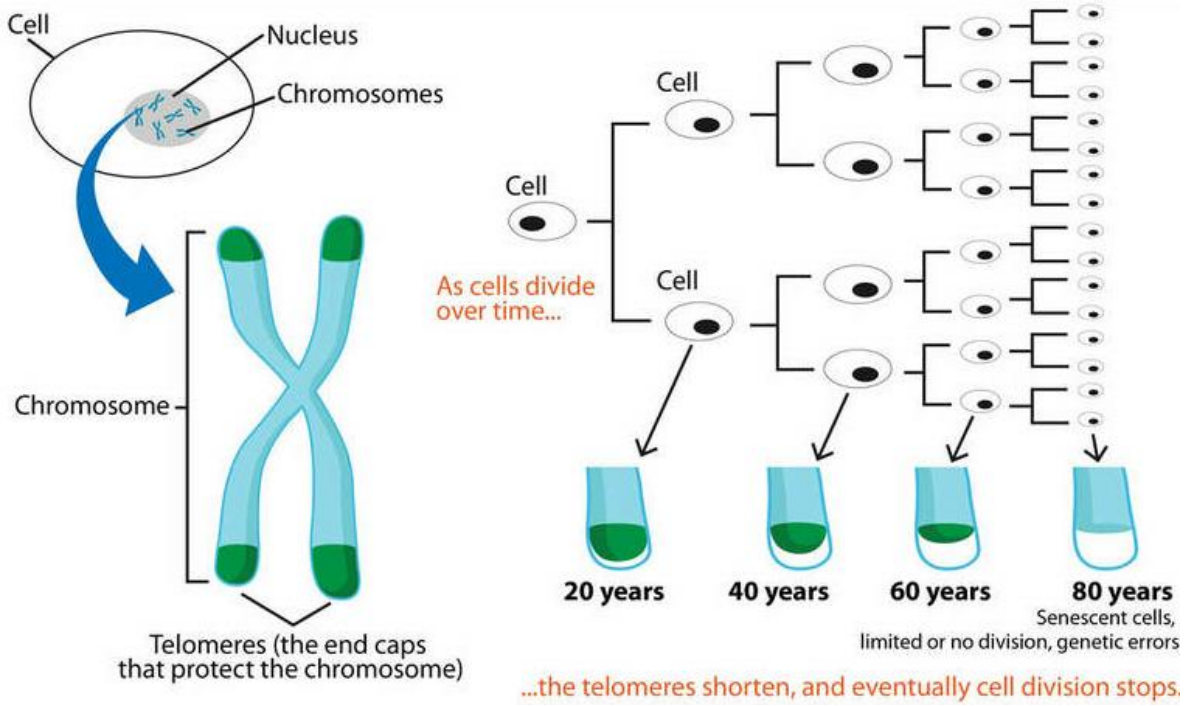
**Introduction of PCR to the pipeline substantially decreased the amount of starting material needed.**



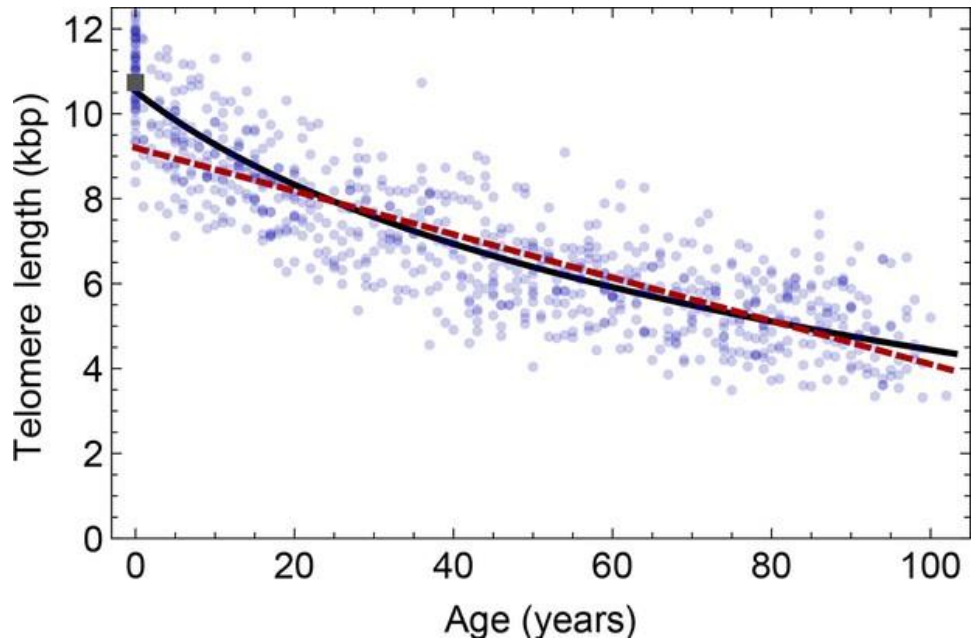

# Study of TERRA transcription in replicative senescence.

# Telomere length and aging

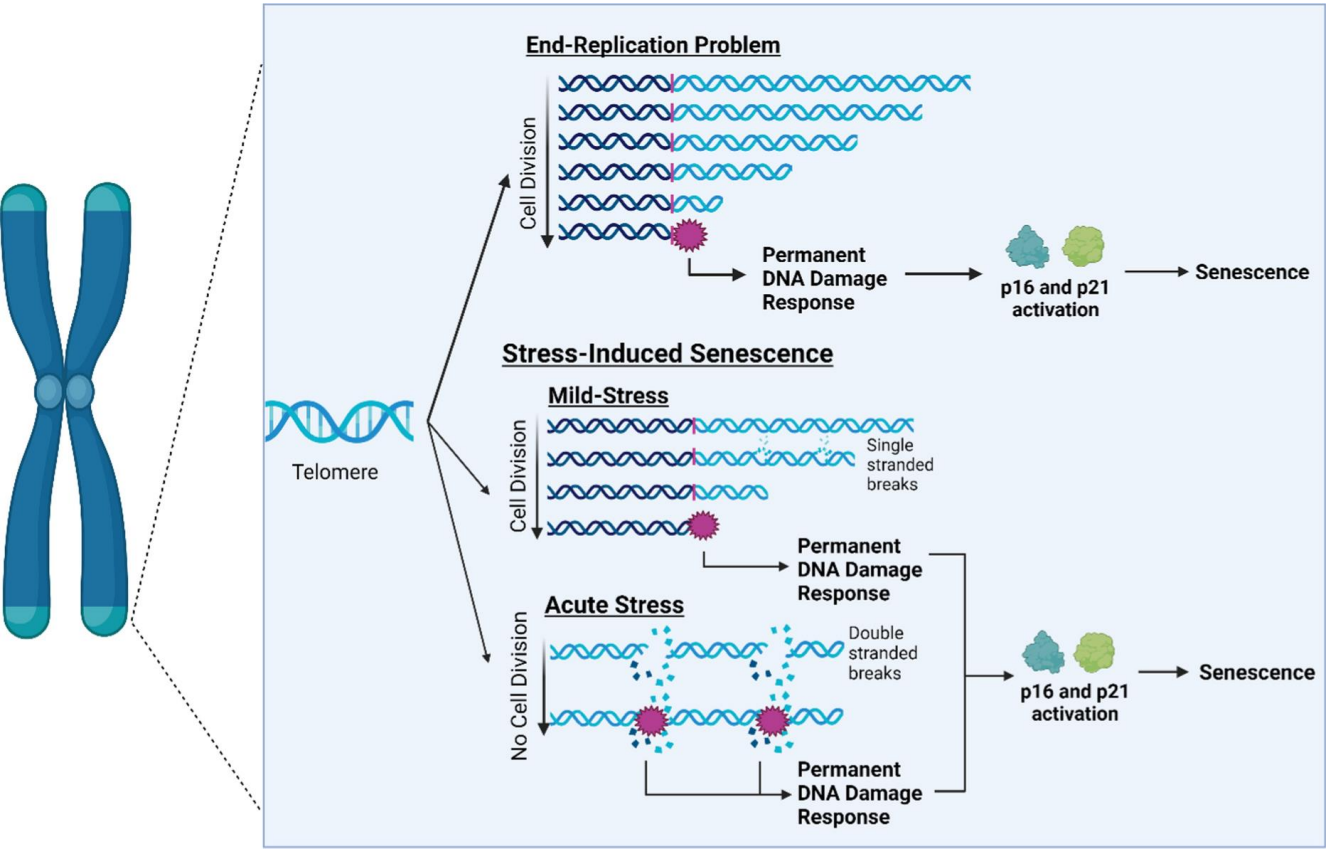
In the absence of telomerase, telomeres get short with every cell division.




Flow-FISH in lymphocytes



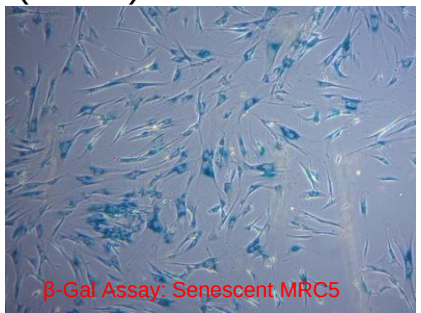
# Replicative senescence



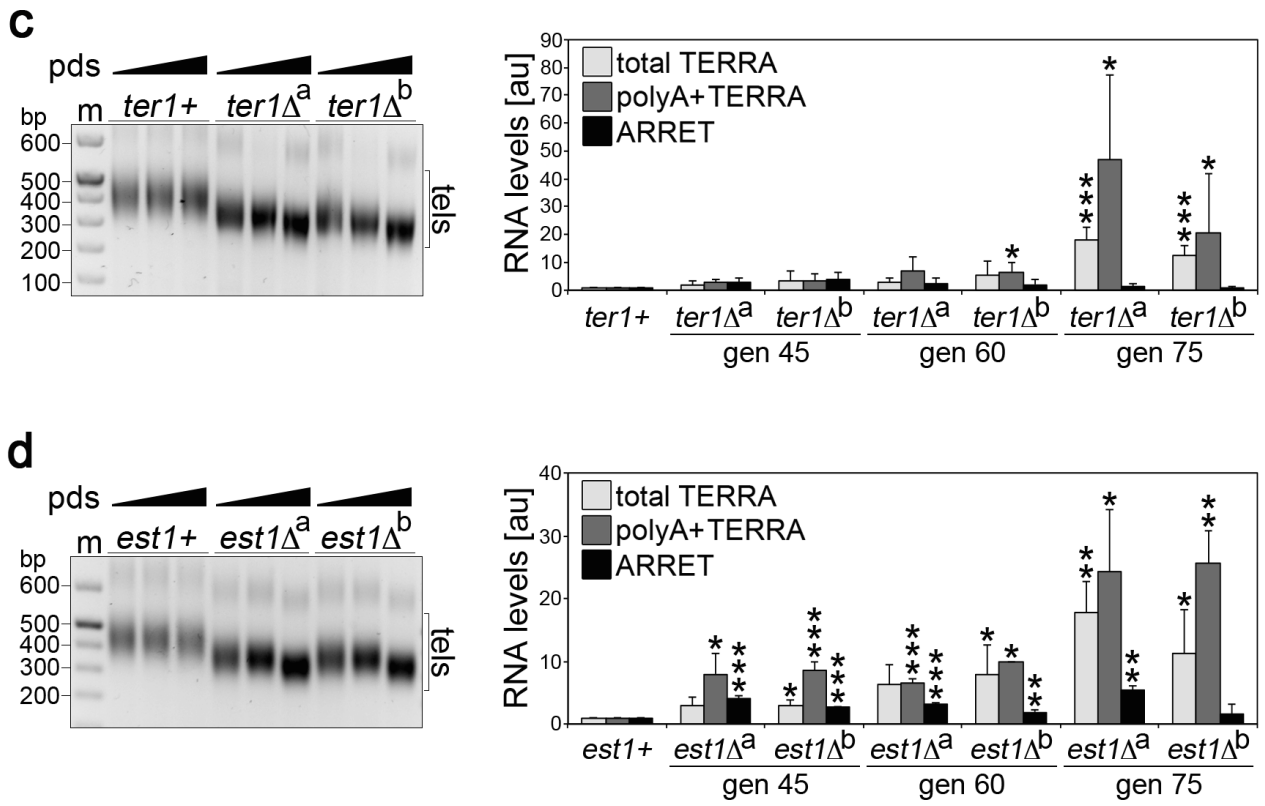

- Telomeric lesions can remain unrepaired for long periods of time.
- Senescent cells “want” to stay senescent:
  - Induced ROS production, leading to DNA lesions.

