How telomeres protect chromosome ends from engaging the DNA damage response

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NIA
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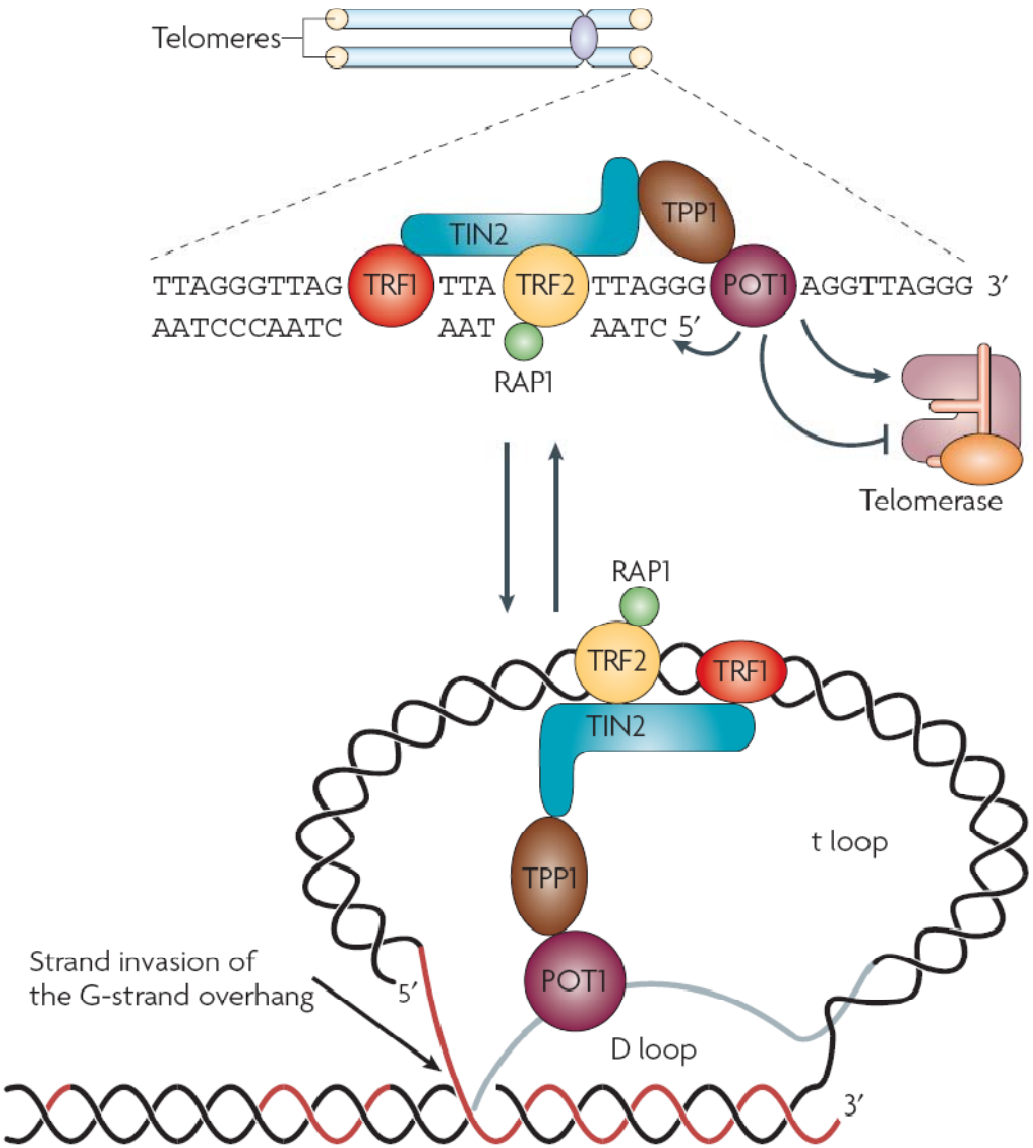
Mammalian telomeres



telomere uncapping

absence of telomerase

telomere dysfunction

DNA damage response

wt p53 | p53 loss

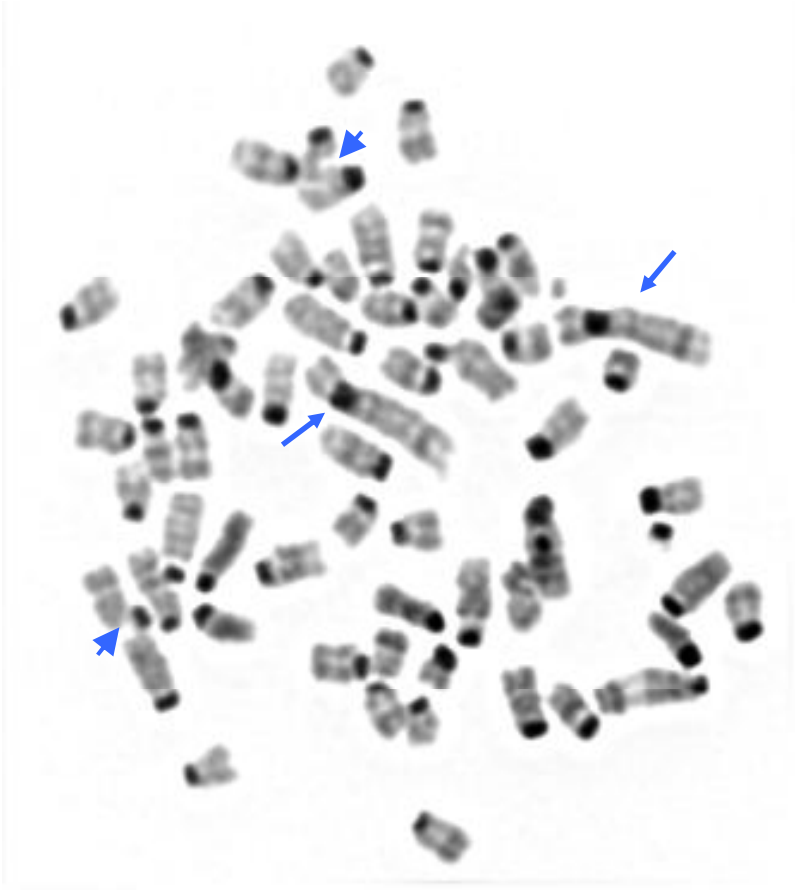
apoptosis senescence

cancer initiation

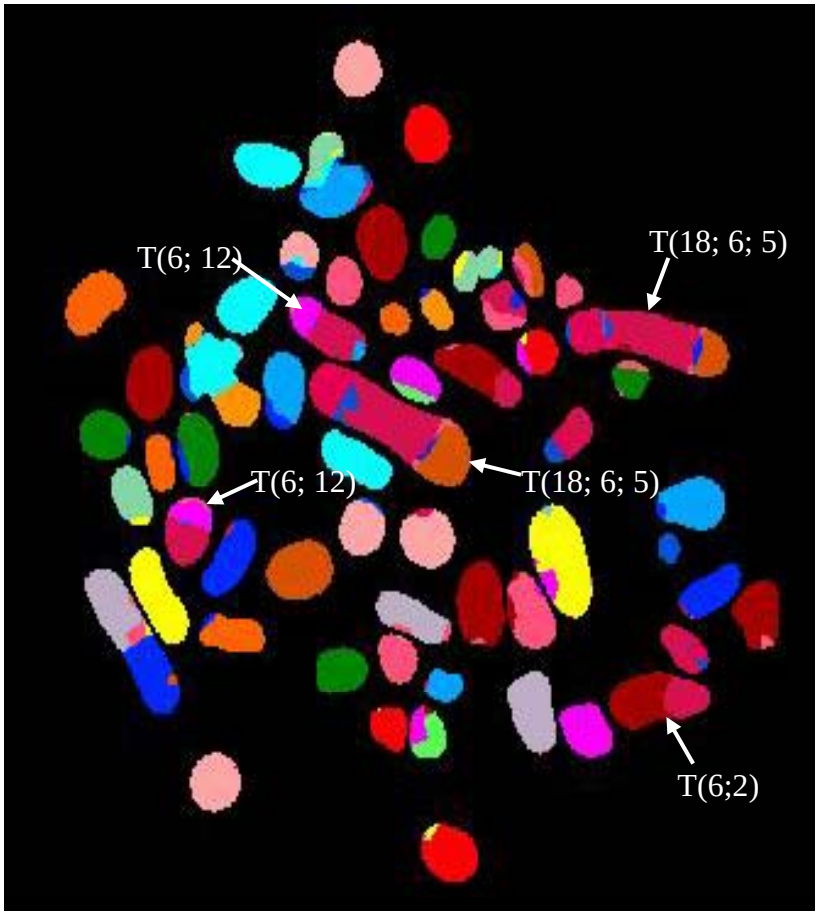
Deng et al., Nat Rev Cancer2008

Consequence of natural telomere attrition: multiple non-reciprocal chromosomal translocations in tumors with short telomeres

