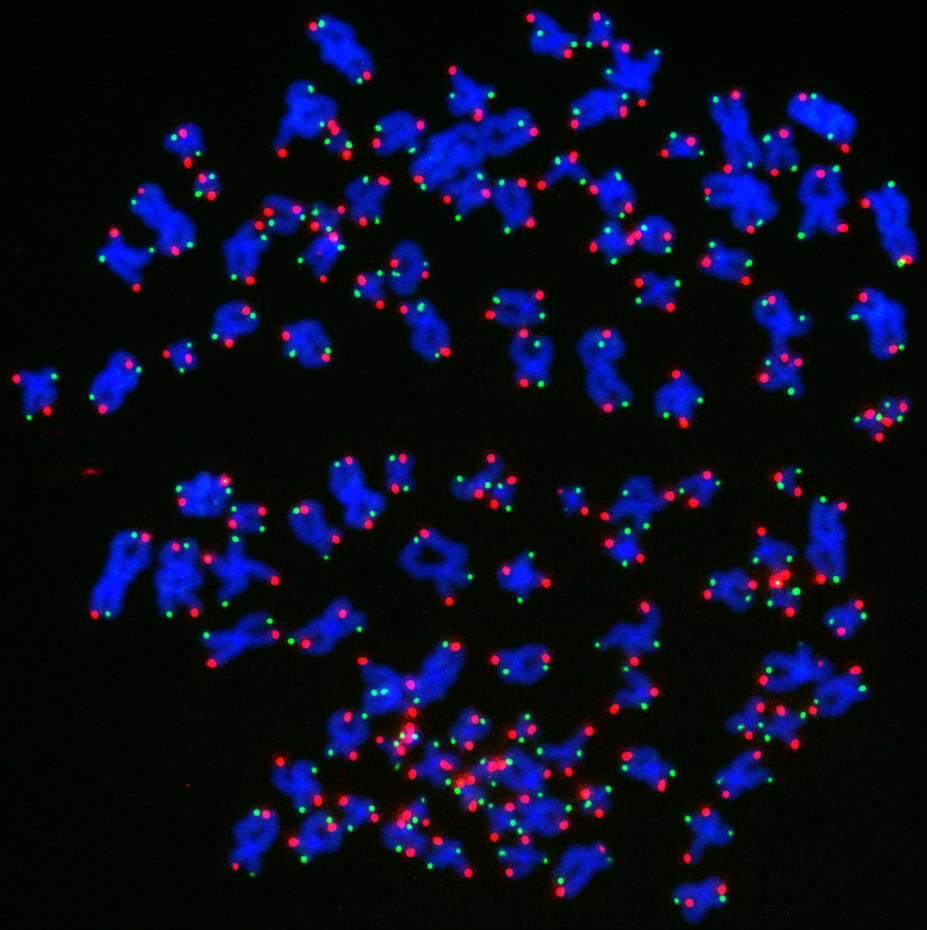
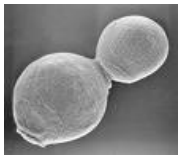


Regulation of telomere homeostasis in human cancer…

…a ncRNA point of view

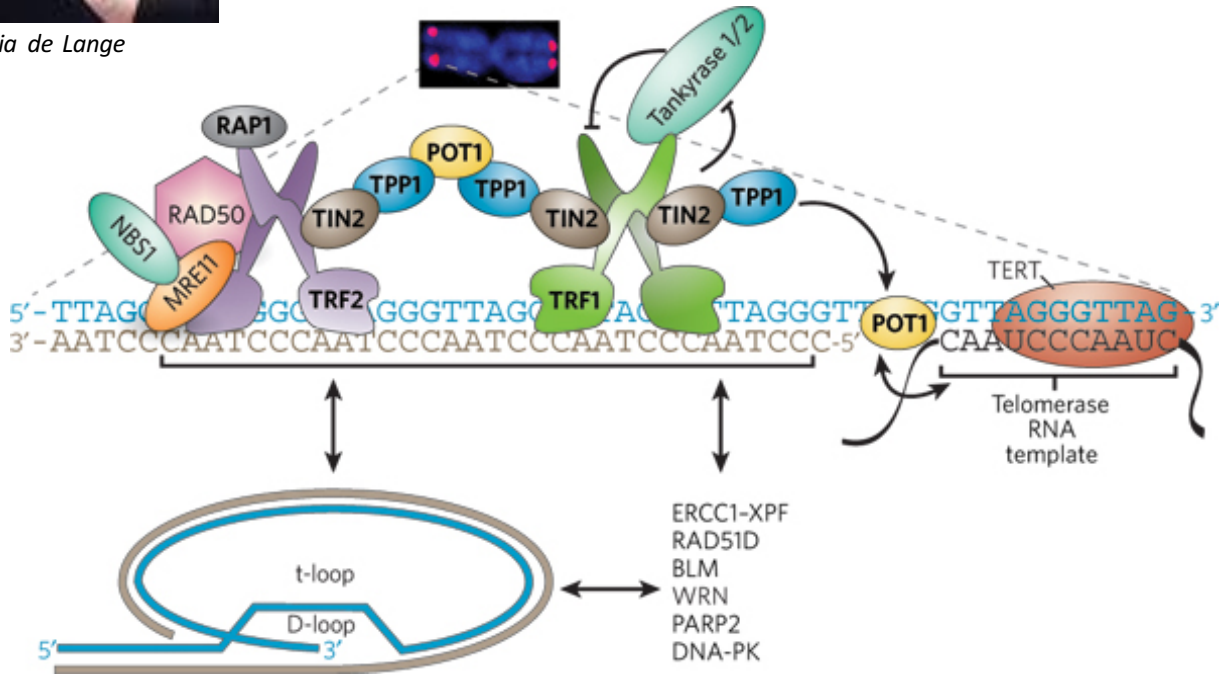
Prof. Stefan Schoeftner
Department of Life Sciences; University of Trieste
Laboratorio Nazionale CIB (Area Science Park, Trieste)

Chromsosome ends consist of tandem repeats

		sequence	*length*
Vertebrates	 	***TTAGGG***	human 10 kb mouse 40 kb
Plants		*TTTAGGG*	2 – 9 kb
Tetrahymena		*TTGGGG*	250 bp
S. cerevisiae		$G_{(2-3)}(TG)_{(1-6)}T$	325-400 bp
S. pombe		$TTAC(A)(C)G_{(1-8)}$	200-300bp
D. melanogaster		Transposons	