Senescence markers:

- **Growth arrest**
- **Senescence-associated beta-galactosidase**
- Senescence-associated heterochromatin foci (SAHF)
- Senescence-associated secretory phenotype (SASP)
- **Enlarged and often irregular-shape nuclei**
- **High p16 and/or p21**
- Increased ROS



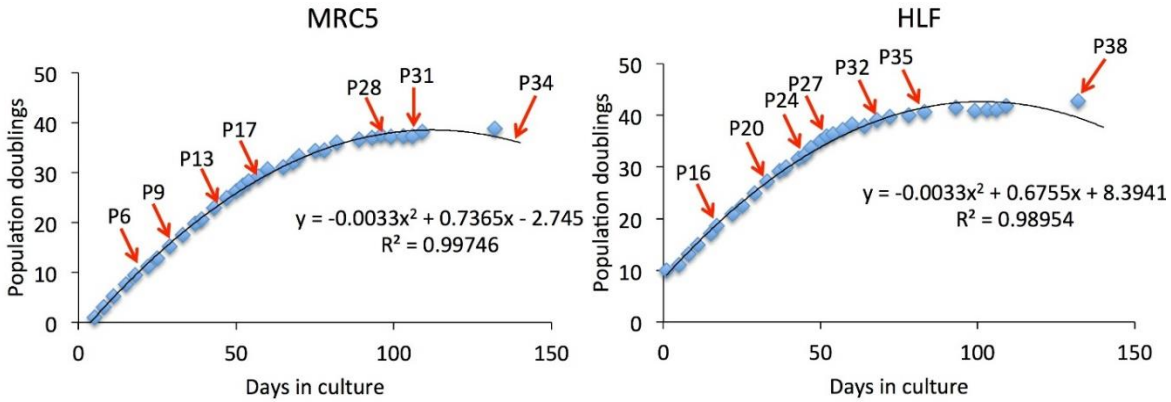
# TERRA is expressed from shortened telomeres in yeast

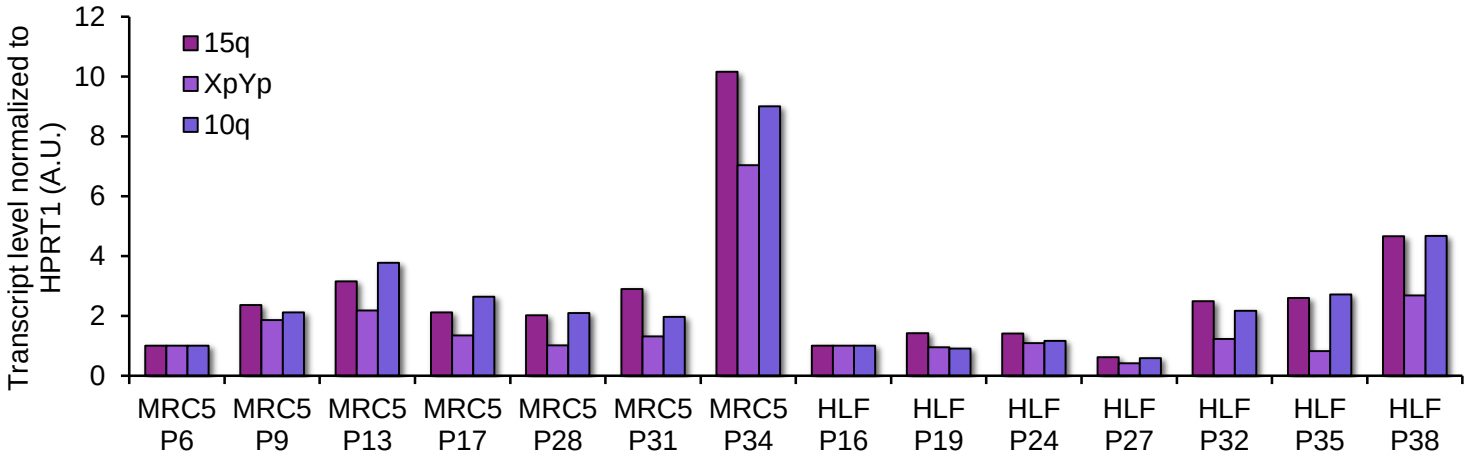


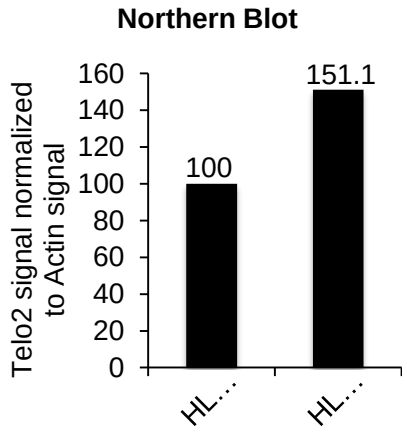

Also observed by Luke and Chartrand's labs

Moravec M. (…) Azzalin CM, *EMBO rep*, 2016

# TERRA accumulates in senescent cells

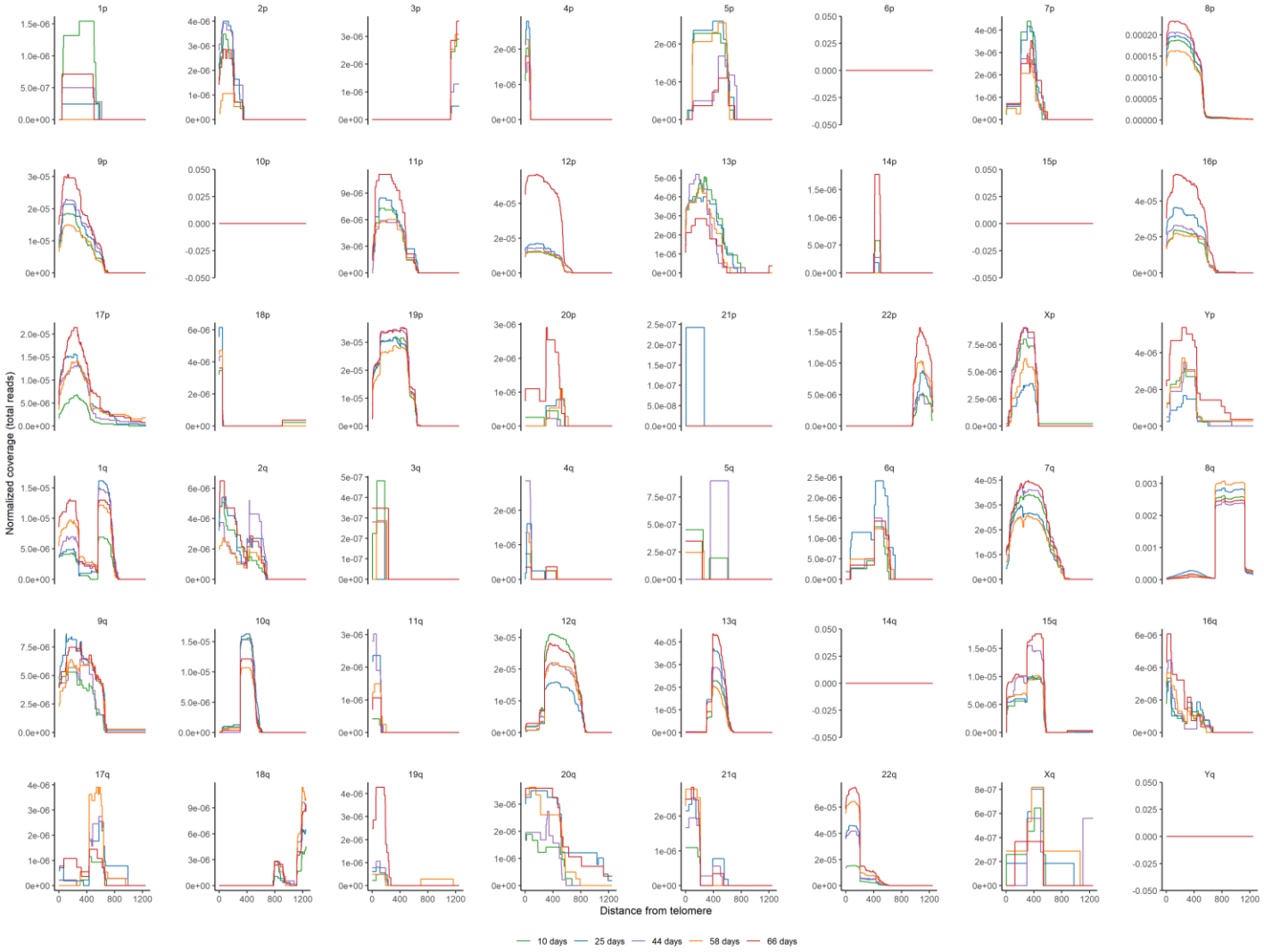


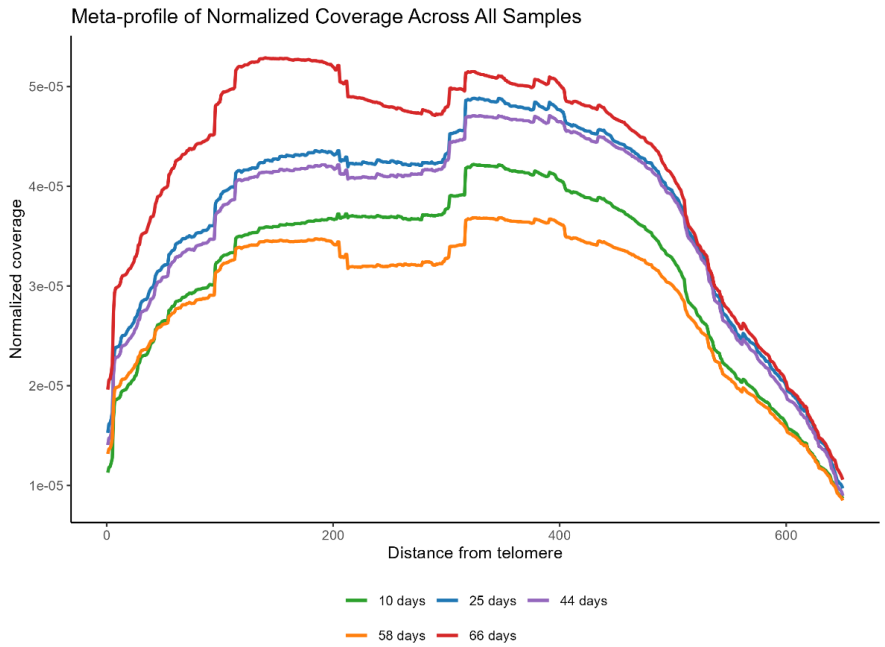

HLF and MRC5: primary lung fibroblasts

# TERRA accumulates as telomeres shorten




Nanopore sequencing of aging HLF:

- 10 days
- 25 days
- 44 days
- 58 days (early senescence)
- 66 days (established senescence)

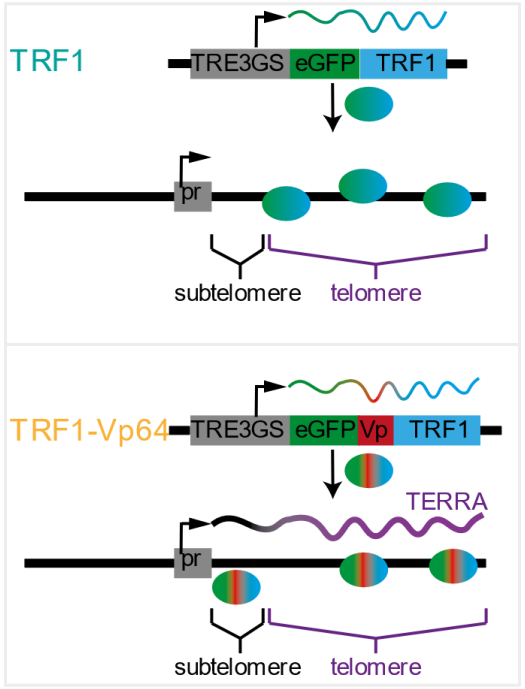


# TERRA and aging

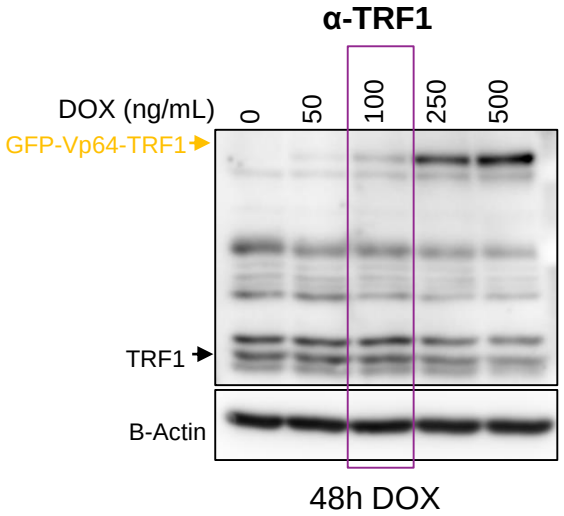
- Telomere shortening leads to increased TERRA expression.
- Aged cells have increased TERRA levels.

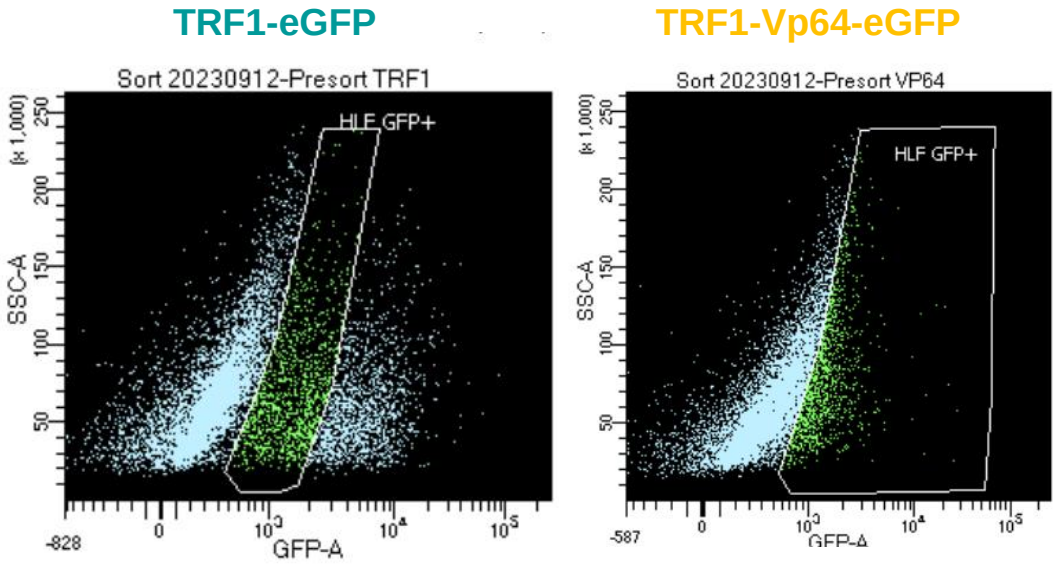
**Does TERRA play a central role in the induction and/or maintenance of senescence?**

# TERRA overexpression system



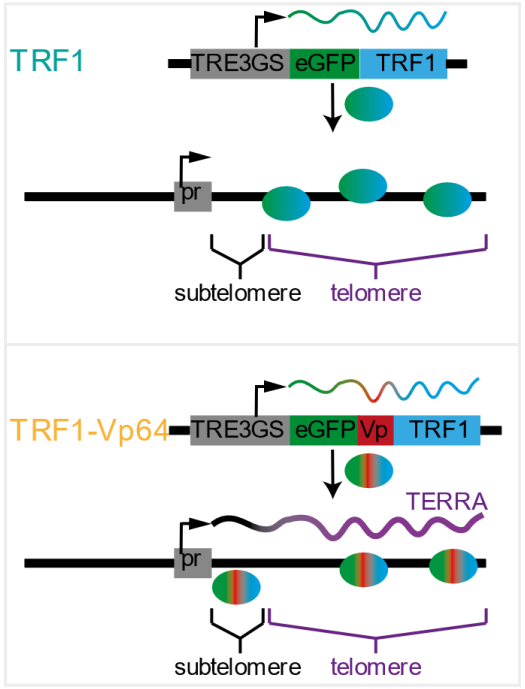

Lentiviral infection, inducible system.