Breast tumor TD 574



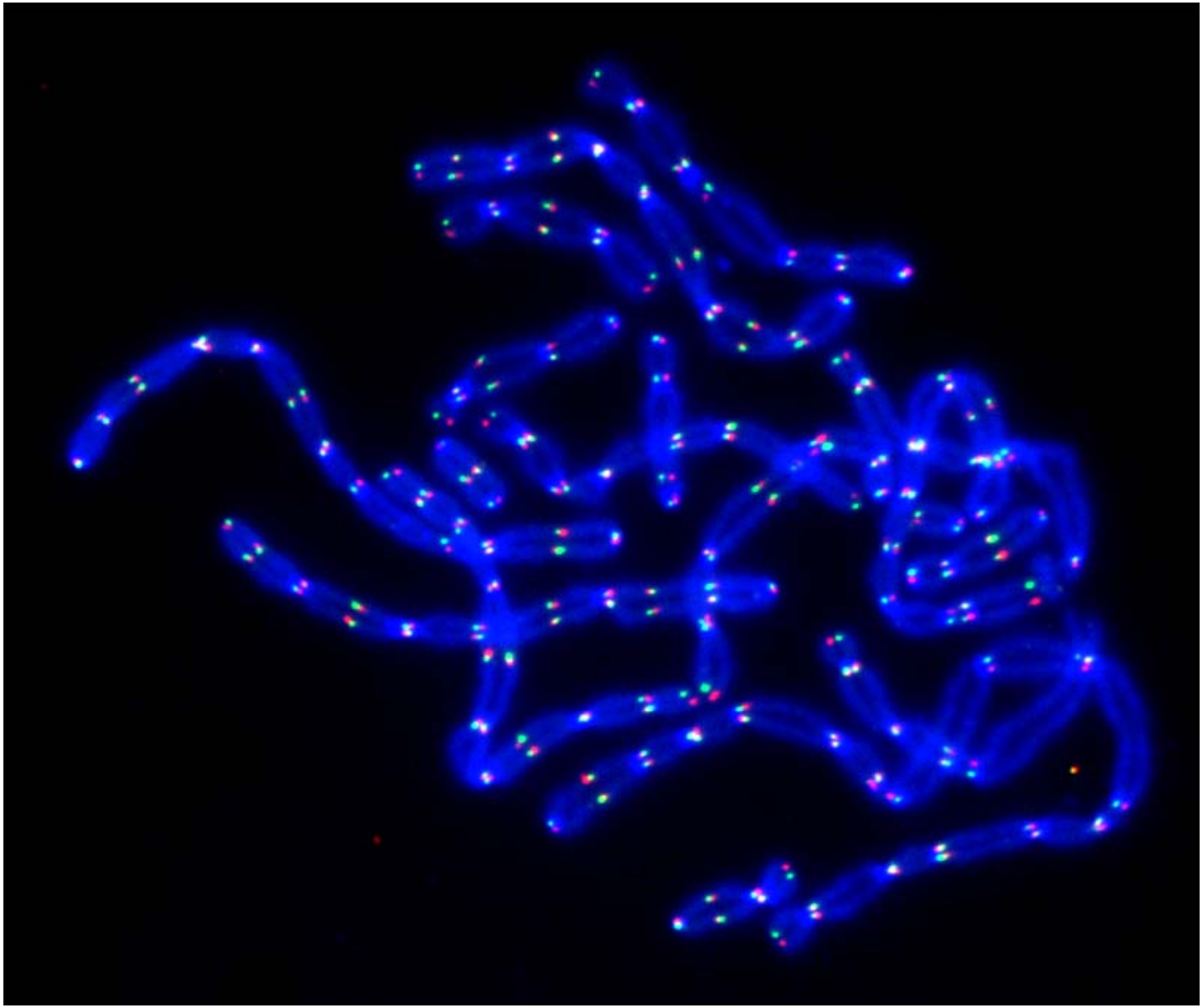
DAPI image



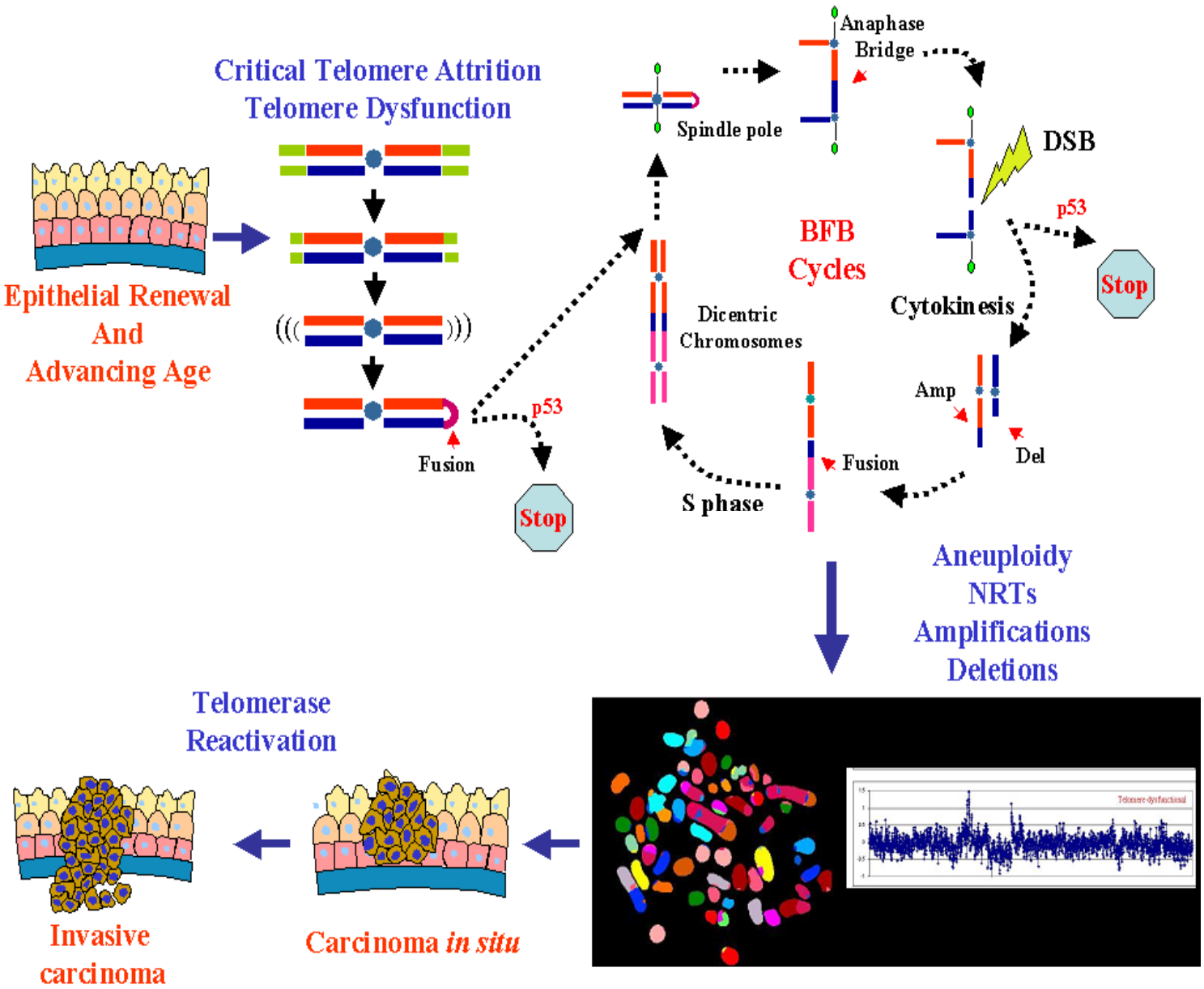
SKY image

Artandi et al., Nature 2000

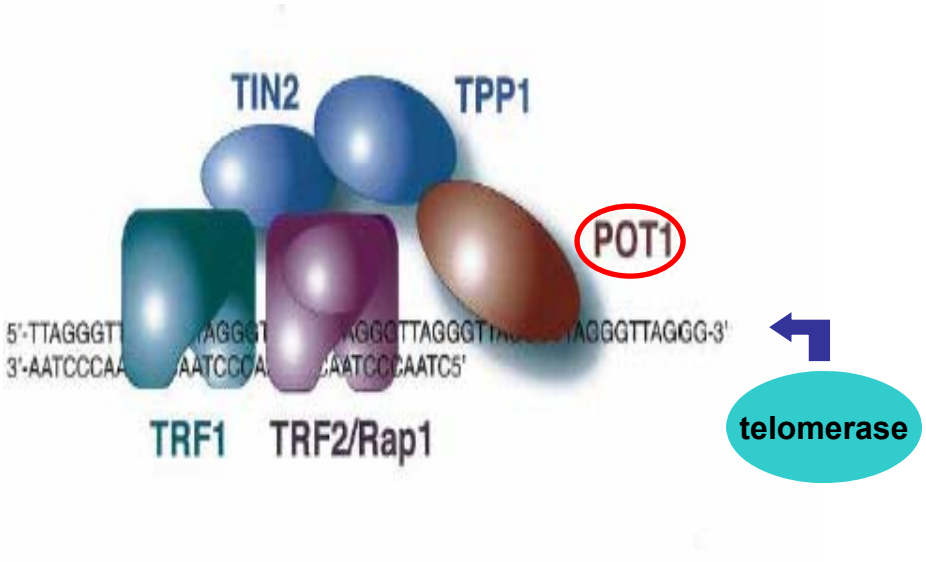
Massive end-to-end chromosome fusions in the absence of Trf2



Dysfunctional telomeres promote genomic instability



Six subunits in shelterin interacts with the t-loop: TRF1,TRF2,TIN2,RAP1,TPP1 and POT1