Titia de Lange

The shelterin complex controls telomere structure and function



Shelterin complex

TRF1
TRF2
bind double stranded telomeric DNA

Pot1
bind single stranded G-strand overhang
Control telomerase access

Tpp1
Rap1
Tin2
Protein-protein interaction

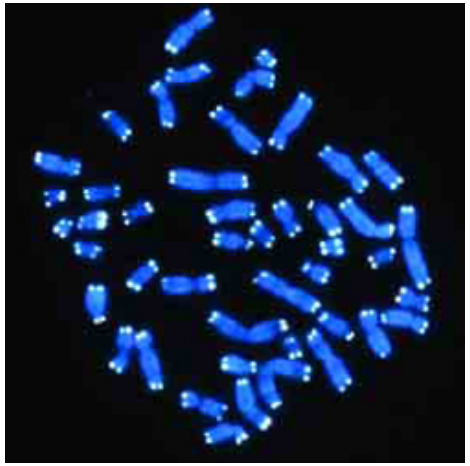
DNA repair factors

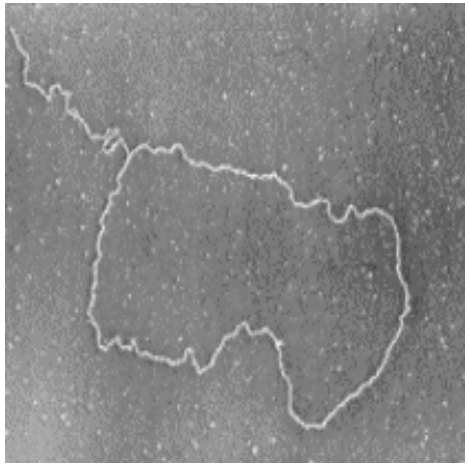
telomere length regulation

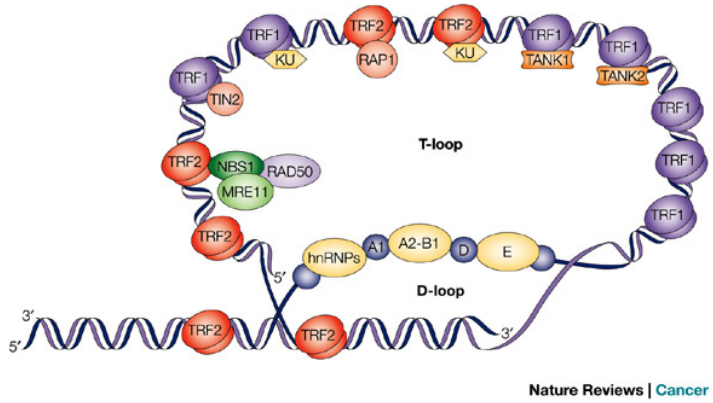
T-loop formation

SUPPRESS DNA DAMAGE AT CHROMOSOME END → *genomic stability*

The structure of mammalian telomeres

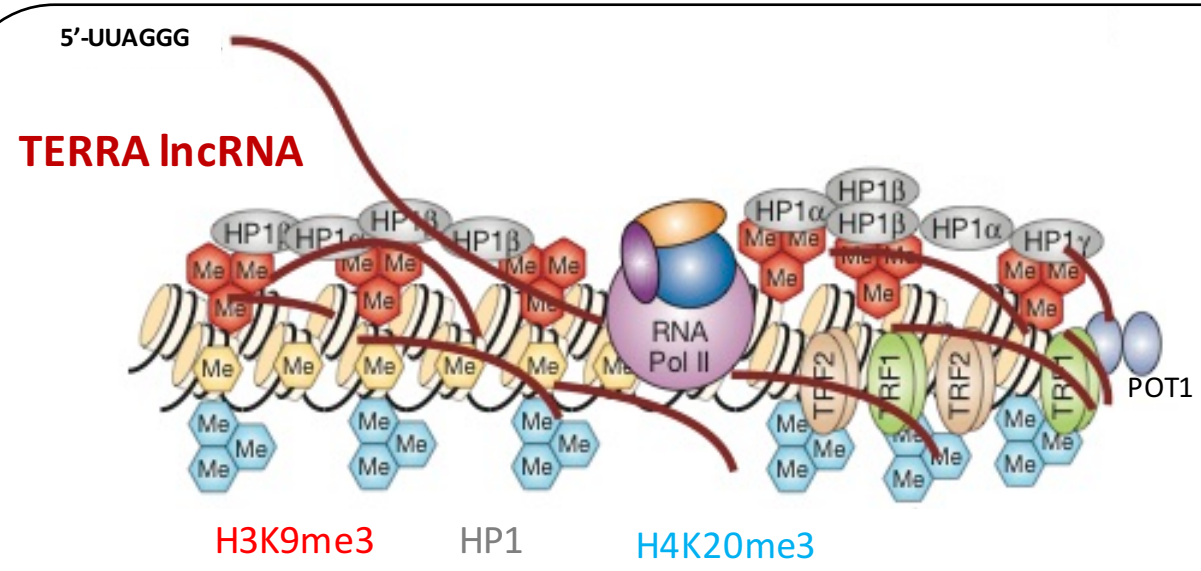




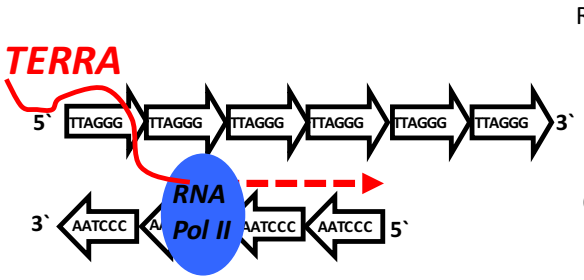


Telomeres form a loop structure, the "T-loop"

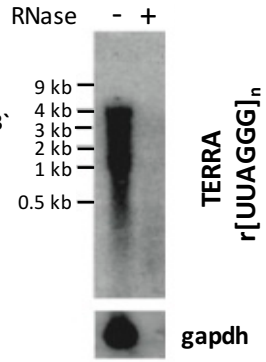
Telomeres are RIBO-nucleo-protein structures

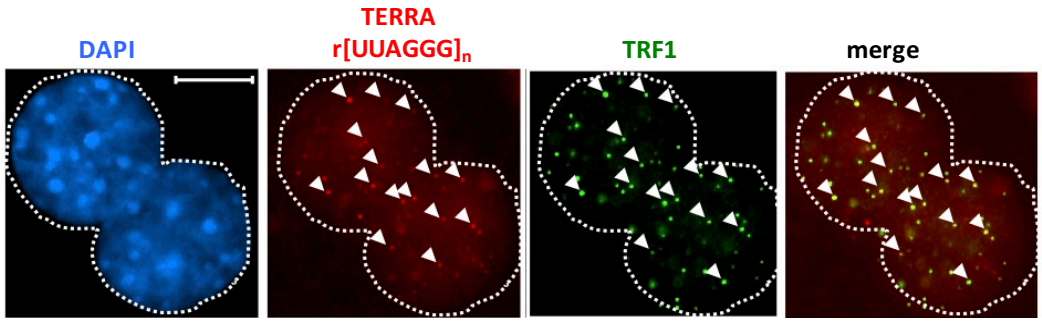


- DNA tandem repeats
- Shelterin complex
- Constitutive heterochromatin
- *long non-coding RNA (TERRA)*



Transcription by RNA Pol II



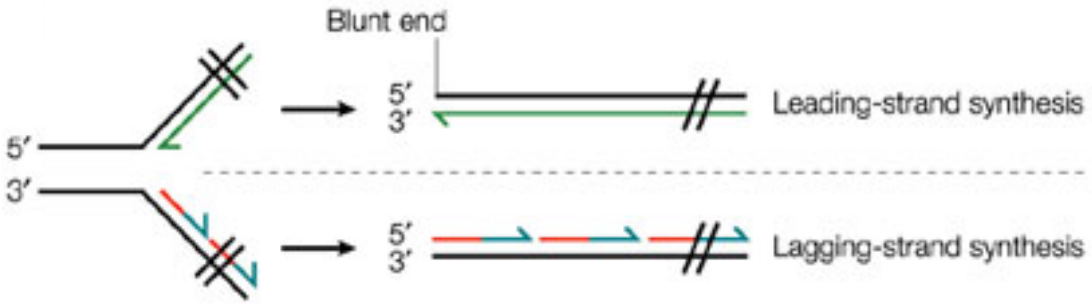


Association with telomeric chromatin

James Watson,
1972

The "Hayflick limit", the "end replication problem" and telomeres

"….DNA replication is uncomplete at the 3`end of the linear DNA molecule of the T4 bacteriophage"

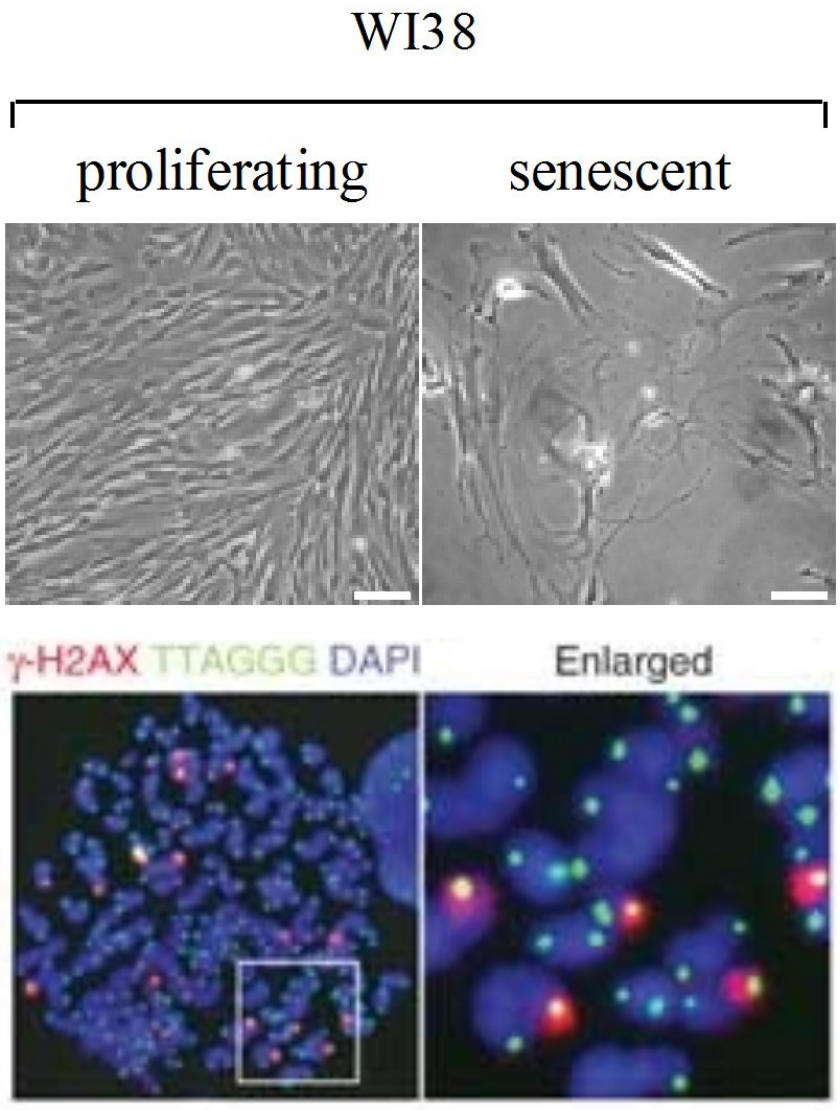


proliferation

loss of chromosome ends,

Viability??