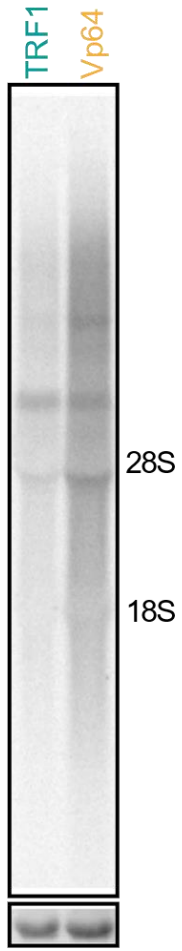


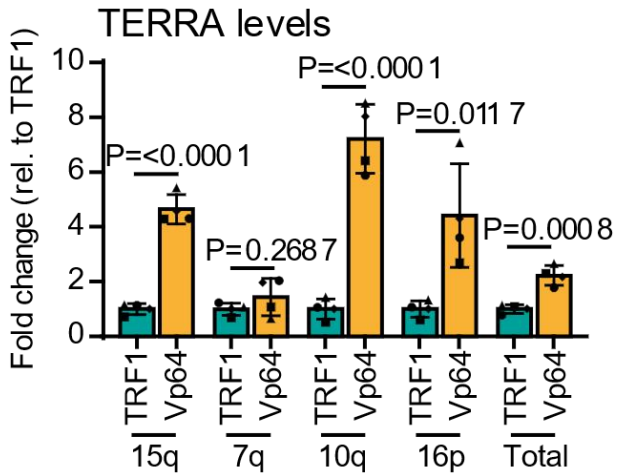




**Cell line: HLF (human lung fibroblast)**

# TERRA overexpression system

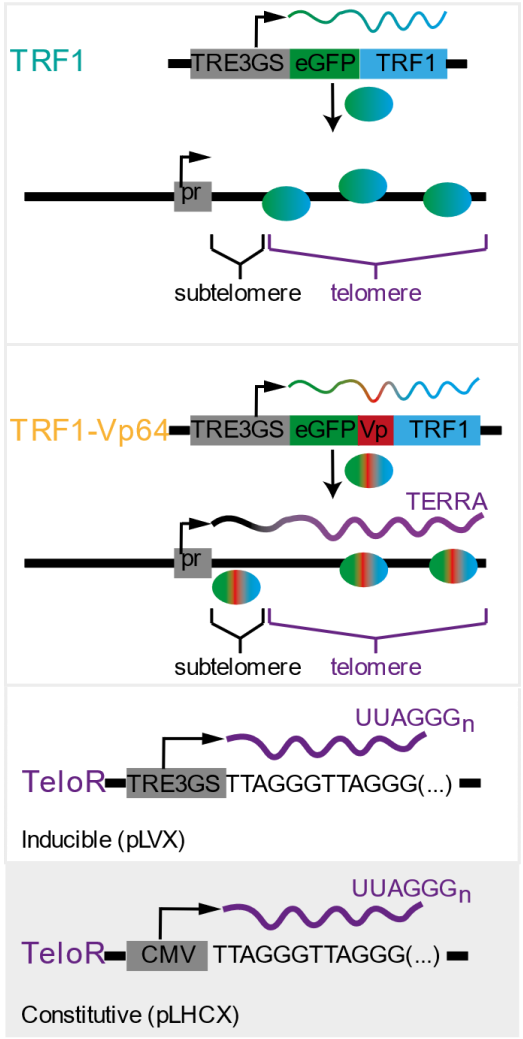


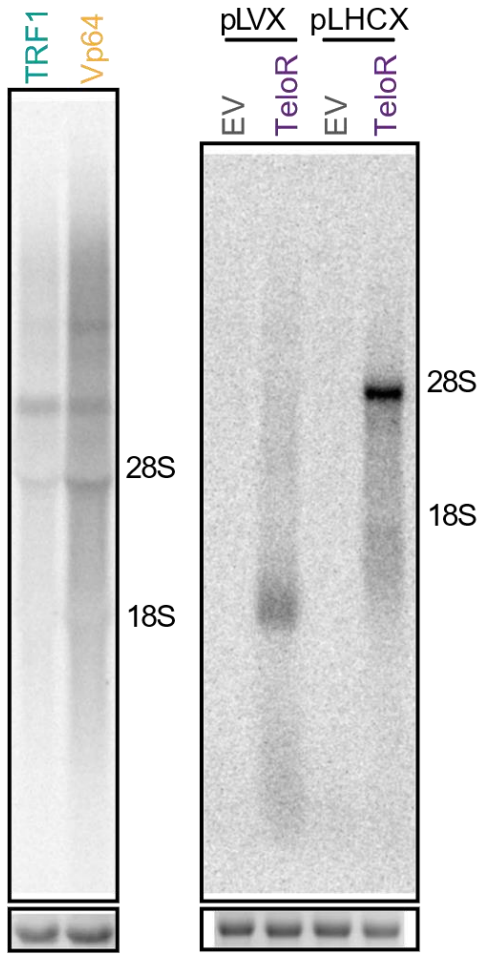



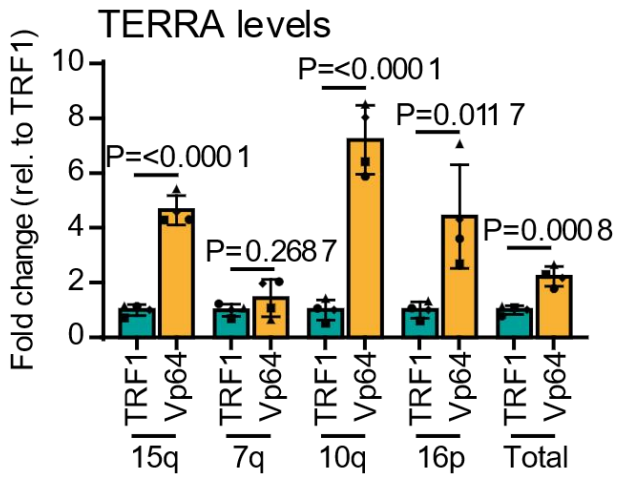



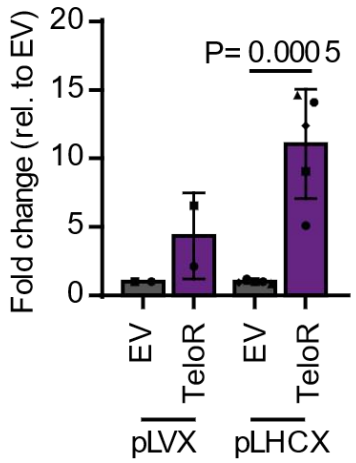

**14 days of expression**

# TERRA overexpression system



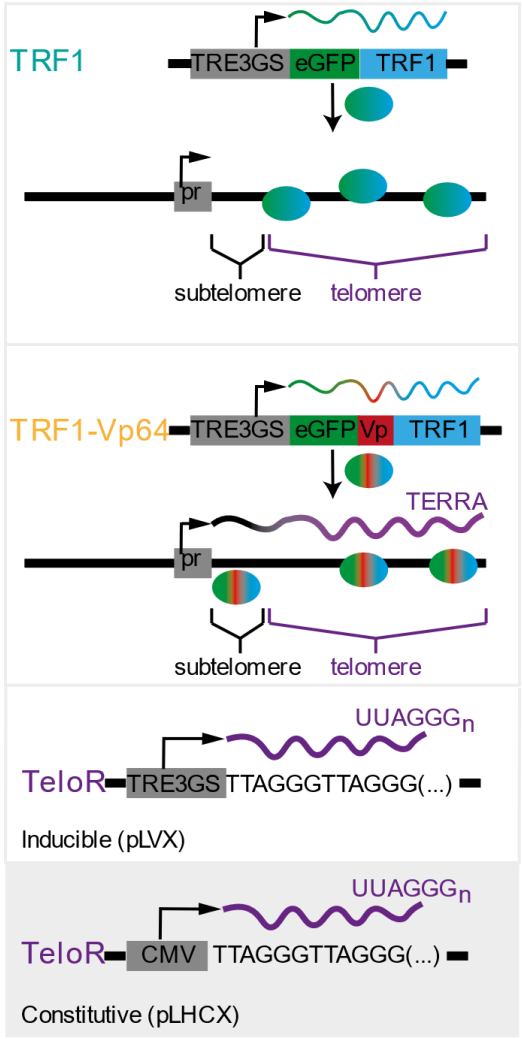



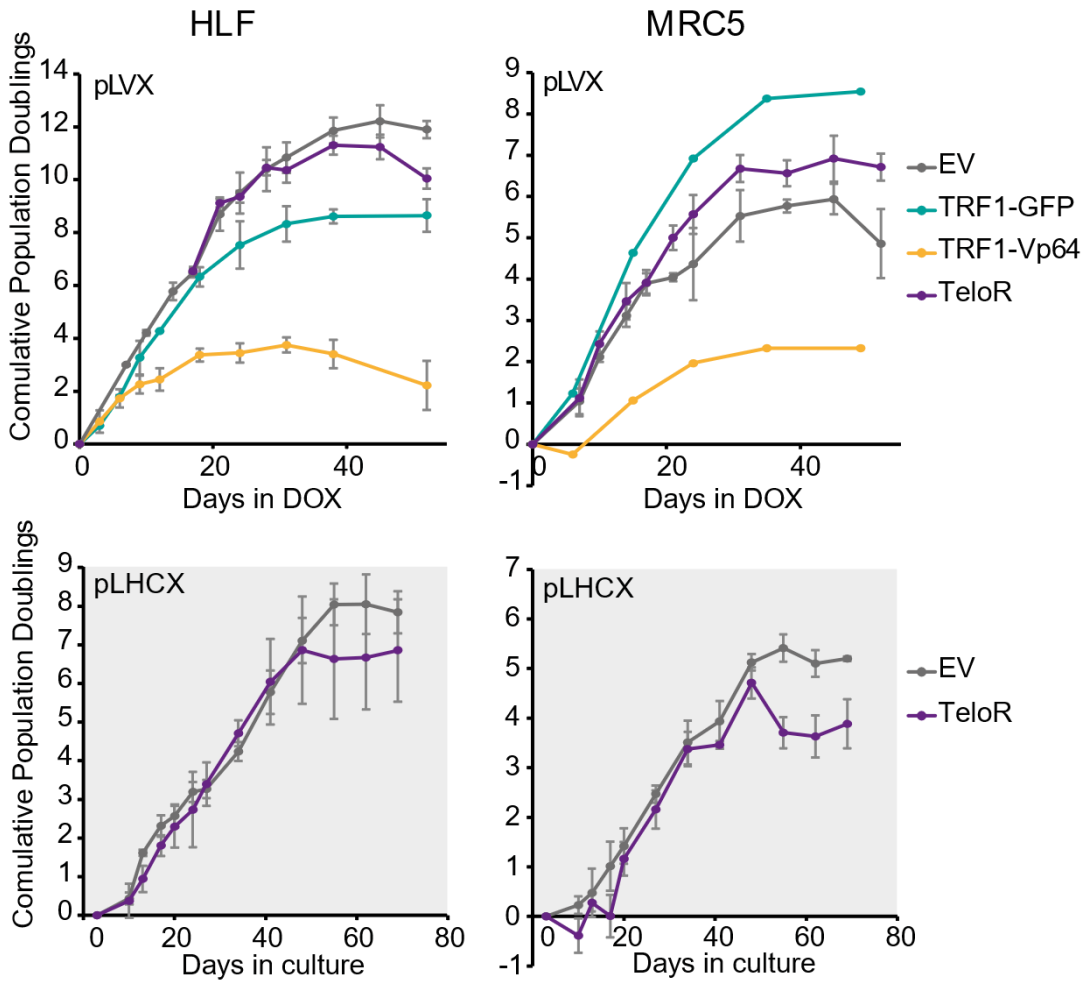



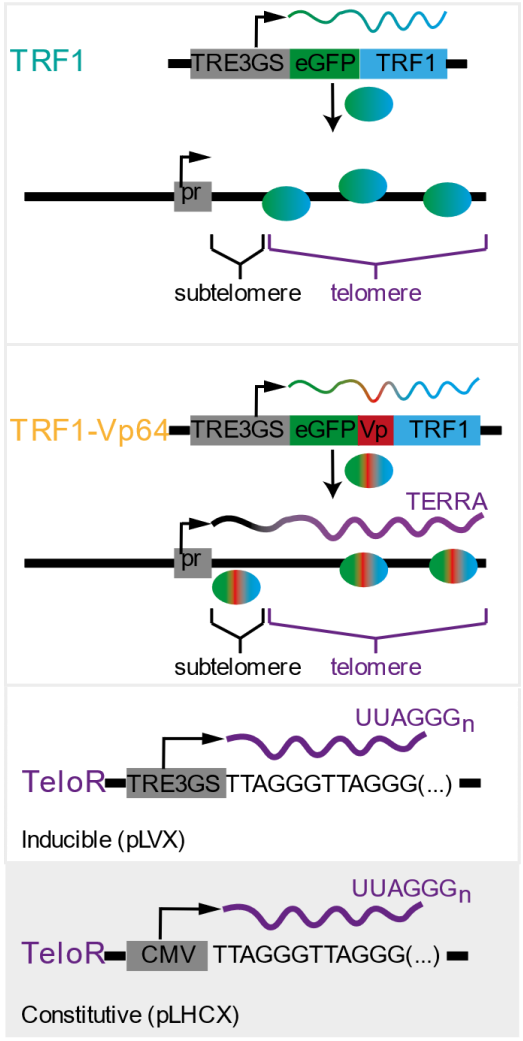

**14 days of expression**

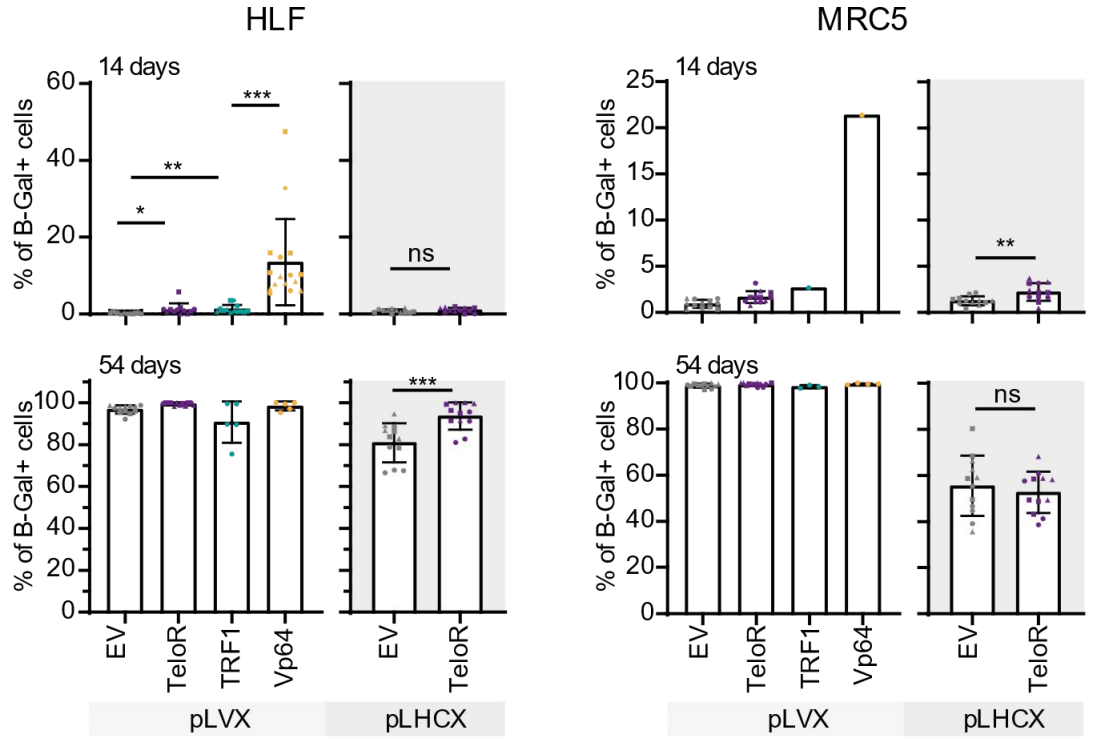
# TERRA induces premature senescence in primary cells