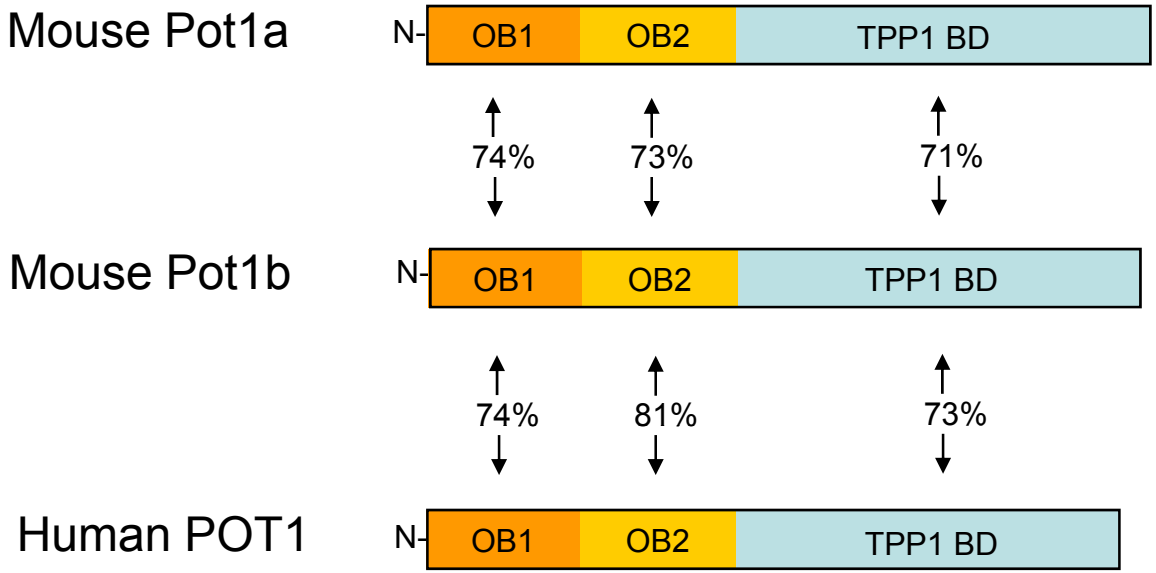


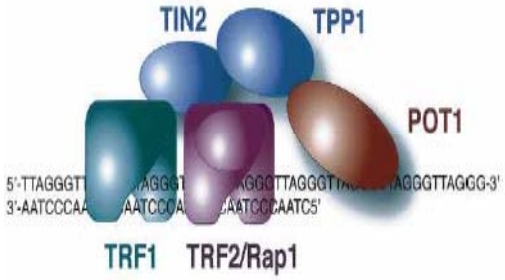
POT1-Protection of Telomere 1

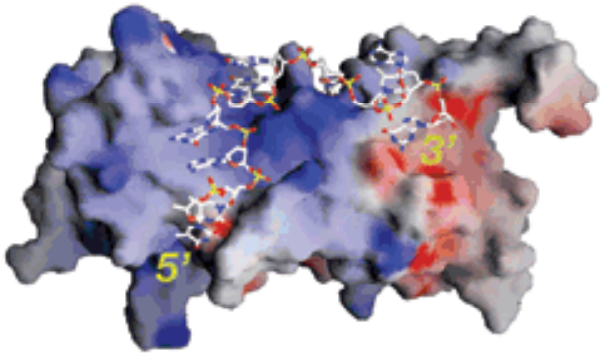
Required for telomere end protection

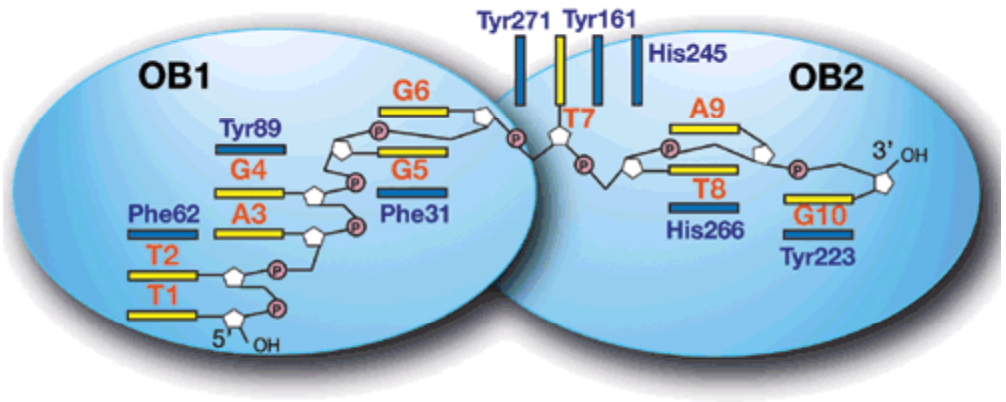
Negative regulator of telomere length

POT1 structure