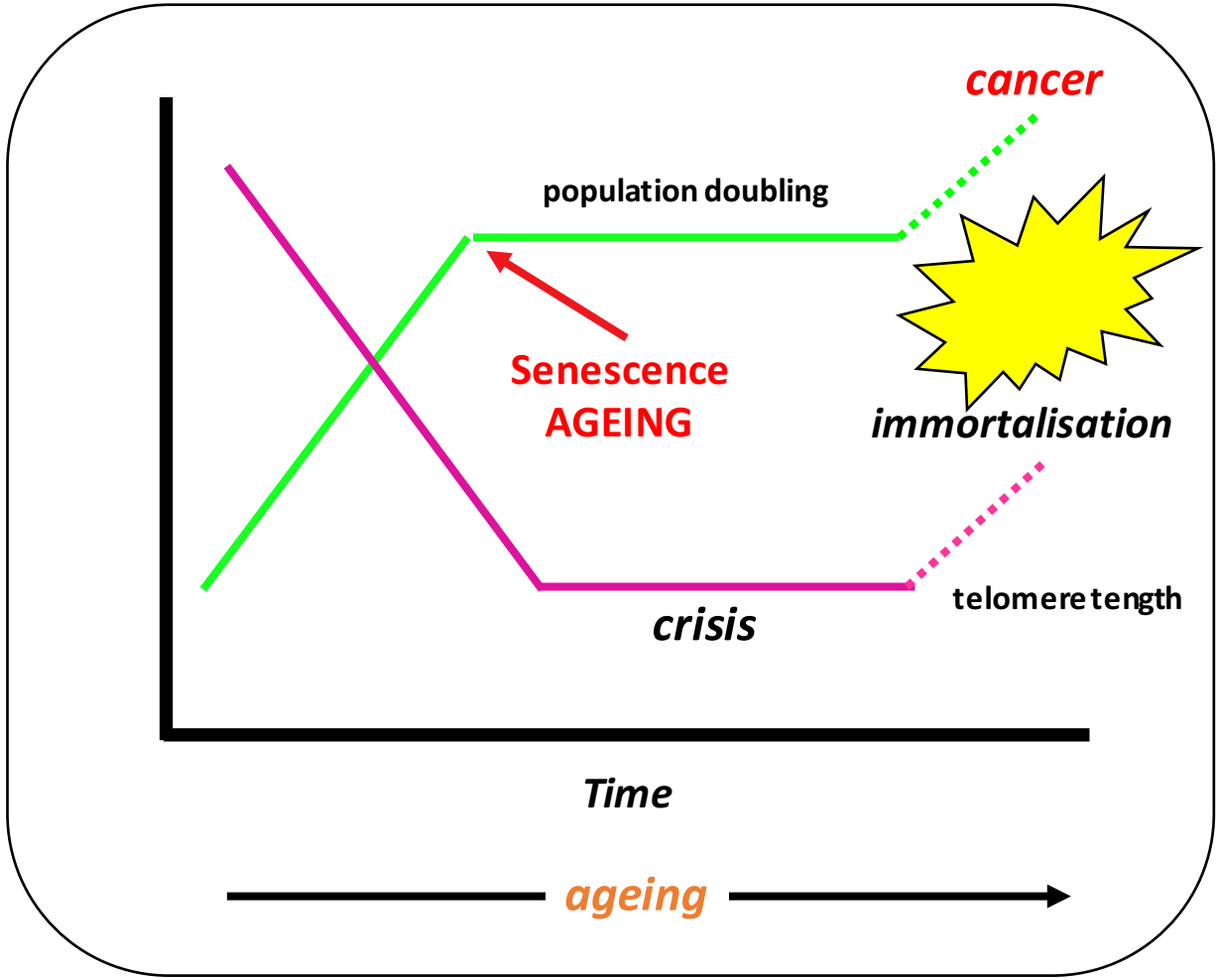
Telomere dysfunction leads to DNA damage at chromosome ends and senescence



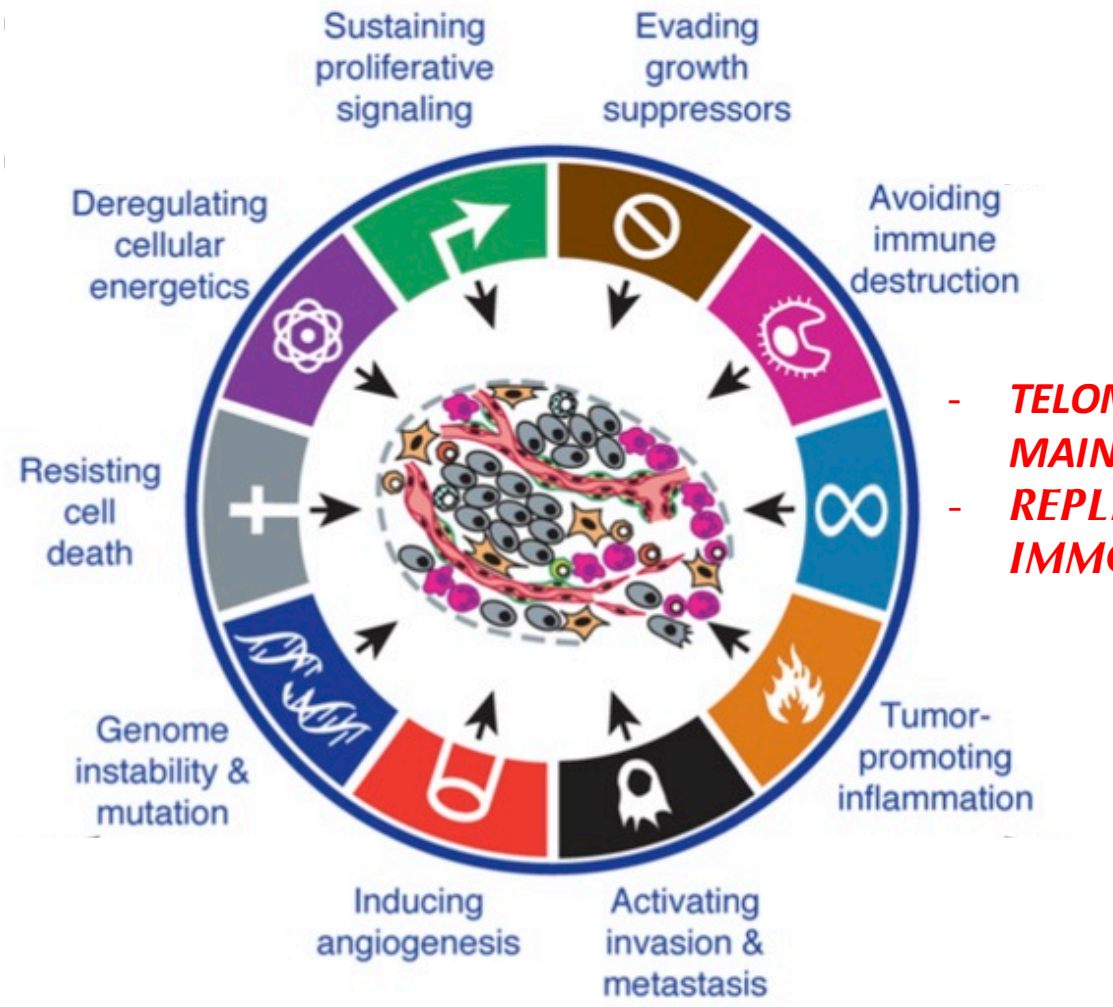
Telomere shortening ->Telomere DNA damage

1. **LOSS OF TELOMERE PROTECTION (shelterin)**
2. **LOSS OF TELOMERE REPEATS (normal aging, accelerated aging – pathology**

The "telomere" hypothesis



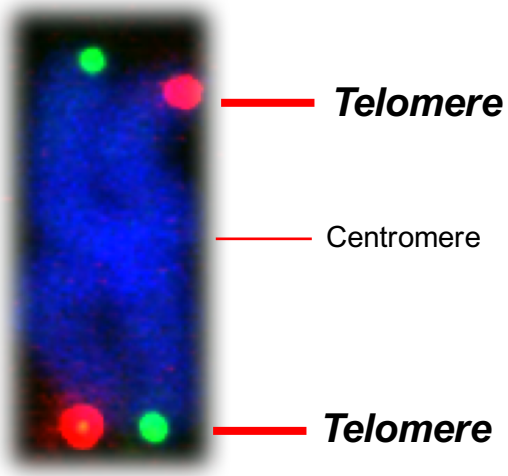
Tumor cells escape from replictive senescecne by re-activating telomere maintenance mechanisms

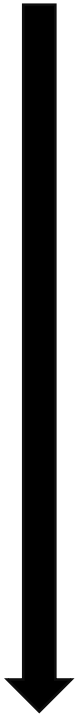


- ***TELOMERE MAINTENANCE***
- ***REPLICATIVE IMMORTALILTY***

1 of the 11 hallmarks of human cancer

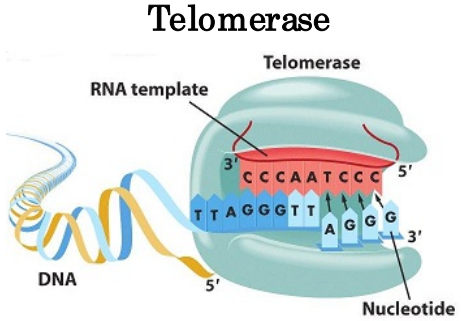
Telomere Maintenance in cancer cells come in 2 flavours





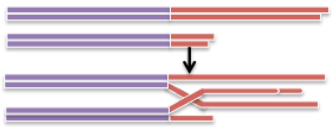
Tumor cells

90%



10%

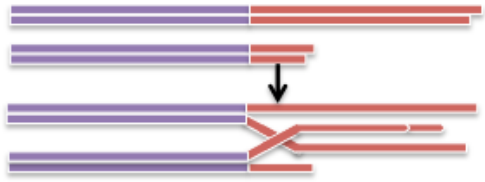
Alternative lengthening of telomeres (ALT)



Reddel R.R. 2003

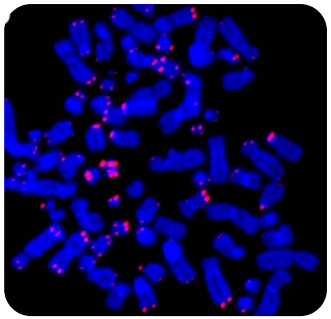
Telomere lengthening mechanisms: ALT mechanism