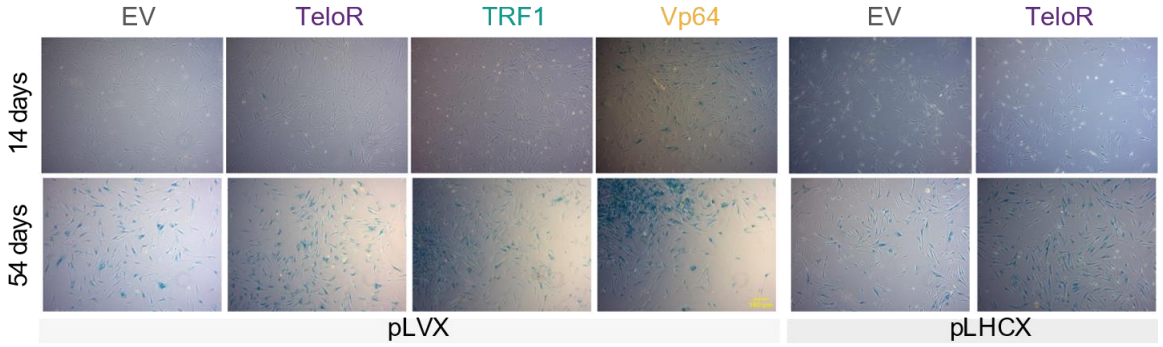


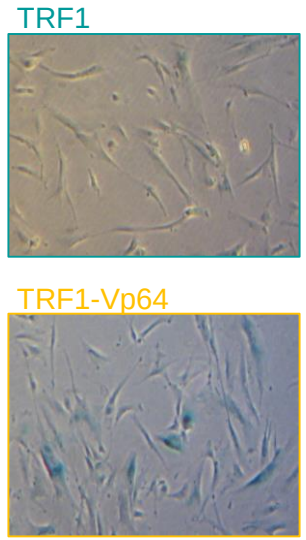



# TERRA induces premature senescence in primary cells

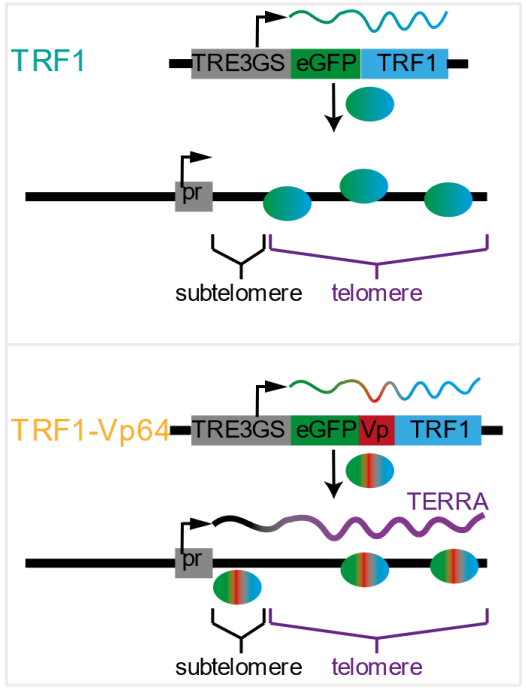


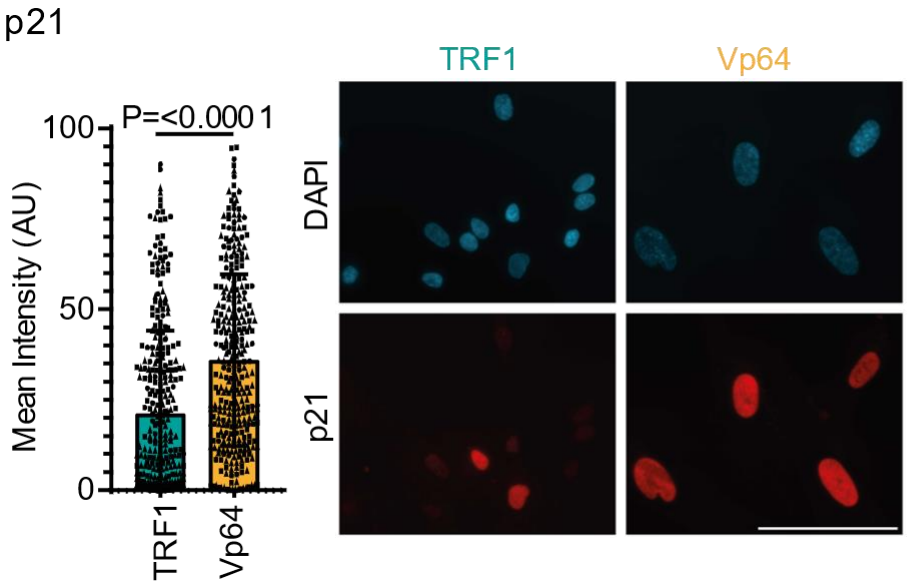



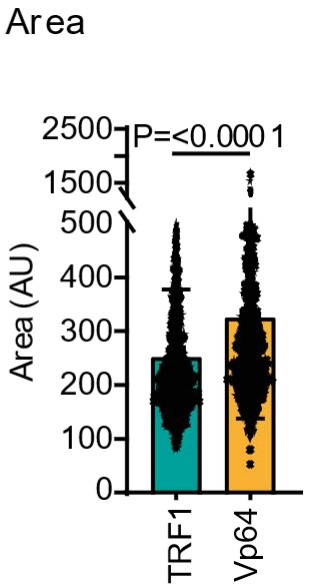



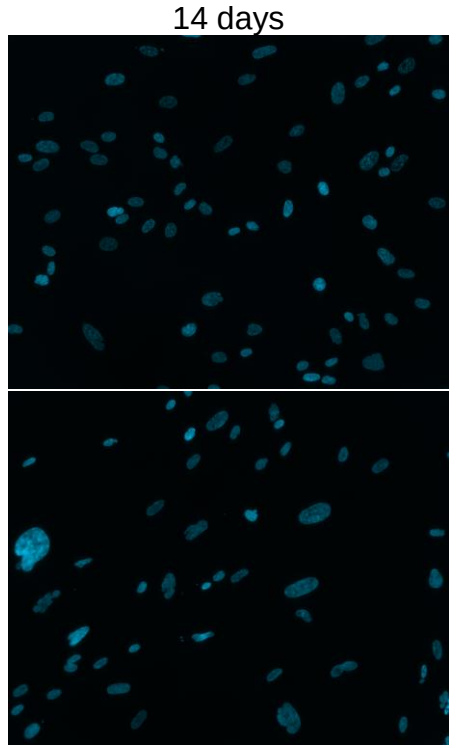



# TERRA induces premature senescence in primary cells



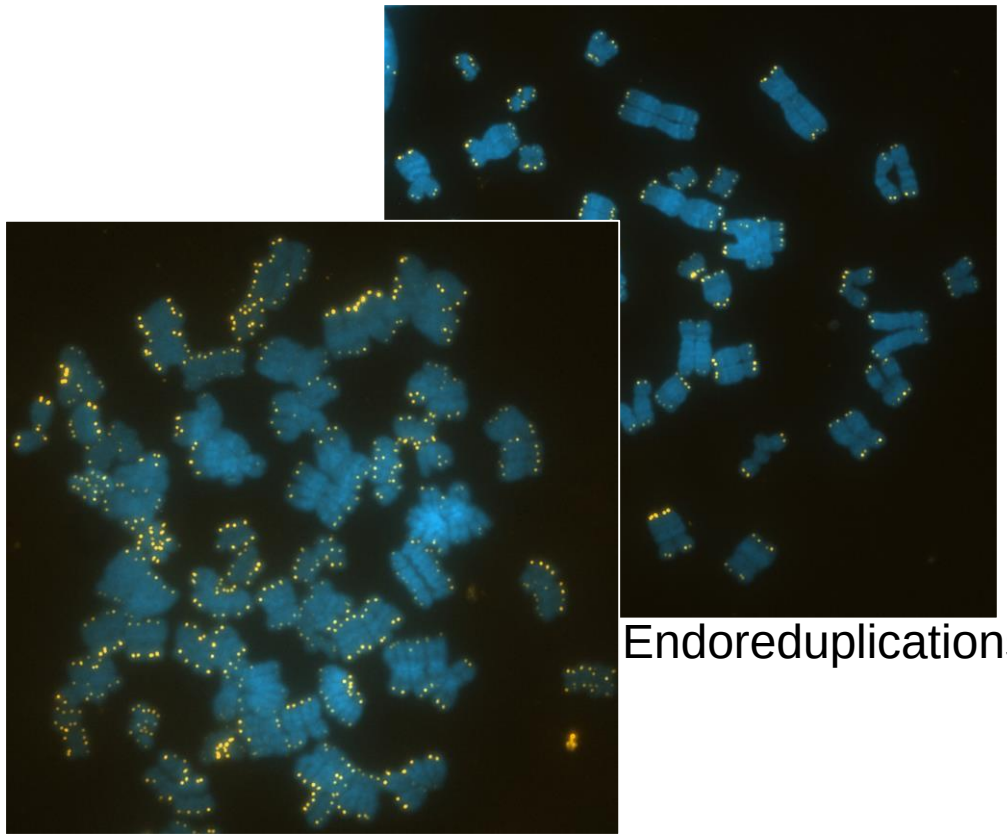