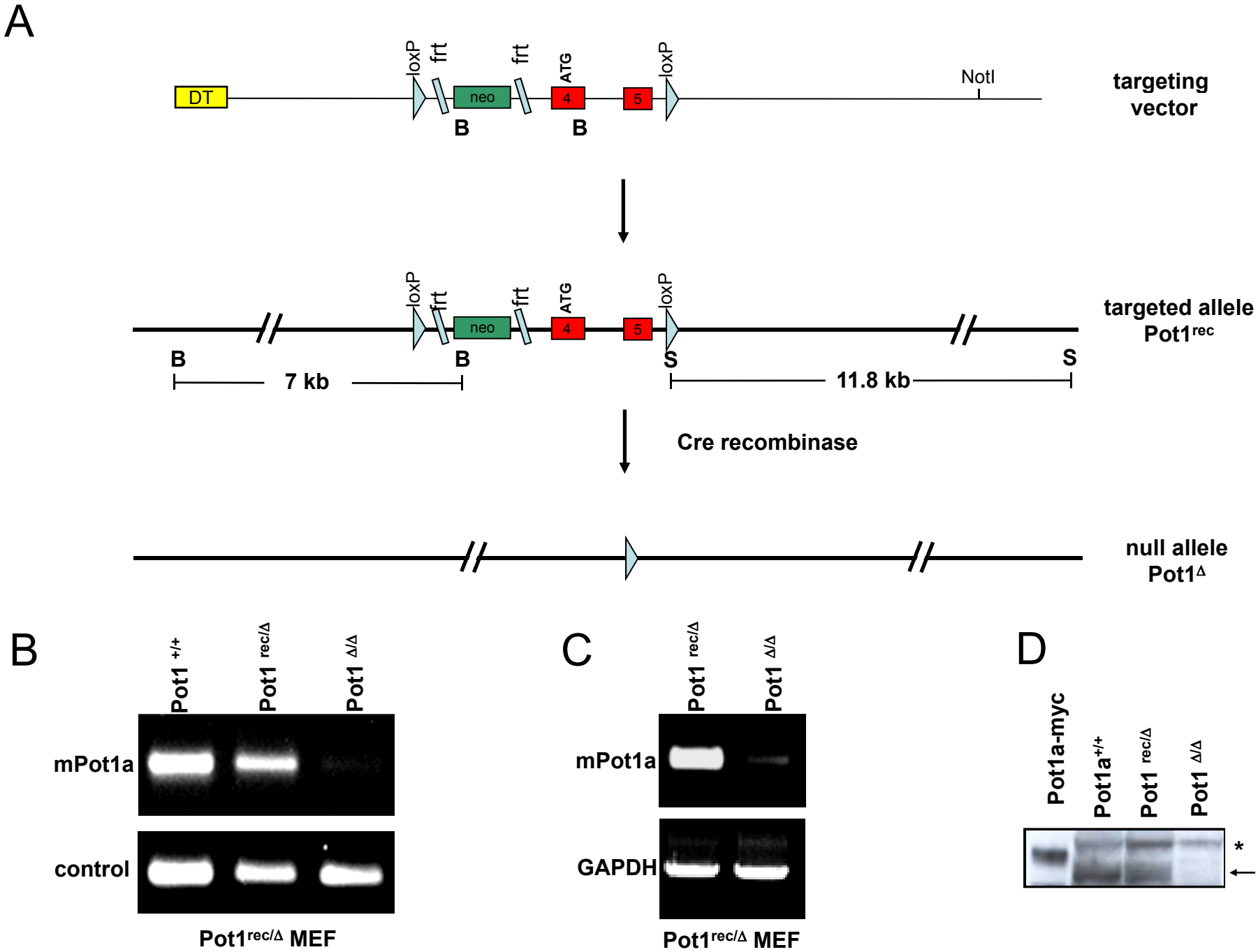






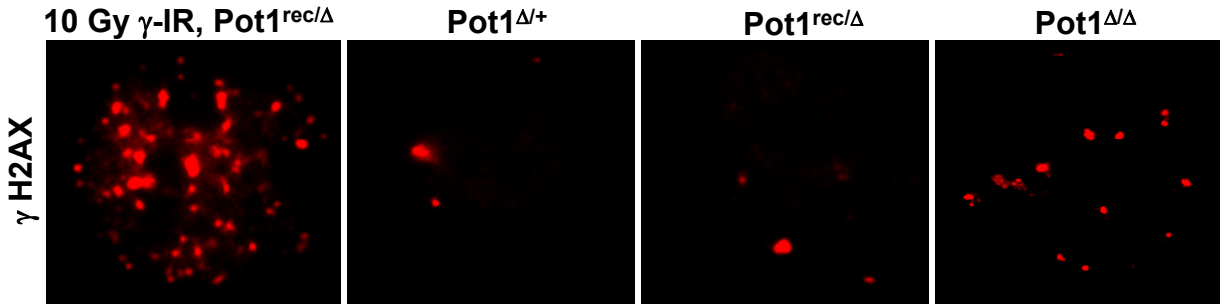
Lei et al., Nature Struc and Mol Biol 2004

Conditional Deletion of Mouse *Pot1a*



Elevated DNA damage response in *Pot1a* deleted cells

A



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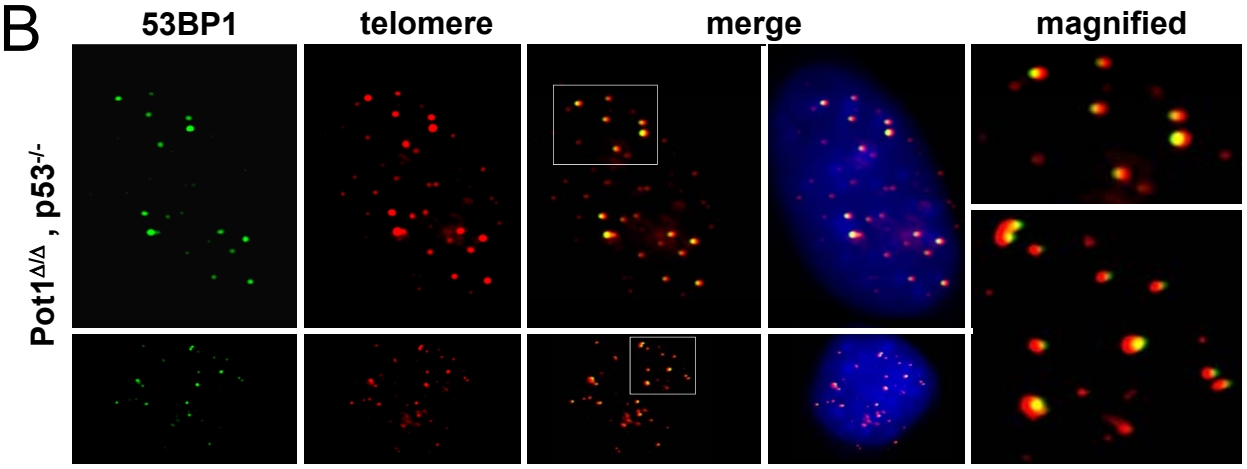