ALT

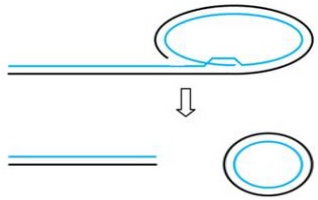


Homology-directed
Recombination-dependent
replication pathway

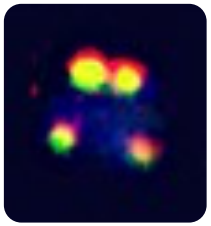
Heterogeneous
Telomere length



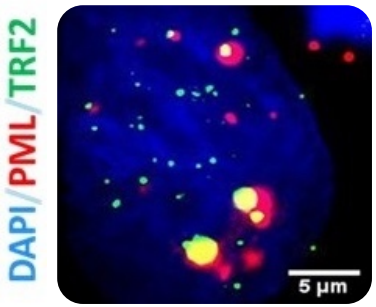
ECTRs
Extrachromosomal
Telomere Repeats

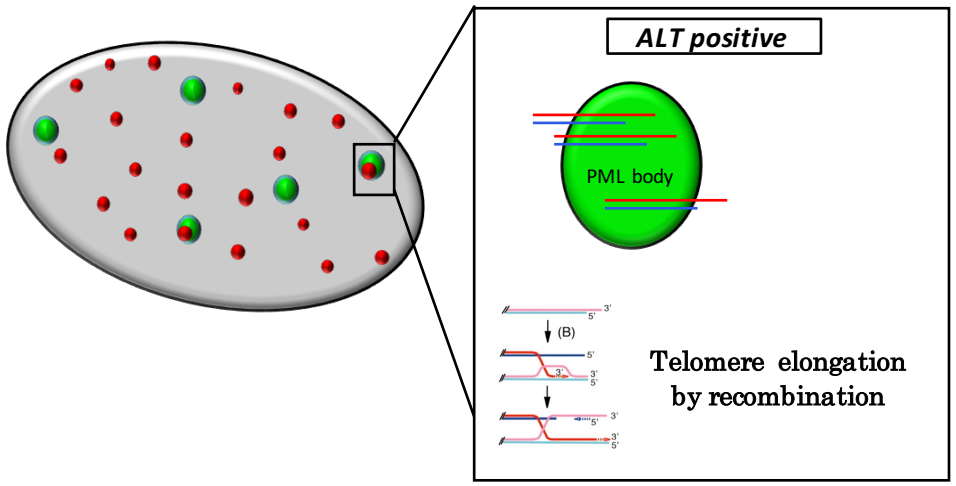


Increased telomeric
recombination (T-SCE)



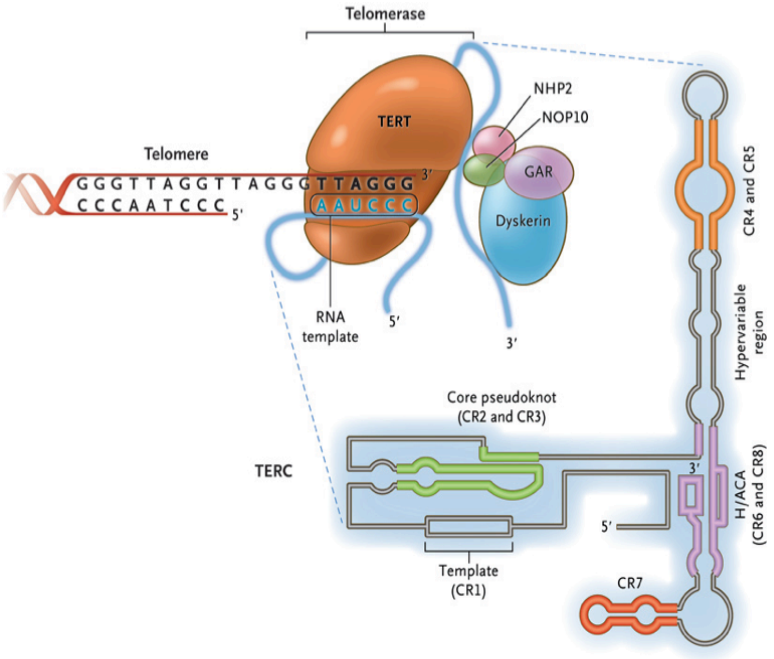
APBs
ALT associated
PML bodies





Modified from Durant S.T. 2012
Cesare A.J. & Reddel R.R. 2013

Telomere lengthening mechanisms: telomerase

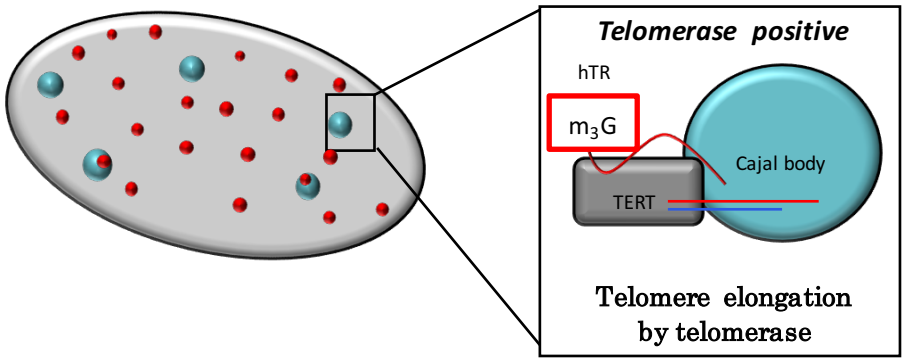


Reverse transcriptase upregulated in 90% of human cancers

The minimal catalytic core consists of the RNA component hTR and the enzymatic subunit hTERT

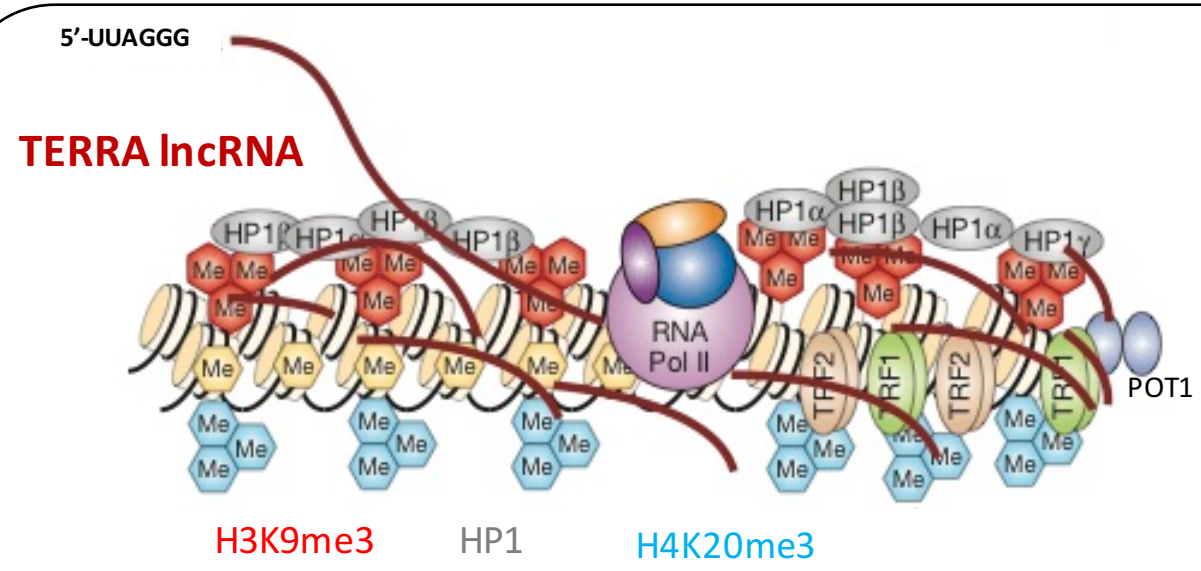
Telomerase complete maturation and assembly occurs in Cajal bodies

Telomeres are elongated by telomerase in Cajal bodies



Calado T. et al. 2009

Telomeres are RIBO-nucleo-protein structures



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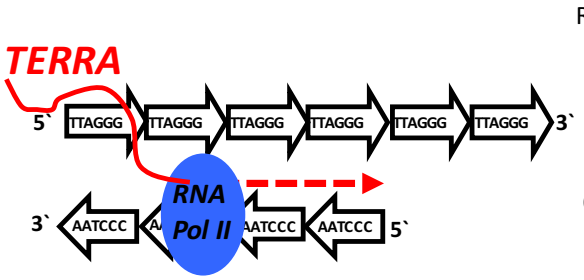
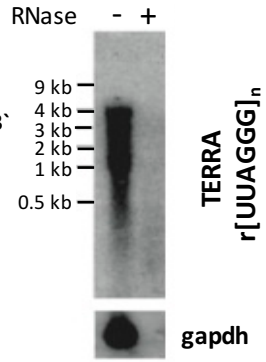
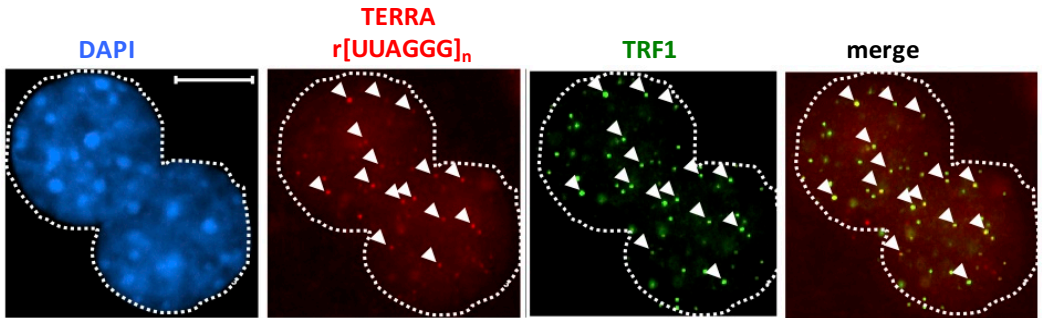