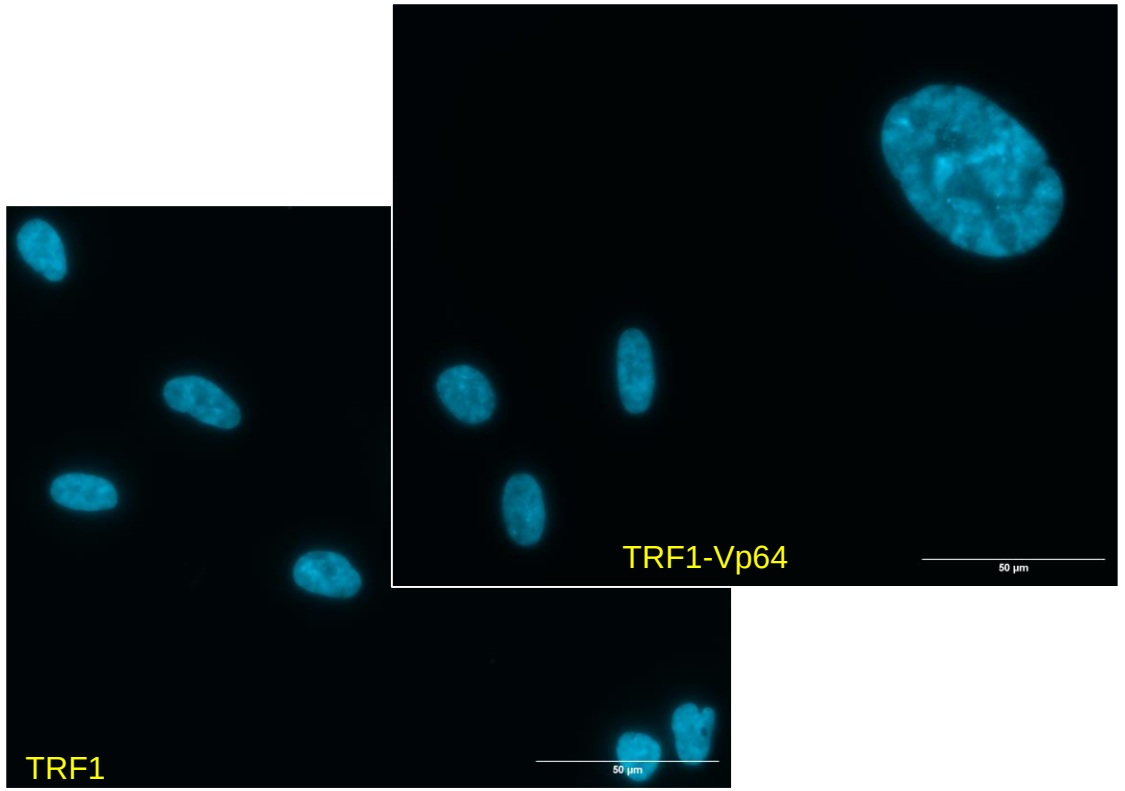

**Increased p21 and nuclear area.**

# TERRA OE leads to senescence

*“Senescent cells are also frequently polyploid.”*

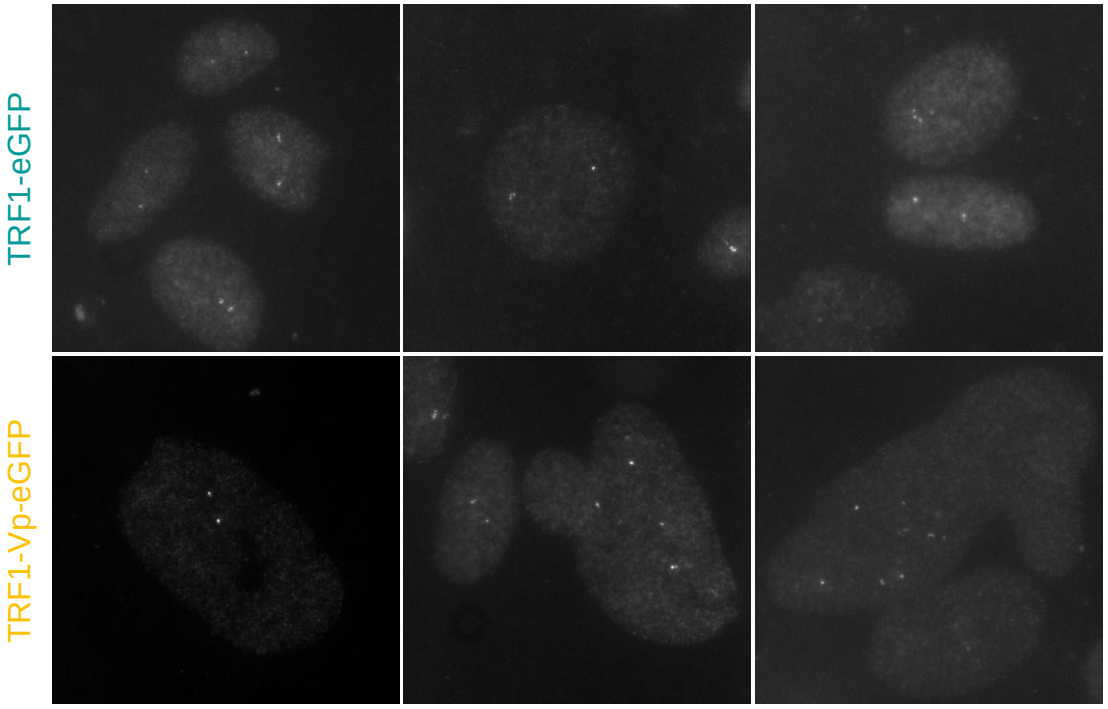


Endoreduplications

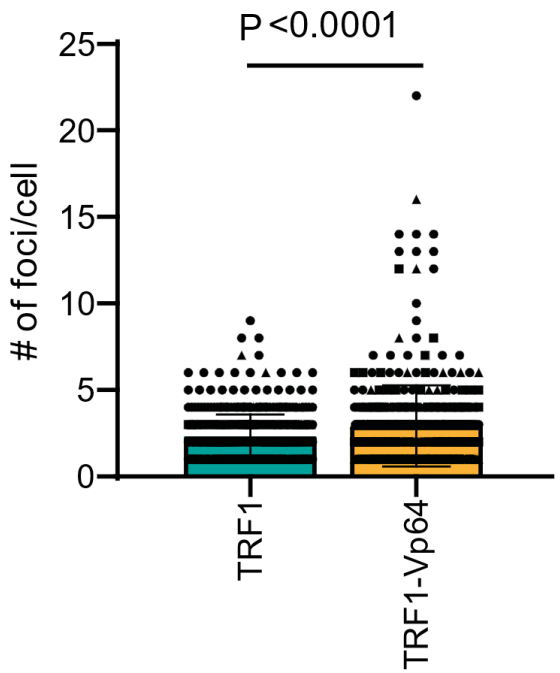


# TERRA OE leads to senescence

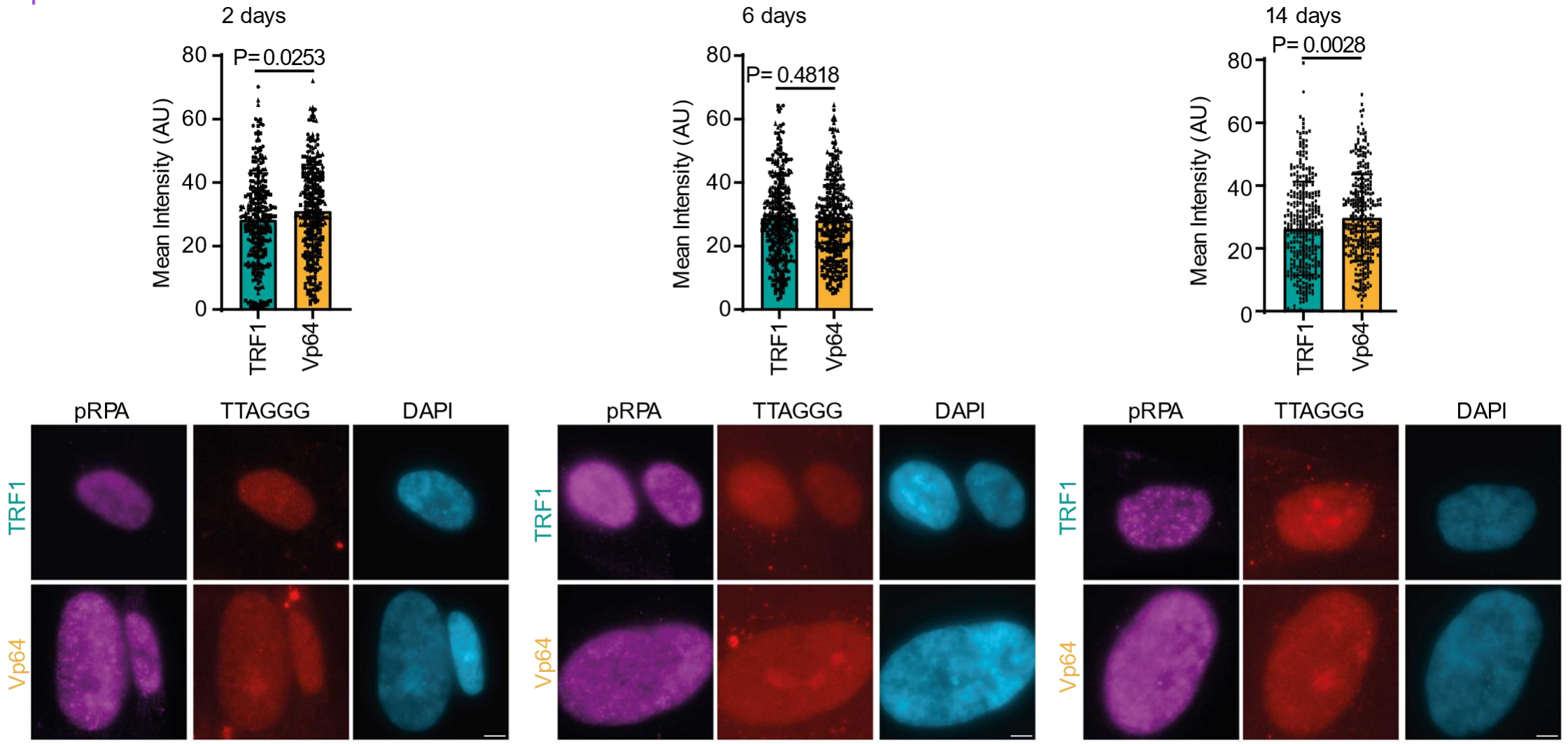
TRF1-Vp-eGFP cells are more frequently polyploid.