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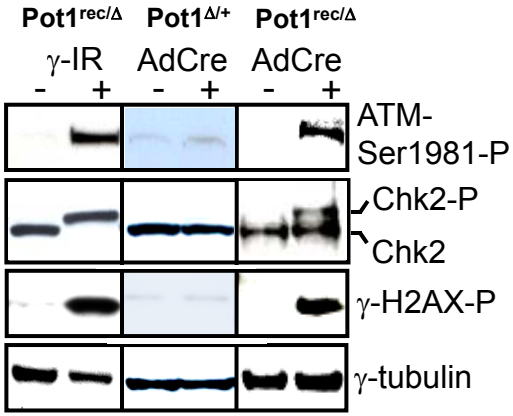
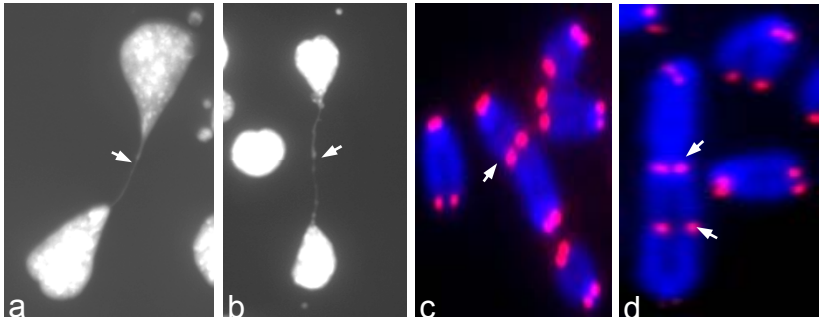


Pot1a loss leads to multiple cytogenetic aberrations and malignant transformation

A



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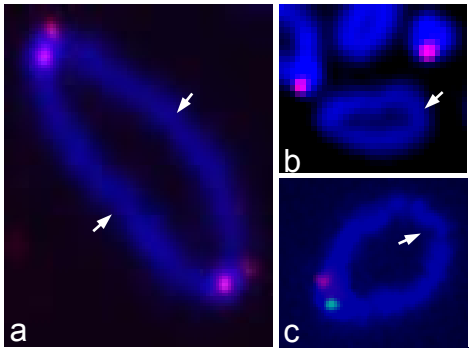

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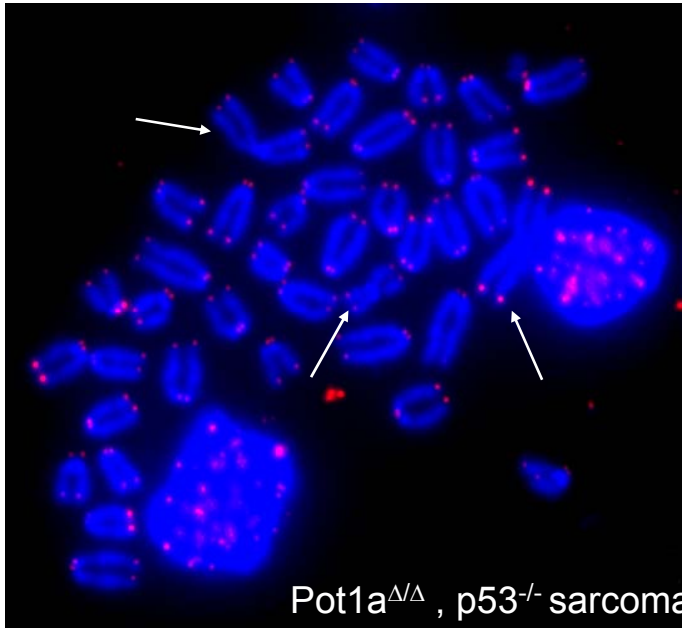

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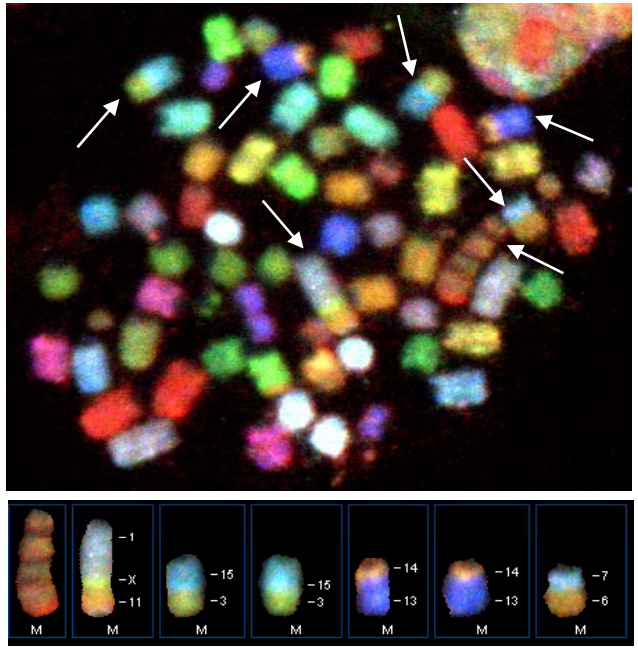
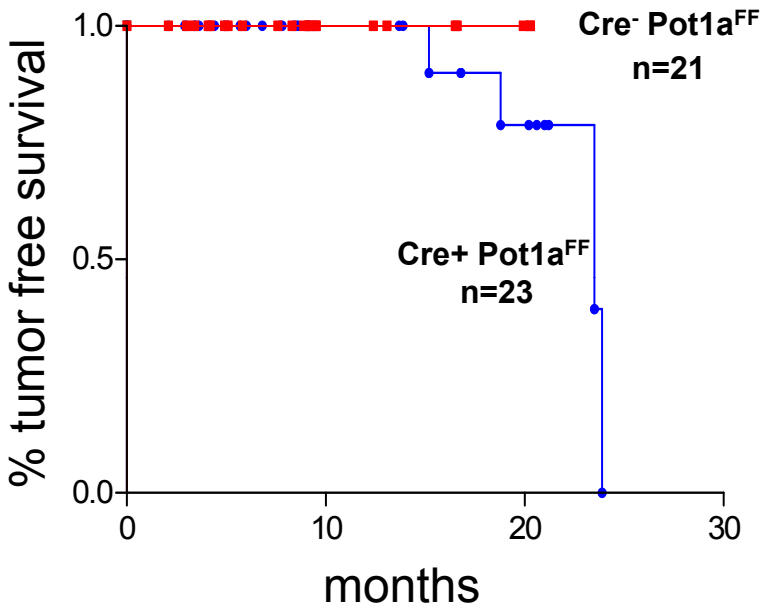
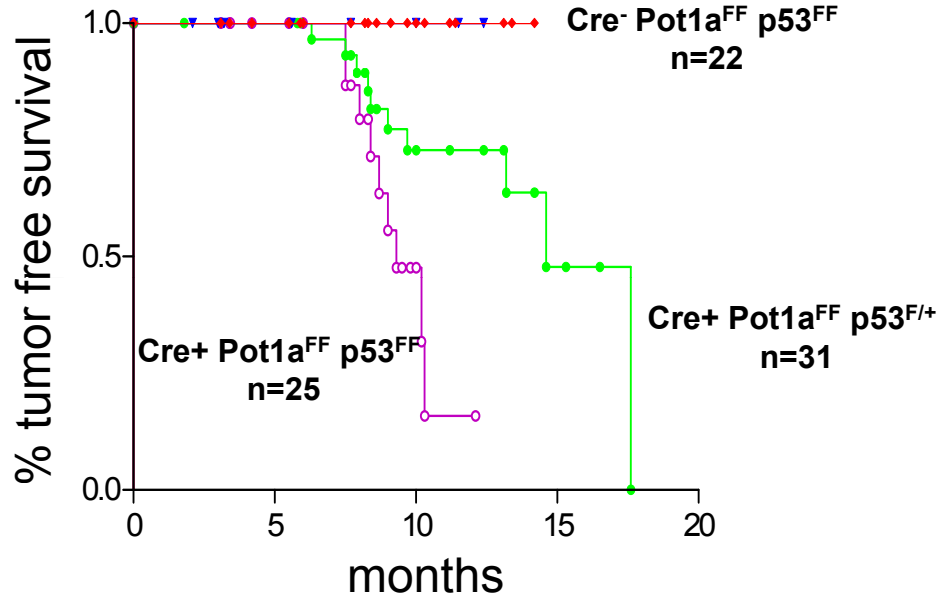
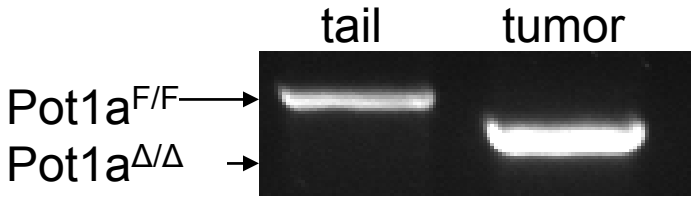