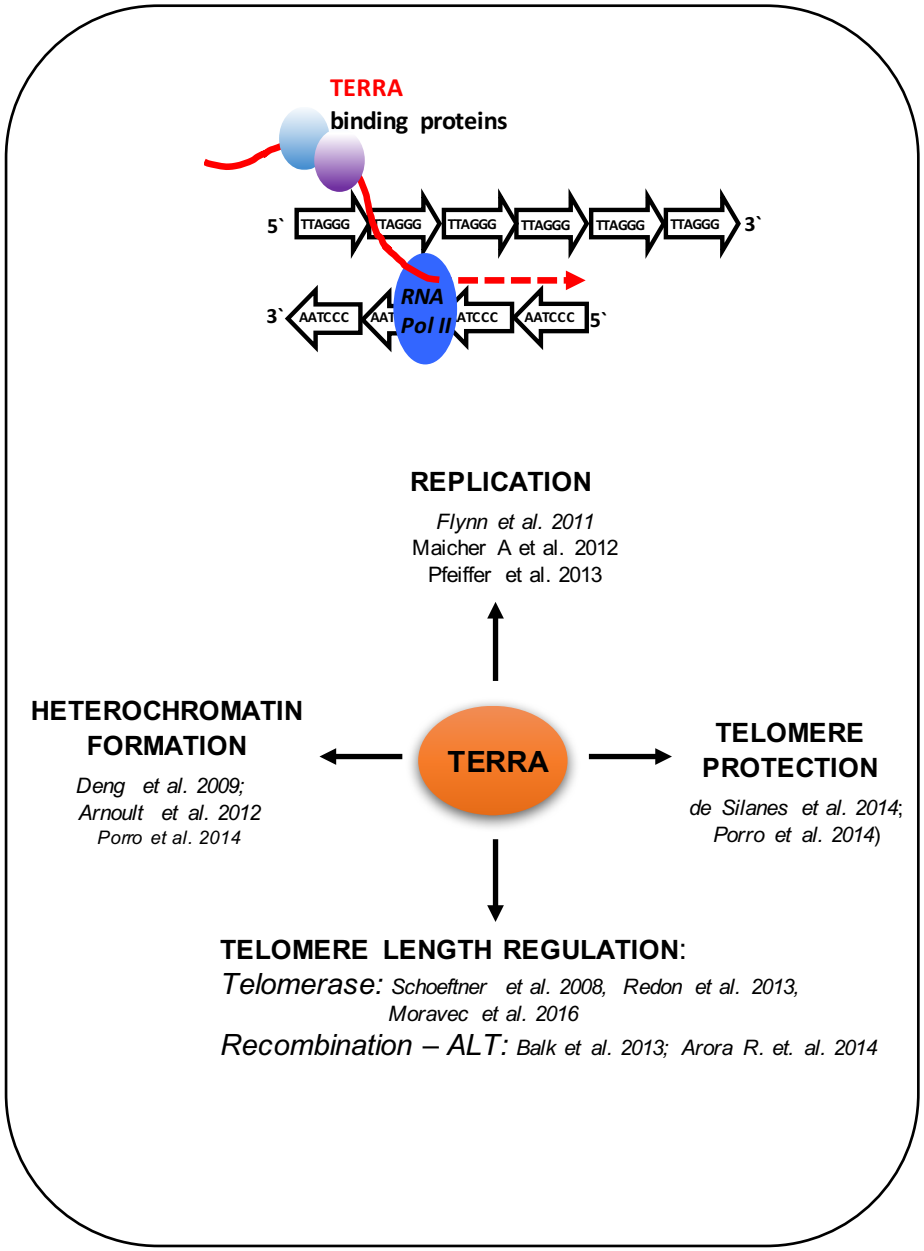
Transcription by RNA Pol II

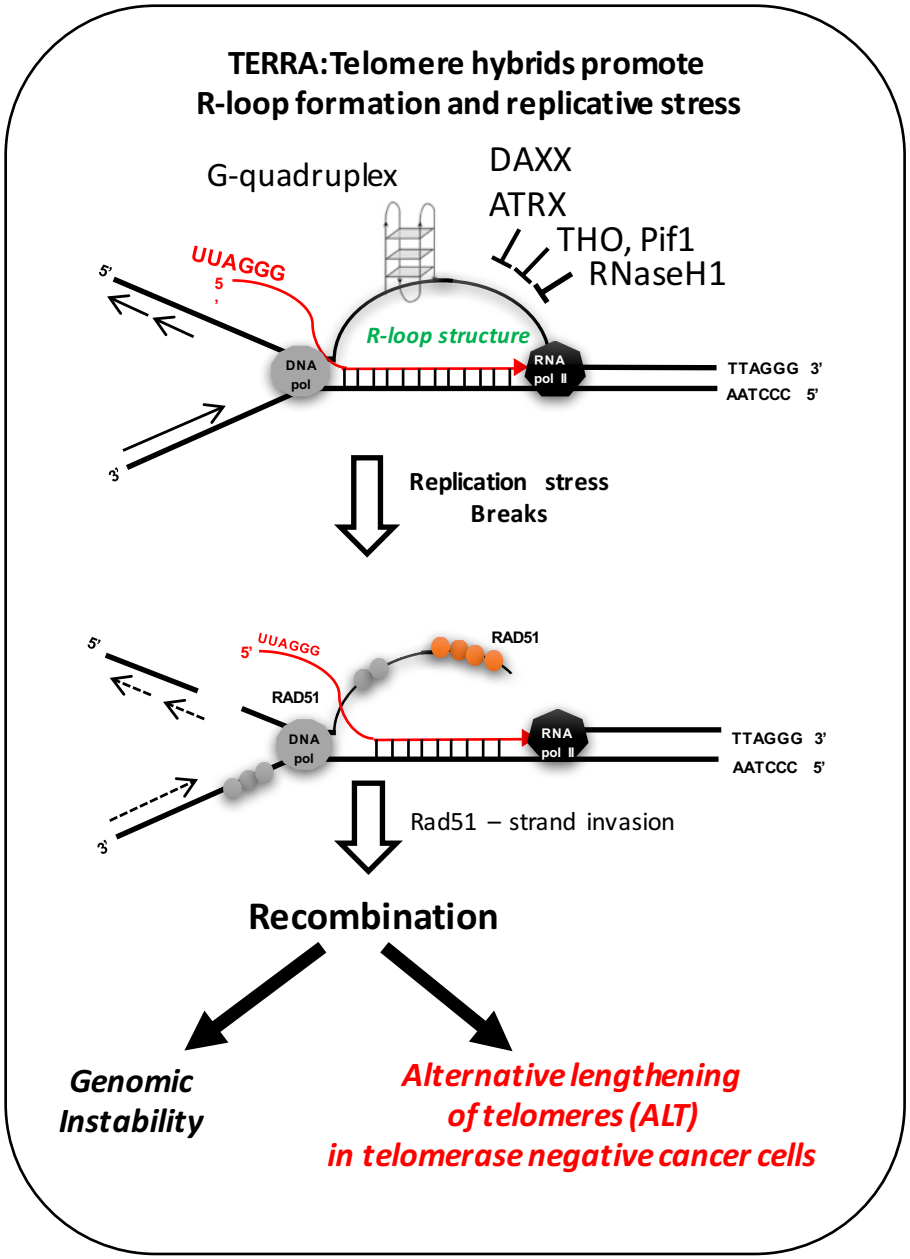




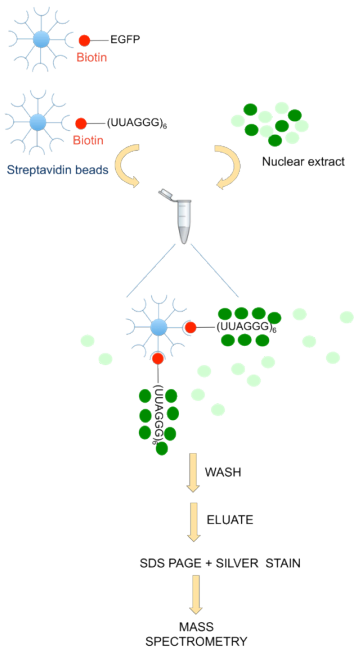
Association with telomeric chromatin

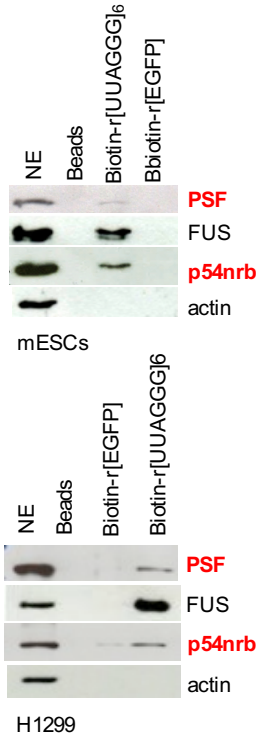
TERRA interferes with telomere function at multiple levels