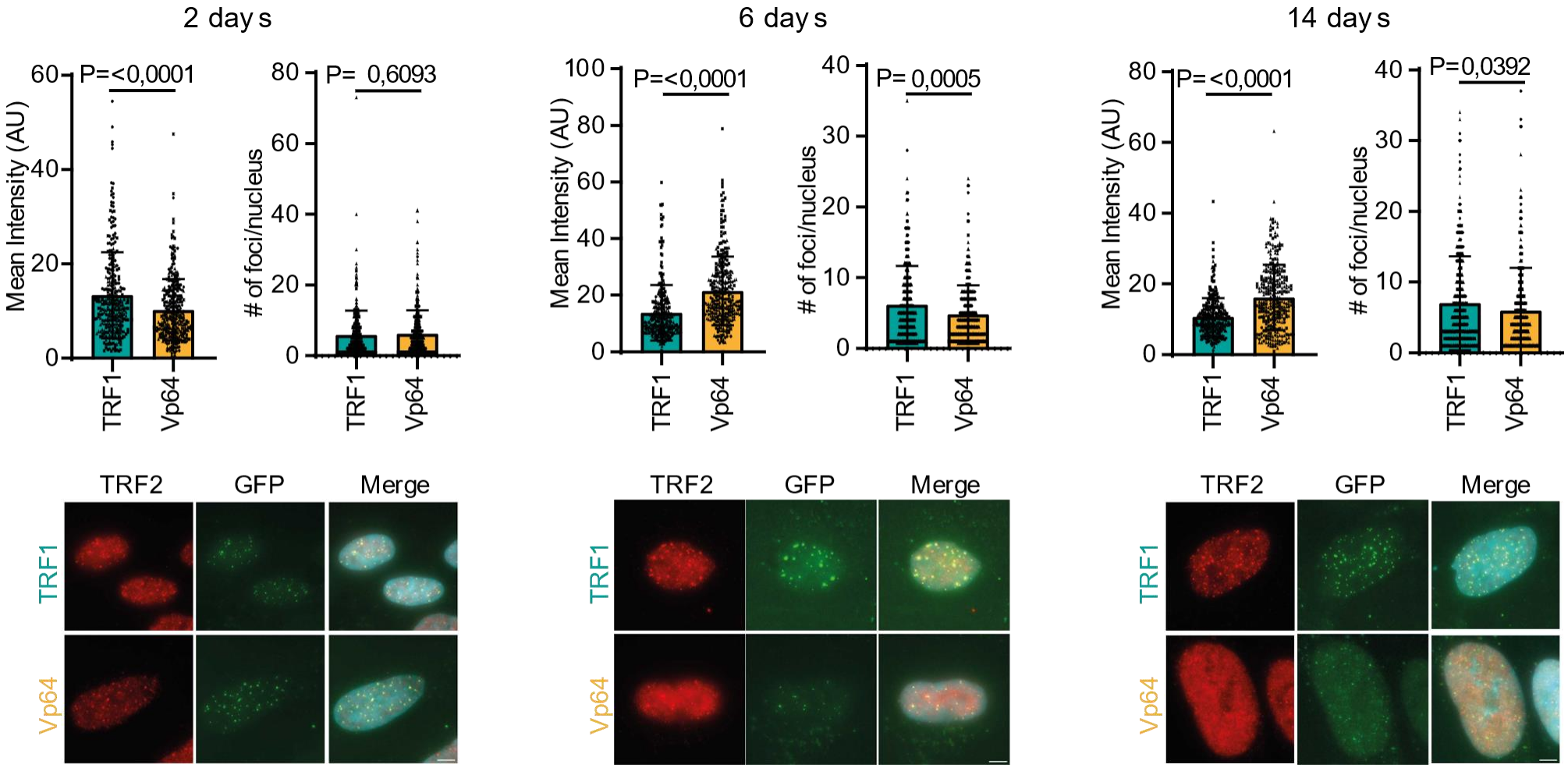

DNA FISH: 16p subtelomere



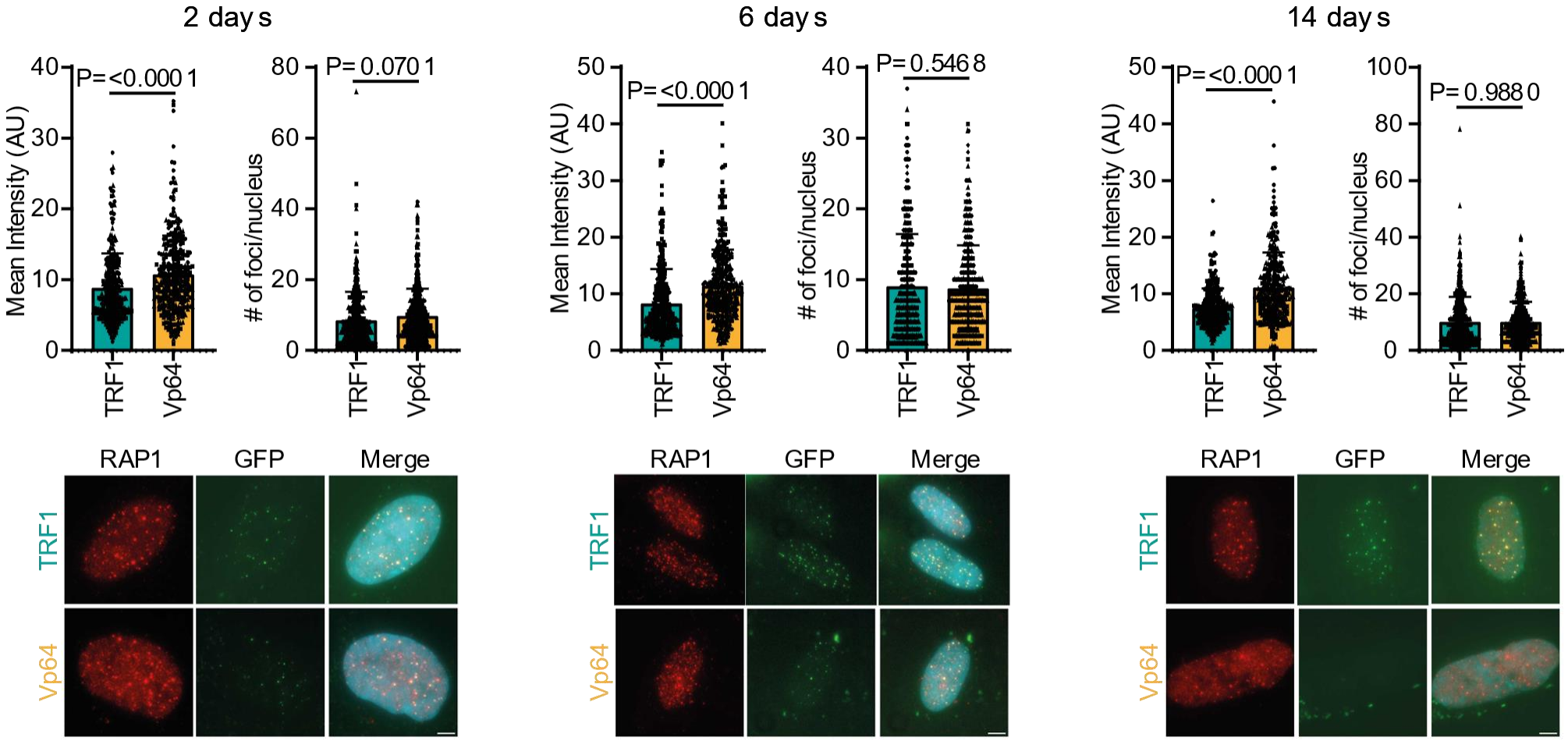
# TERRA OE slightly induces DNA damage



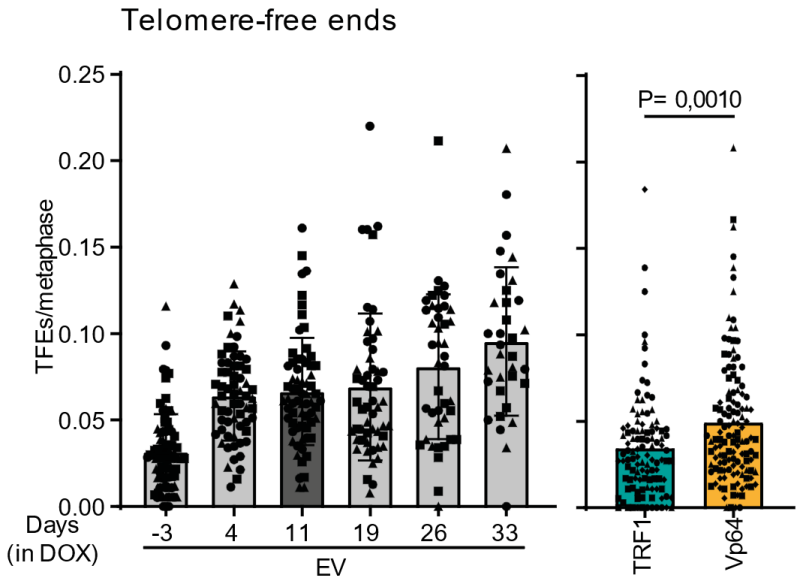
# TERRA

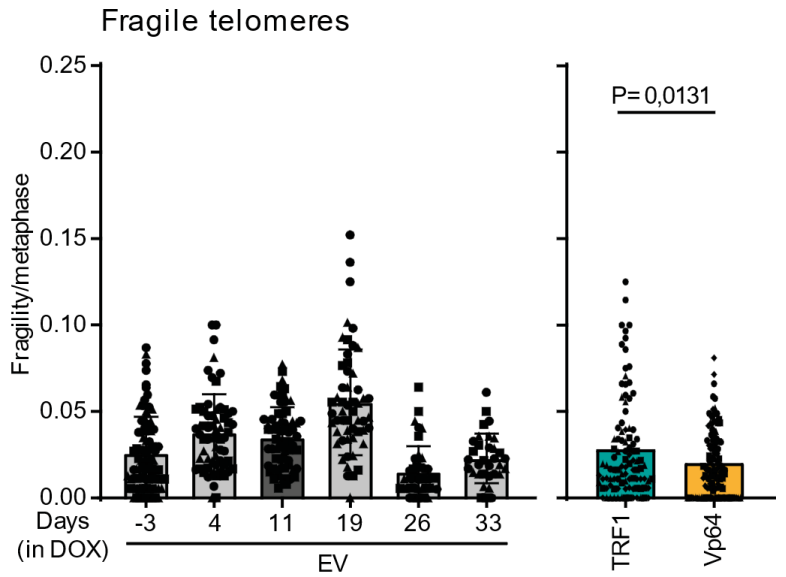

2 days
6 days
14 days
Mean Intensity (AU)
# of foci/nucleus
P=<0,0001
P= 0,6093
P= 0,0005
P=0,0392
TRF1
Vp64
TRF2
GFP
Merge