Pot1a$^{\Delta/\Delta}$, p53$^{-/-}$

Acceleration of MMTV-Cre^{+} ; Pot1a^{FF} breast tumors in the absence of p53

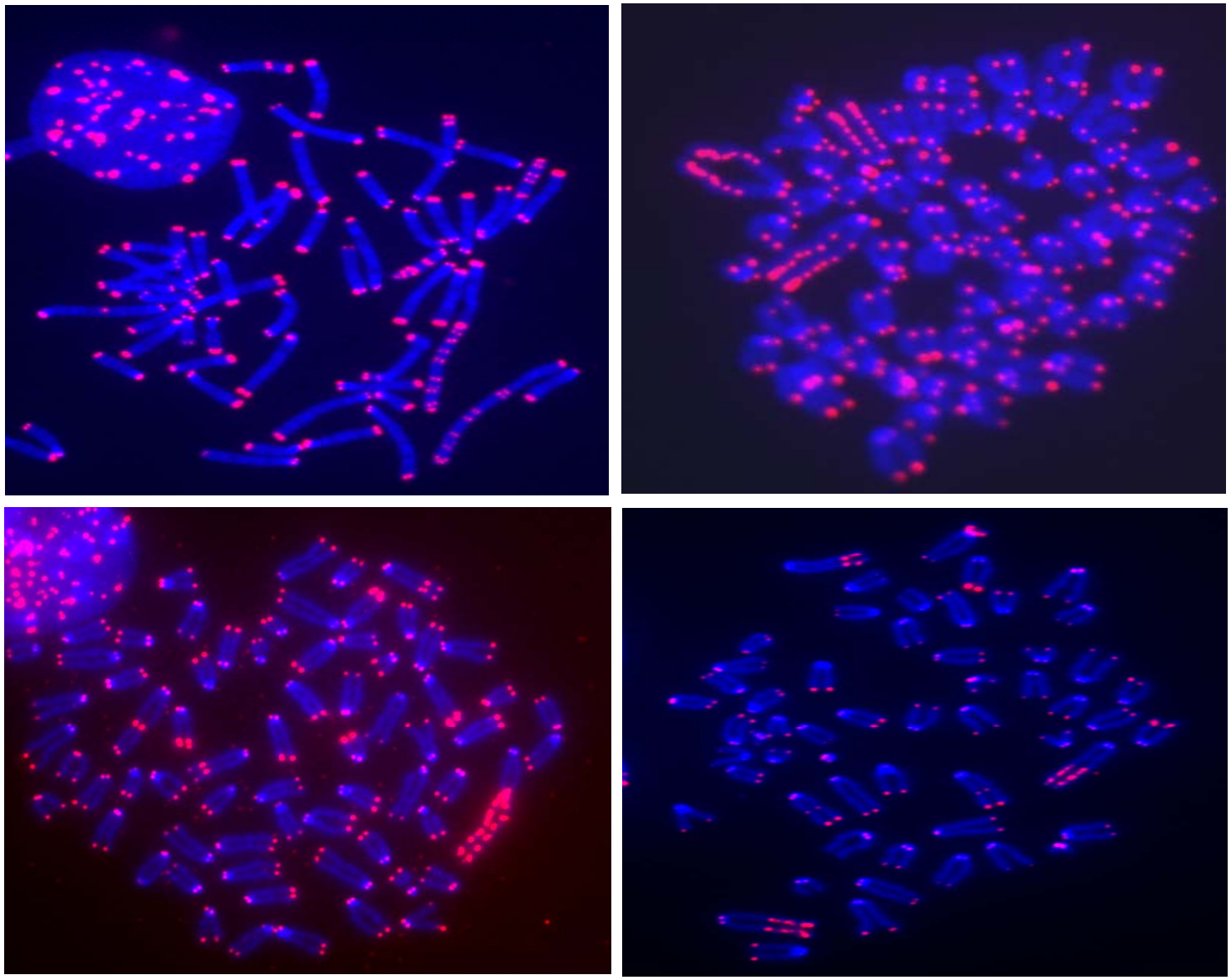




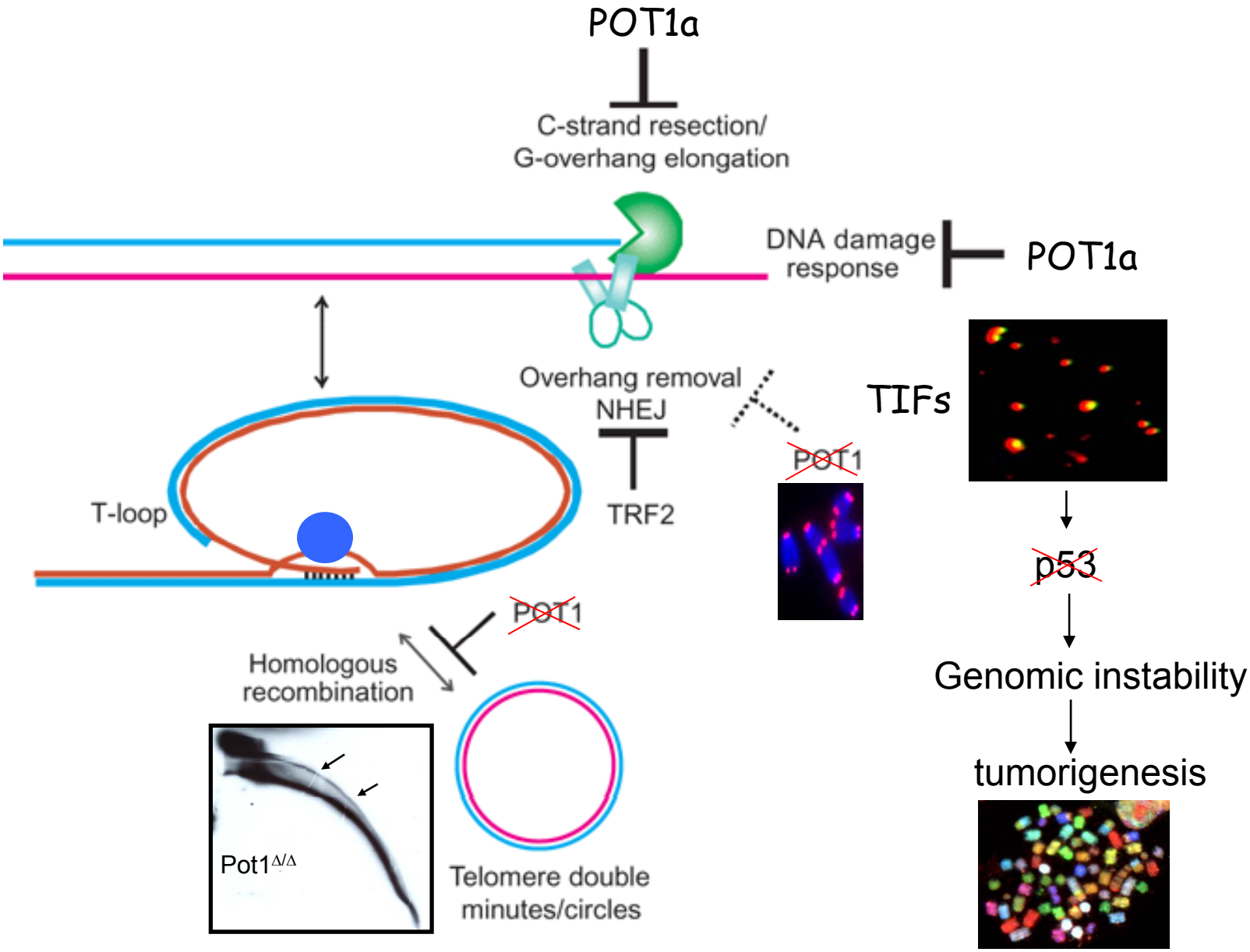


Ling Wu

Massive telomere amplifications in MMTV-Cre^{+} Pot1a$^{\Delta/\Delta}$ breast tumors