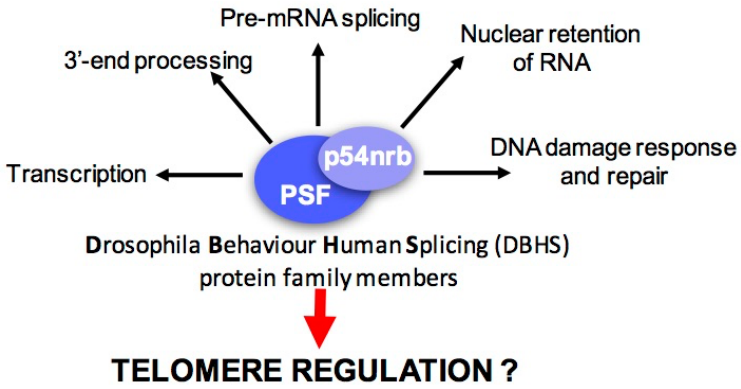


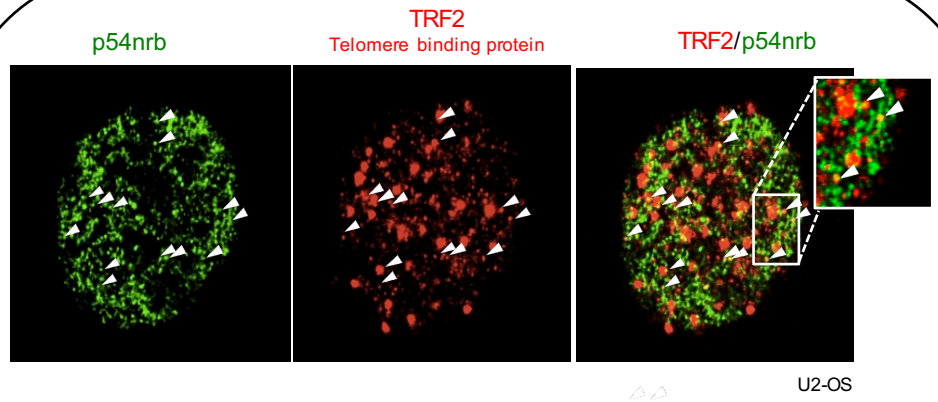


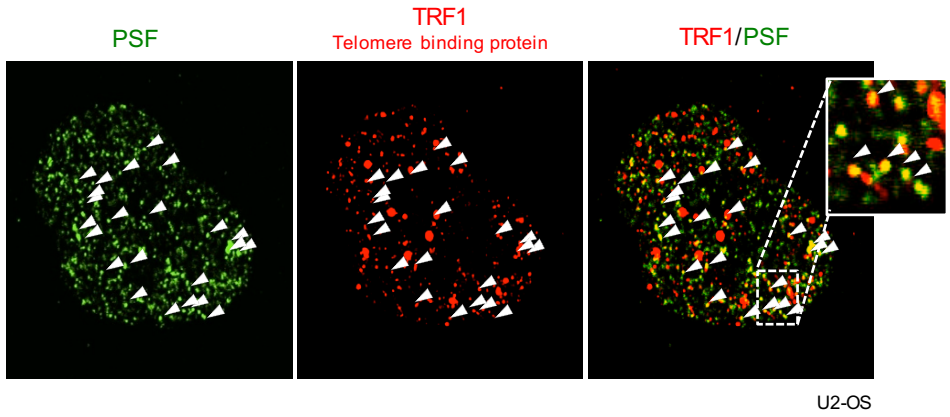
TERRA RNA pull-down identifies p54nrb and PSF as novel telomere proteins