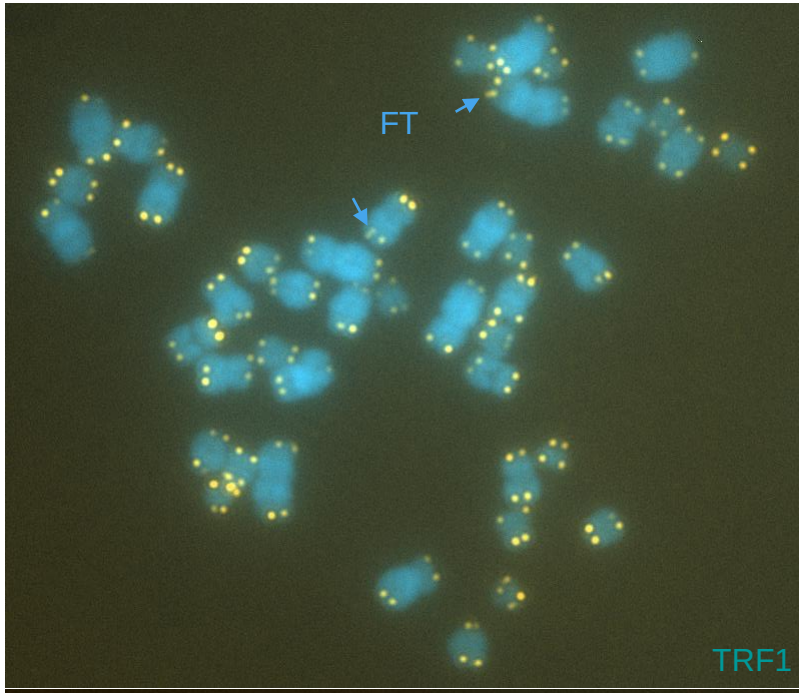
# TERRA displaces TRF2 from telomeres

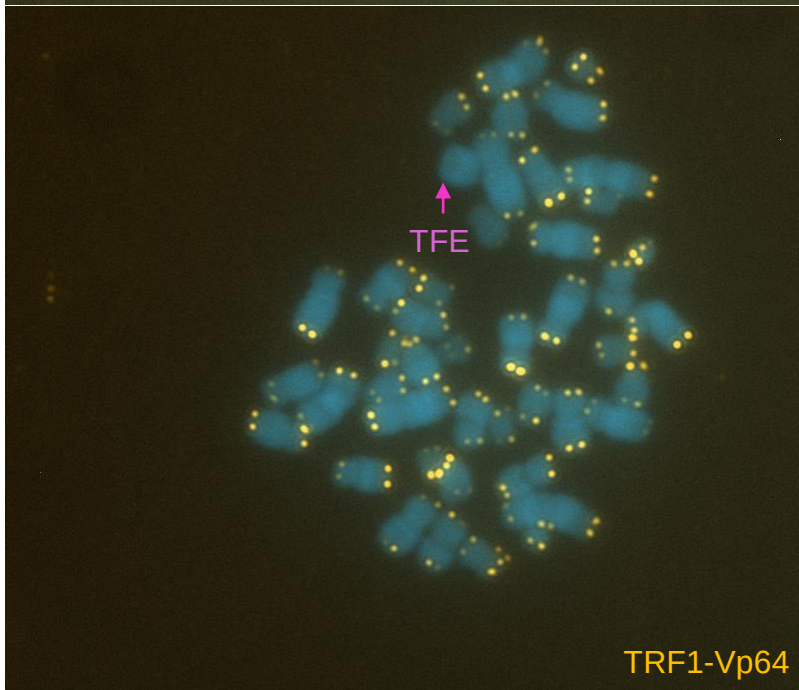


# TERRA OE does not induce TFEs or FT



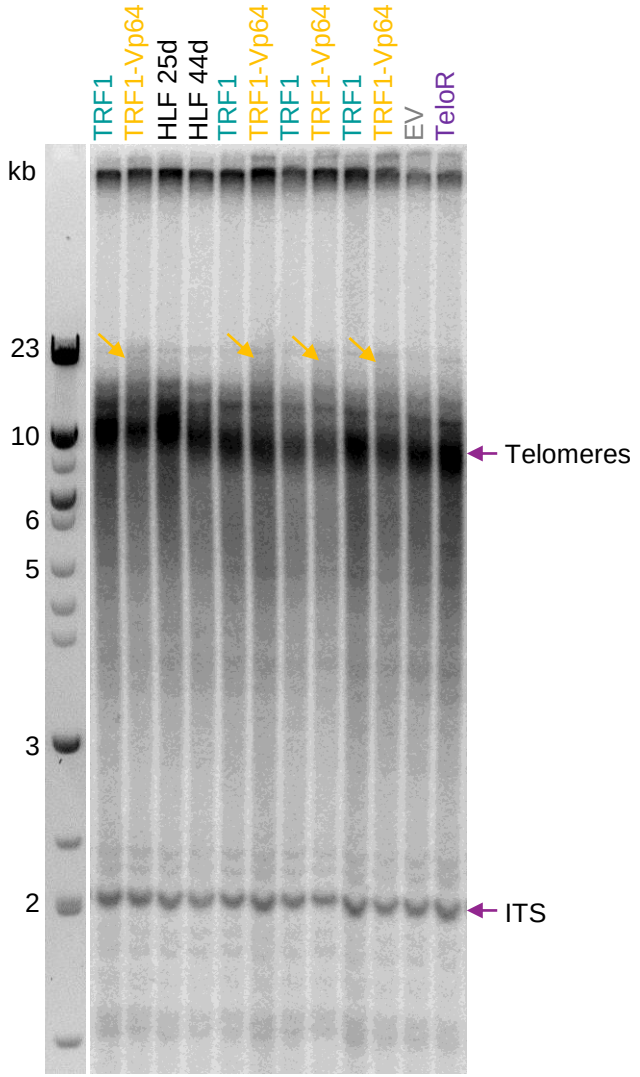



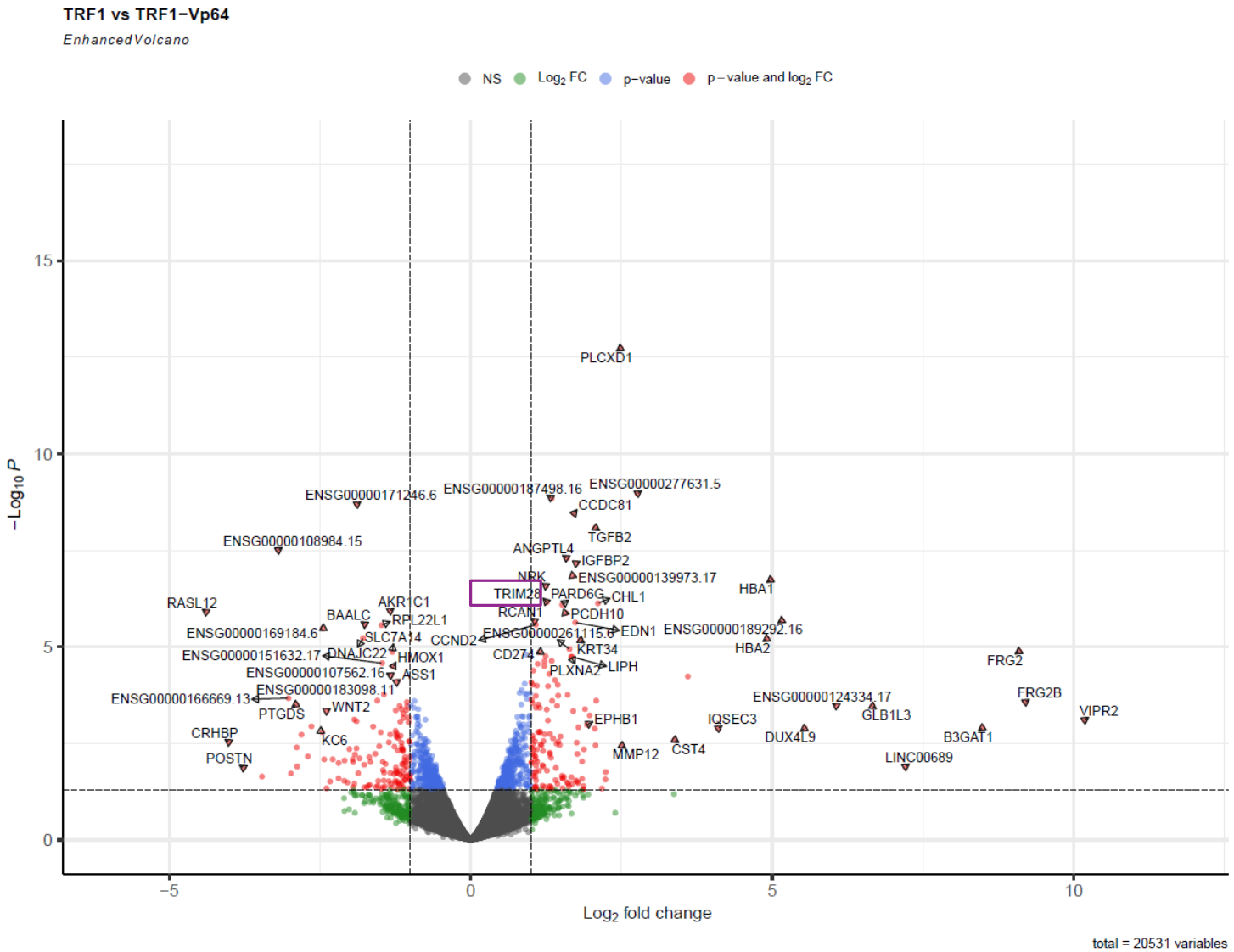
# TERRA OE does not lead to telomere shortening

TERRA-induced senescence is not directly connected to decreased telomere length.

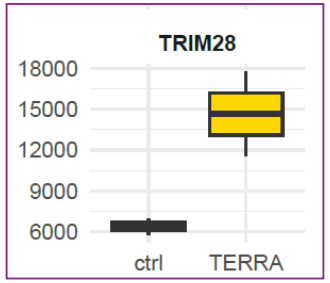



Southern blot (TTAGGG)

# RNAseq: TERRA overexpression




Does TERRA mediate Trim28/KAP1 dependent senescence?



# Conclusions

Telomere shortening leads to increased TERRA expression.

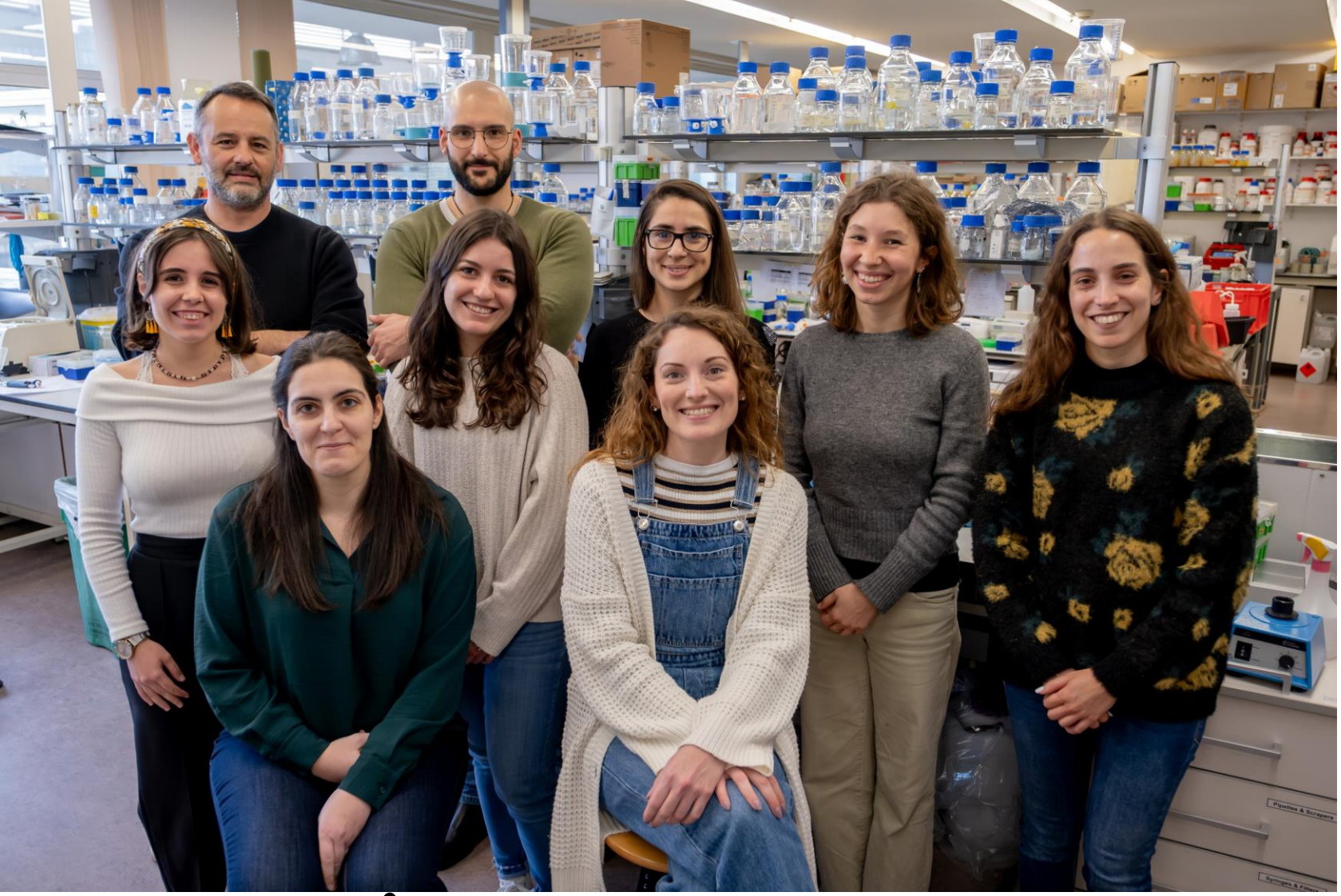
Increased TERRA transcription from telomeres induces senescence in primary lung fibroblasts:

- Telomere damage does not seem to be significantly increased.
- Do increased TERRA levels activate an oncogene-like response mediated by Trim28 to induce senescence?

# Future

CRISPR-Cas9-knockout screen:

- Find the genes required for TERRA induced senescence. Inherent positive controls: p53, CDKN2A, RB1.



# Thank you!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**Bioimaging Unit**

**Flow Citometry Facility**


GIMM Gulbenkian Institute for Molecular Medicine

FCT Fundação para a Ciência e a Tecnologia

EMBO excellence in life sciences

"la Caixa" Foundation

TRIAD