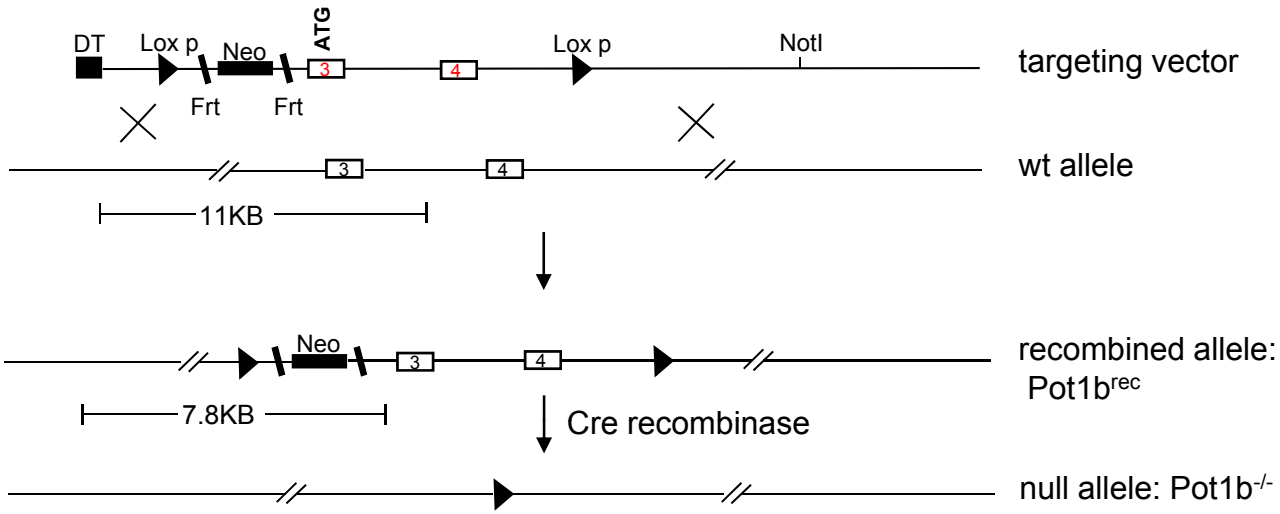


POT1a is required for telomere end protection



Conditional deletion of mouse *Pot1b*

A



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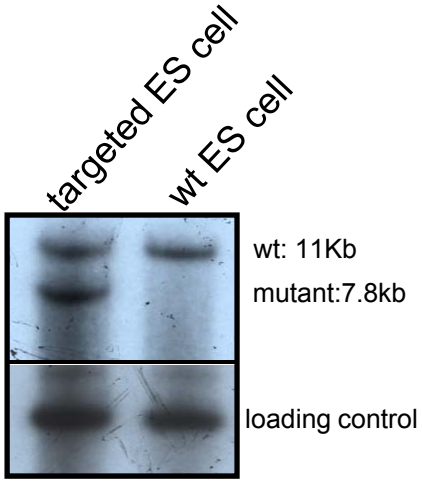

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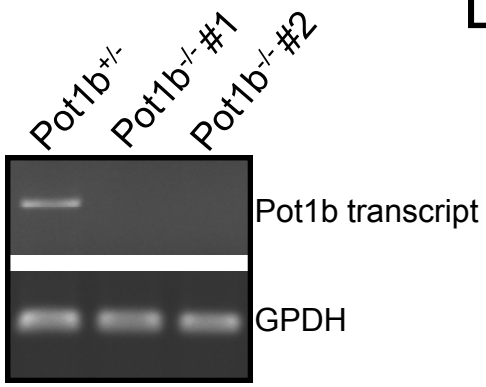

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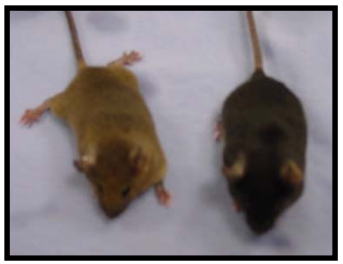
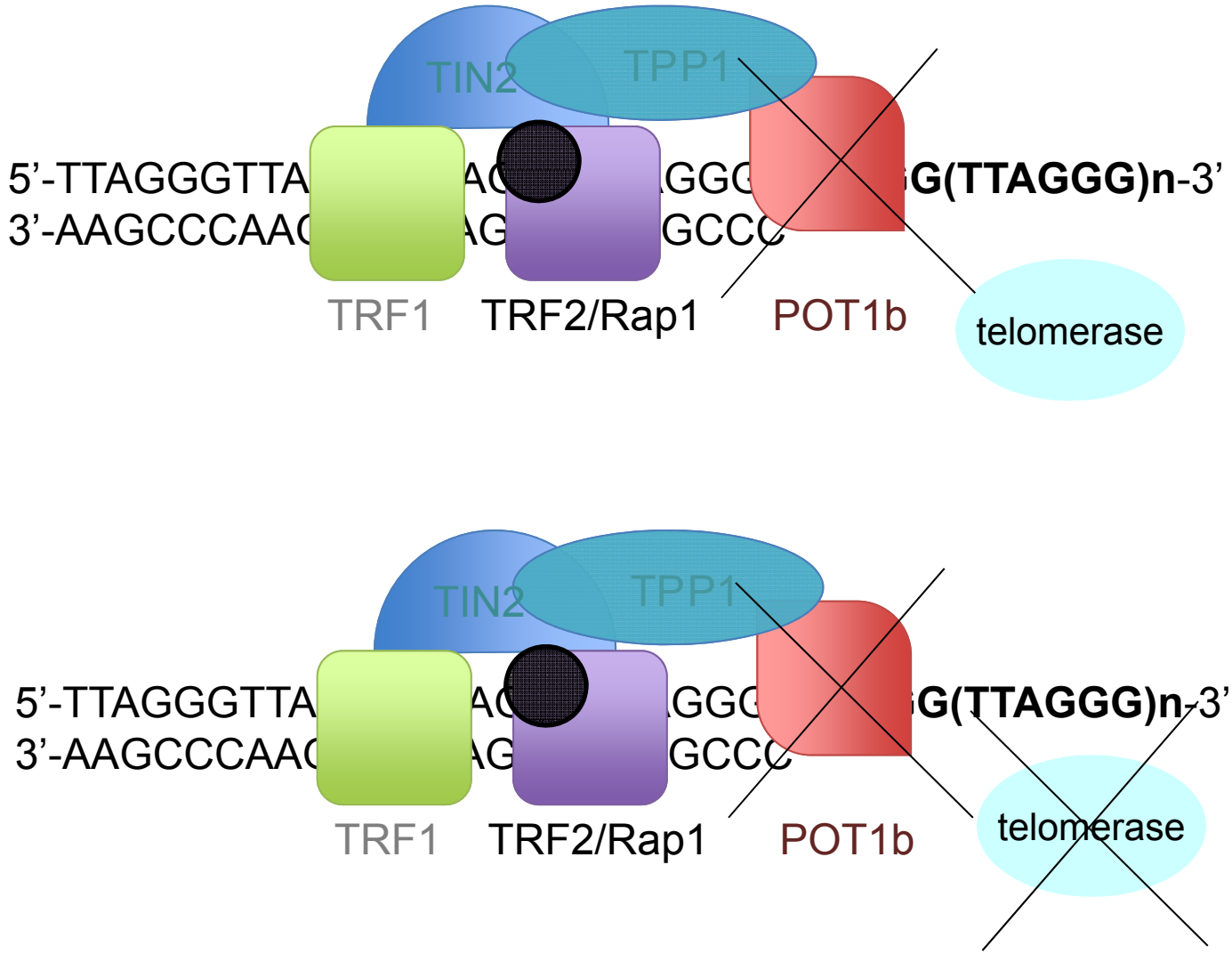
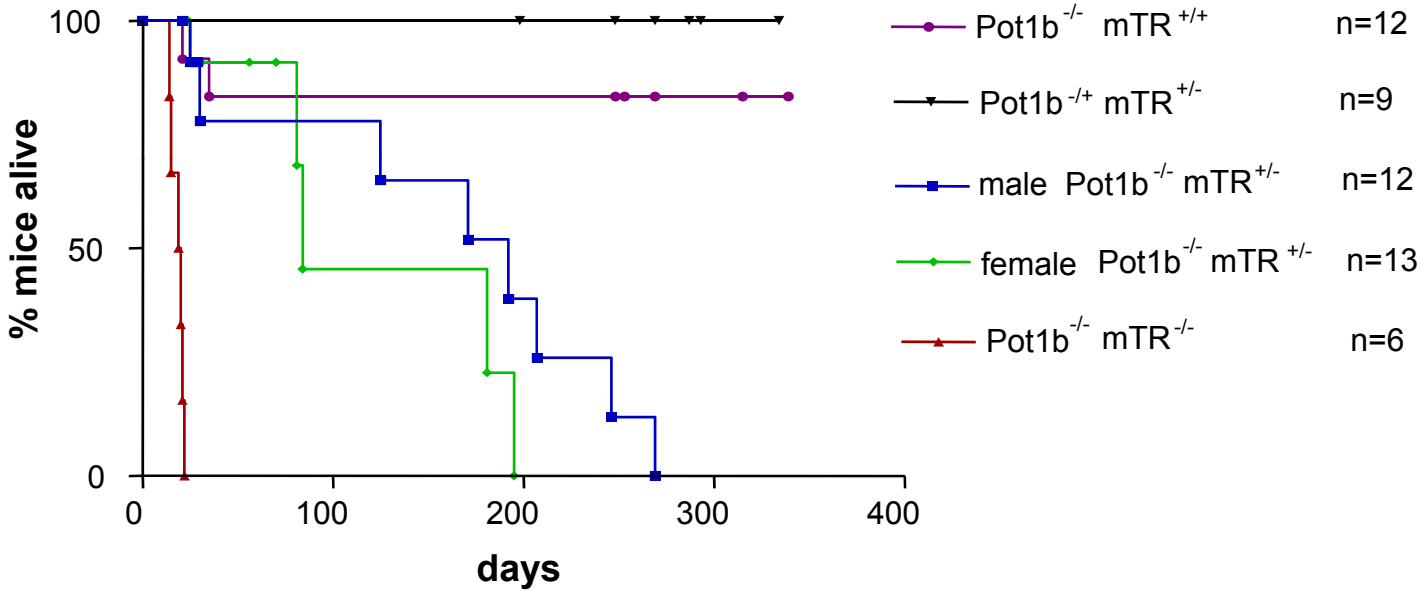