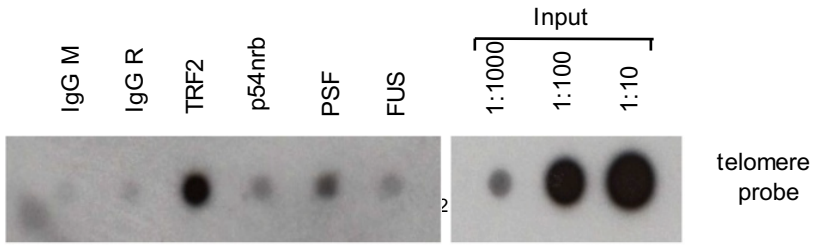




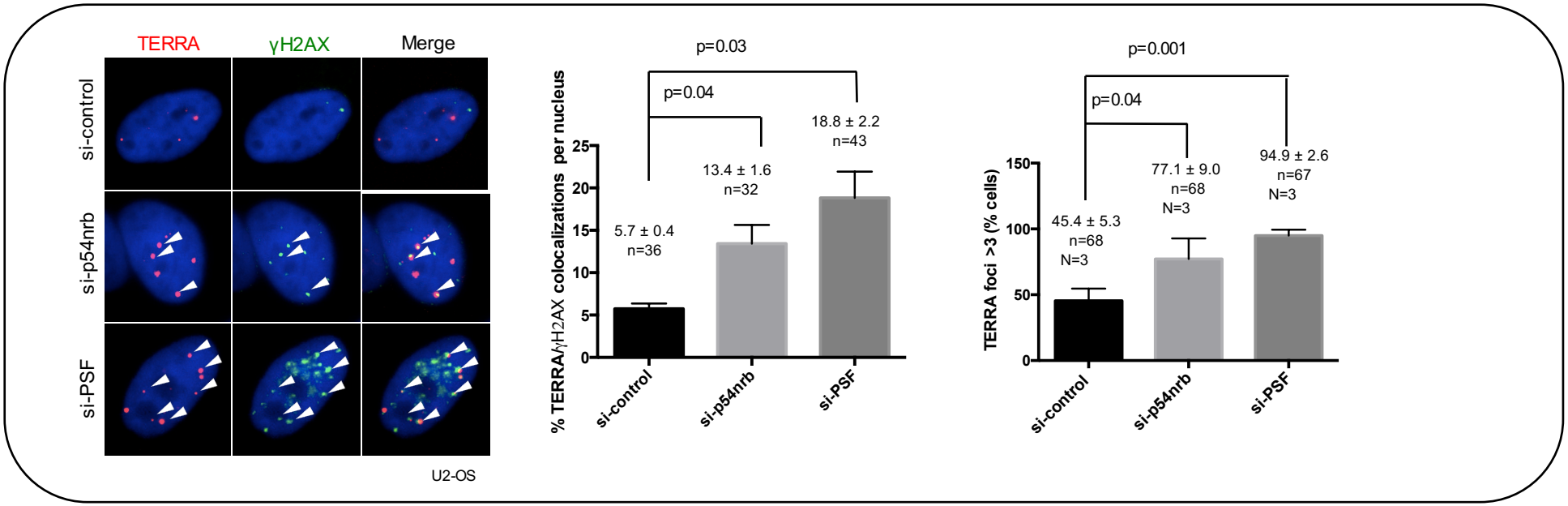


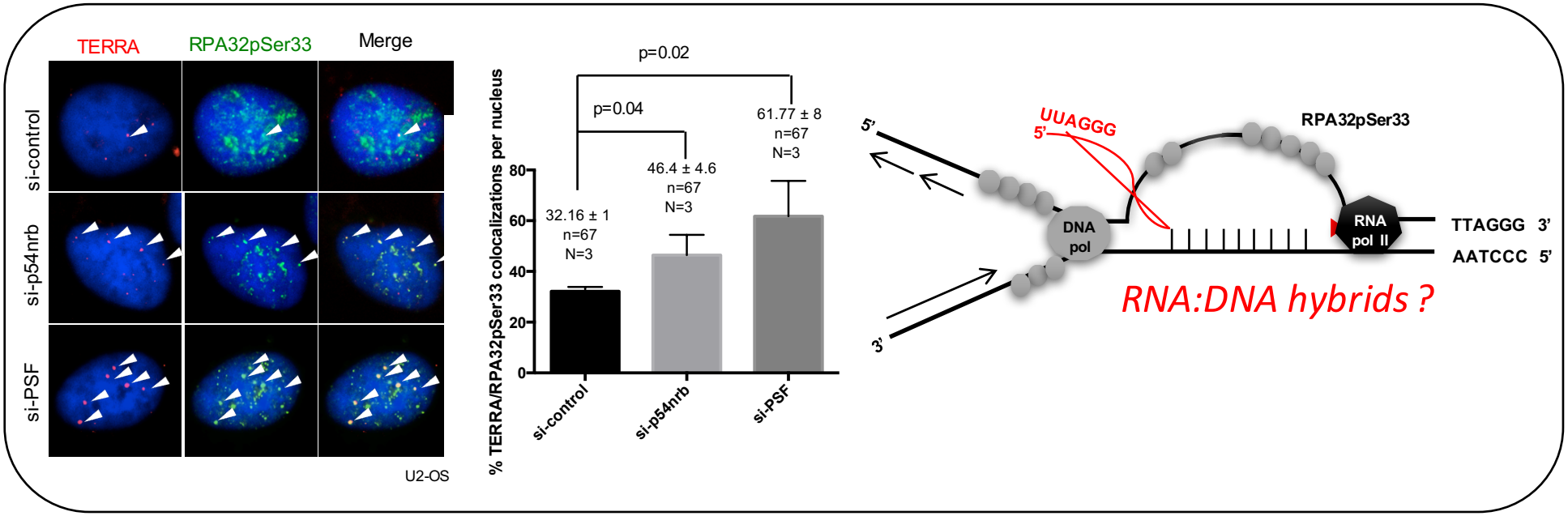




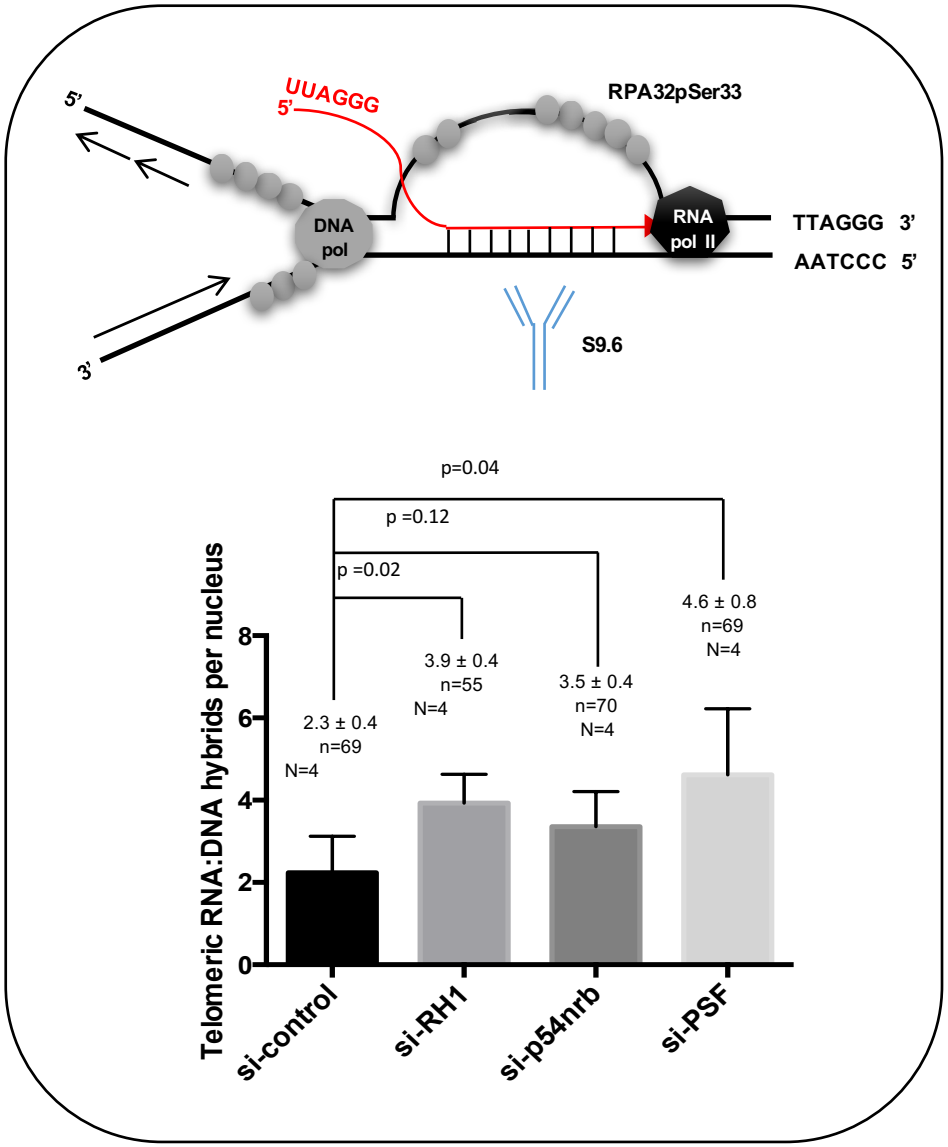


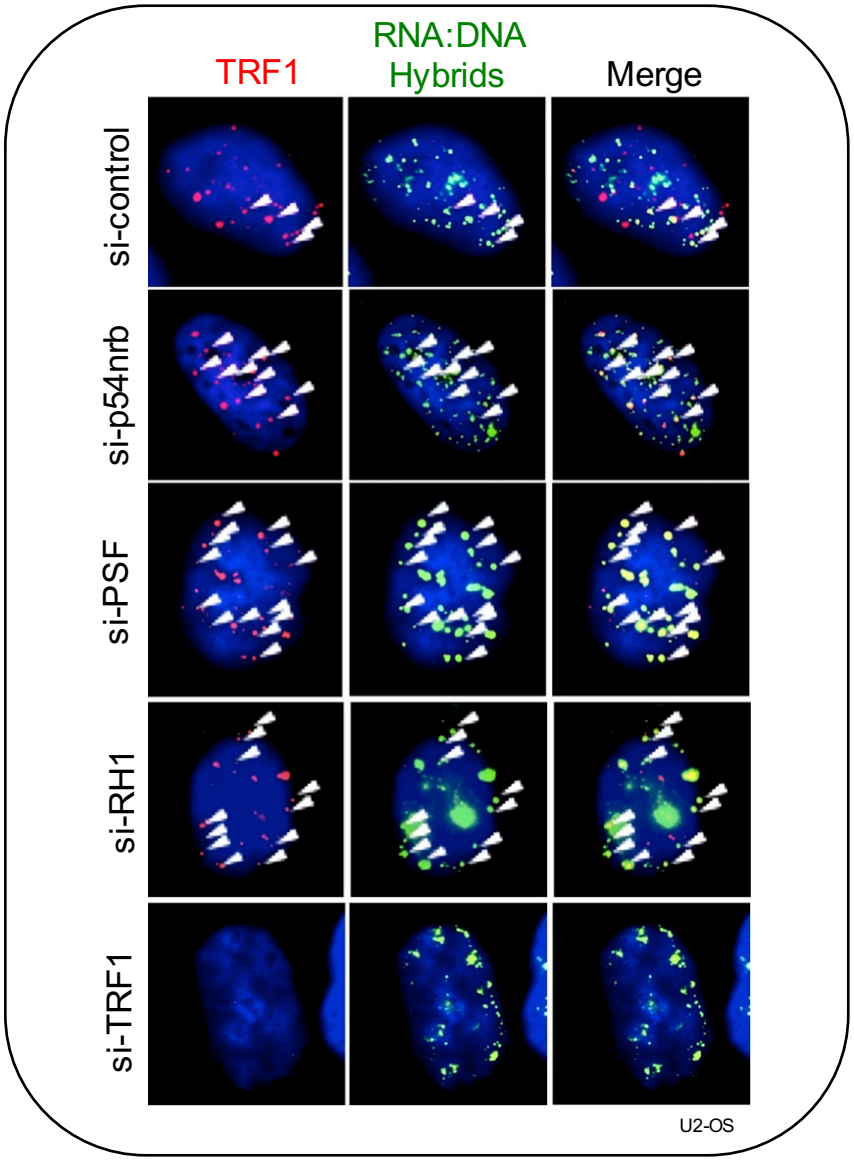
Loss of p54nrb and PSF induces replicative stress





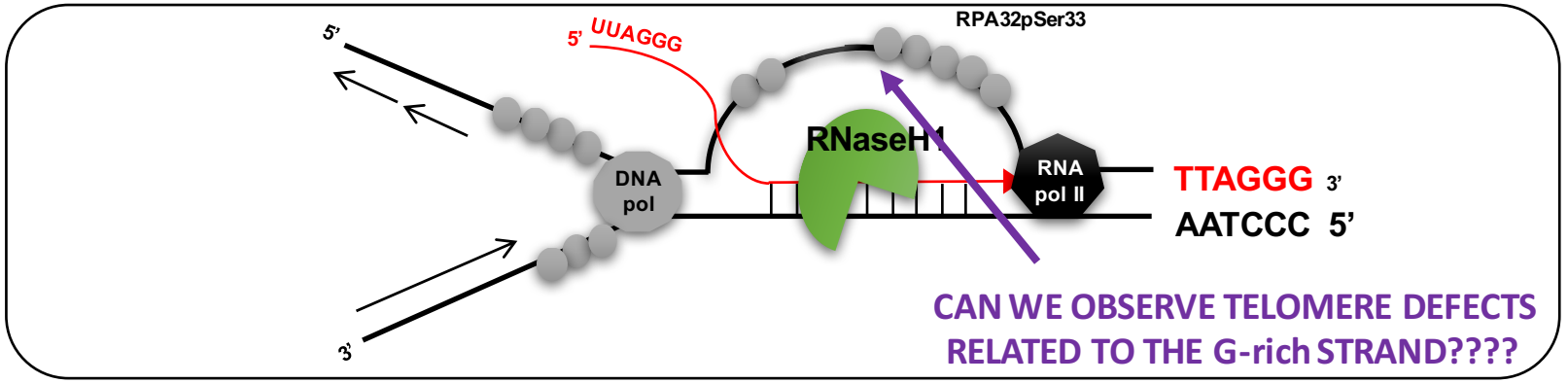
p54nrb and PSF repress RNA:DNA hybrids formation at telomeres

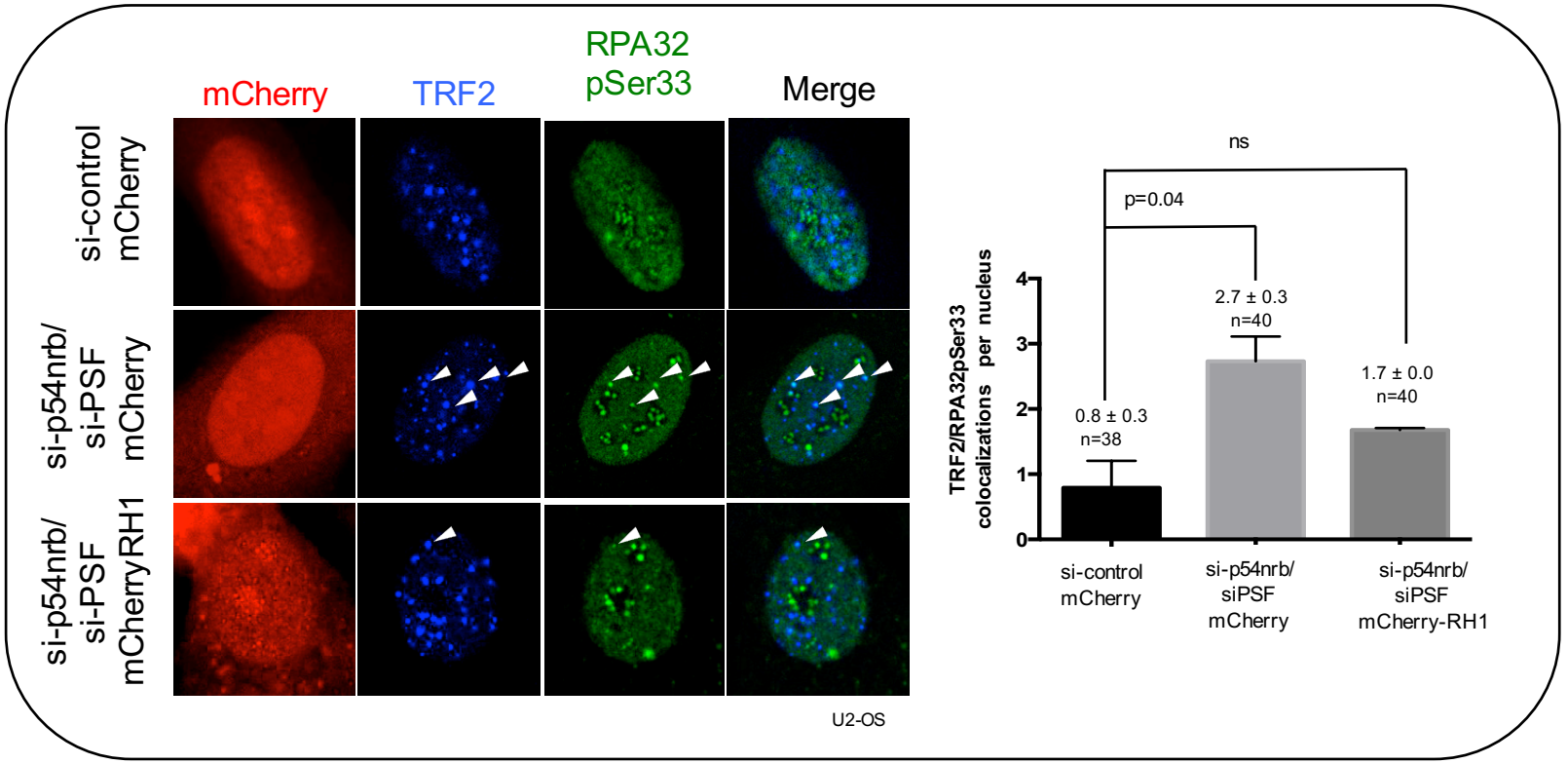




p54nrb and PSF suppress formation of telomeric RNA:DNA hybrids

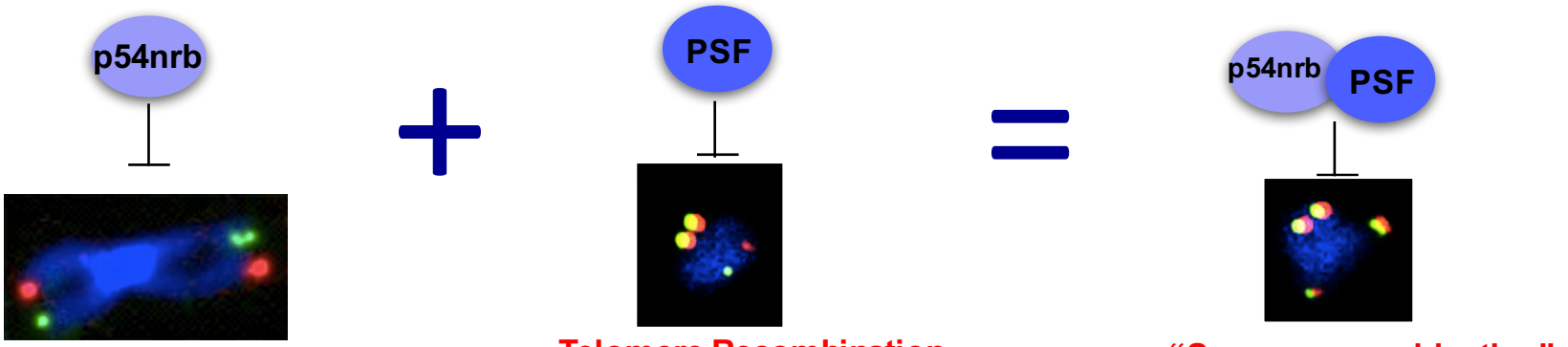
Overexpression of RNaseH1 rescues replication stress at p54nrb/PSF deficient telomeres





Replicative stress induced by PSF/p54nrb depletion is caused by telomeric RNA:DNA hybrid formation

Telomere CO-FISH identifies p54nrb/PSF are suppressor of fragility and recombination

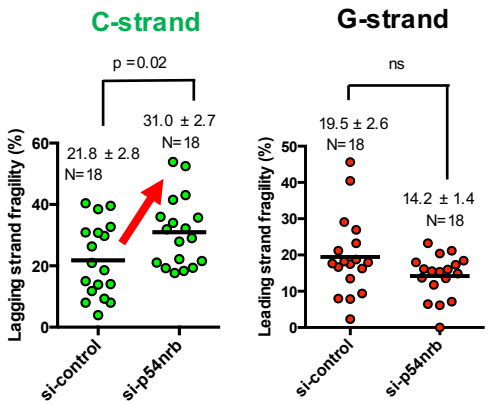


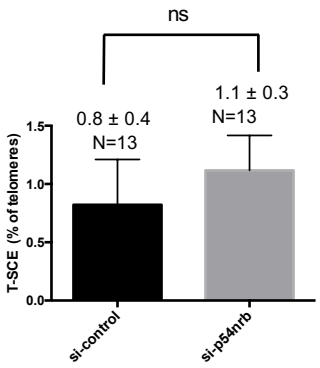
C-strand fragility (CCCTAA)

Telomere Recombination

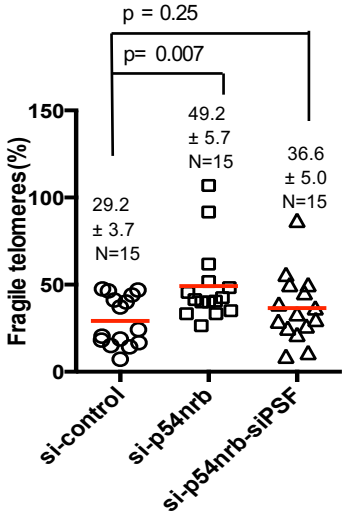
"Super – recombination"

si-p54nrb

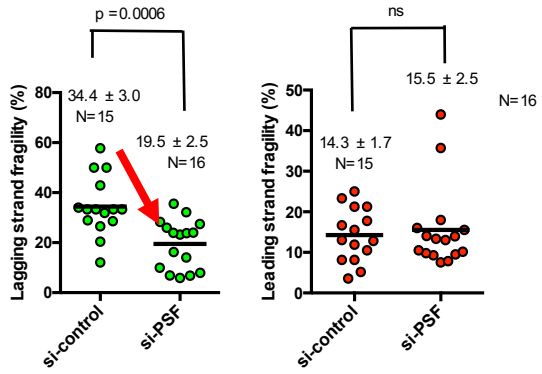


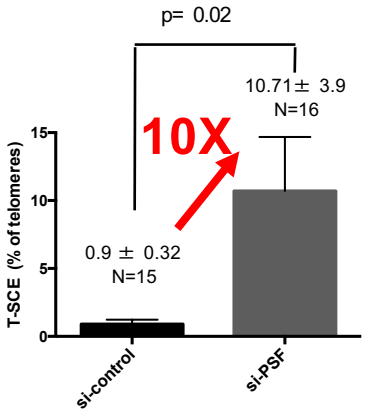


si-p54nrb si-PSF

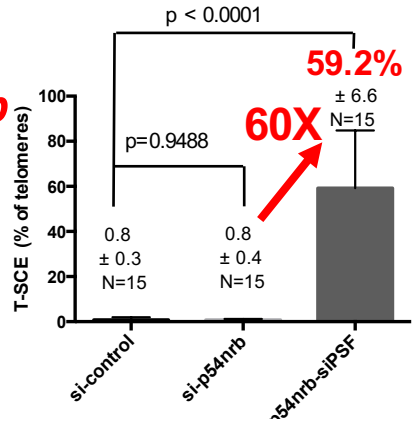


si-PSF





si-p54nrb si-PSF

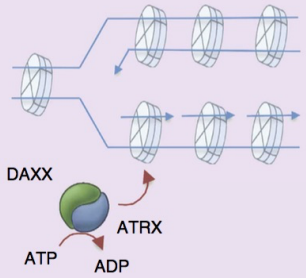


ATRX and DAXX localize to PML bodies and suppress telomere recombination

DAXX: Death-Domain Protein 6
H3.3 nucleosome assembly factor

ATRX: Alpha Thalassemia/Mental Retardation Syndrome X-Linked
ATPase / helicase domain; SWI/SNF family of chromatin remodeling proteins

ATRX/DAXX Wild-type
→ Suppression of ALT

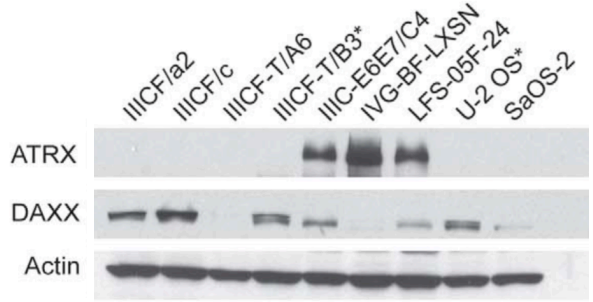


-Replication-independent H3.3 nucleosome assembly at telomeres, centromeres and repeat sequences
- Maintenance of DNA in B-form

Modified from Clynes et al. Nat. Commun. 2015

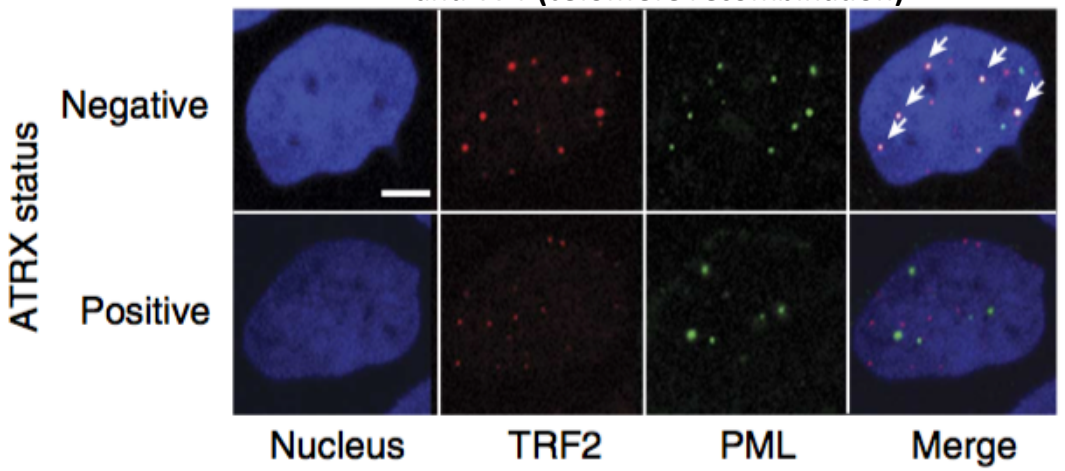
Telomerase positive cancer cells
→ Suppression of ALT

ALT cancer cells lack ATRX/DAXX expression



Lovejoy et al. 2012

Loss of ATRX (DAXX) results in increased APB numbers and ALT (telomere recombination)



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