Generating Pot1b null alleles

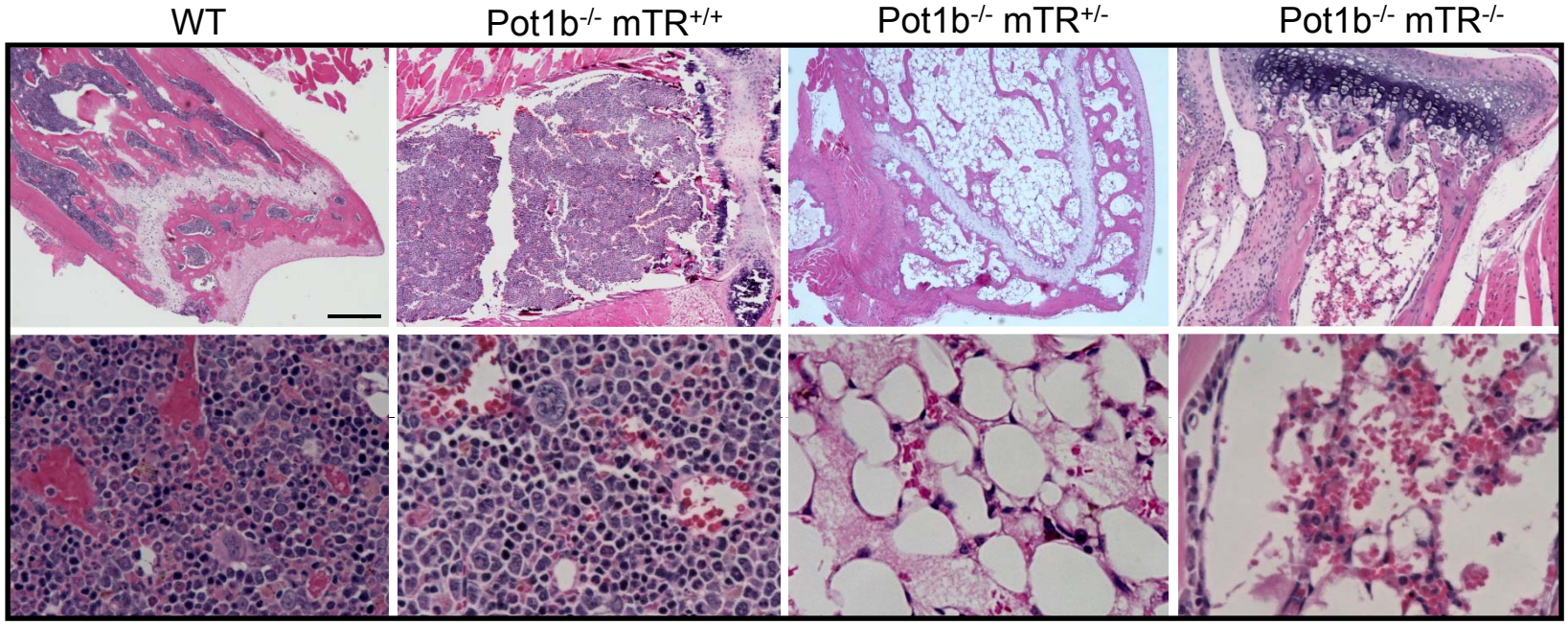


Deletion of Pot1b in the setting of telomerase deficiency results in diminished lifespan



Complete bone marrow failure in Pot1b$^{-/-}$ mTR$^{+/-}$ mice

A



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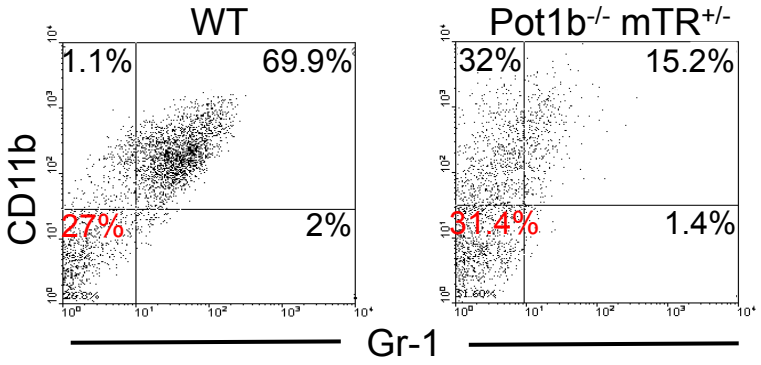
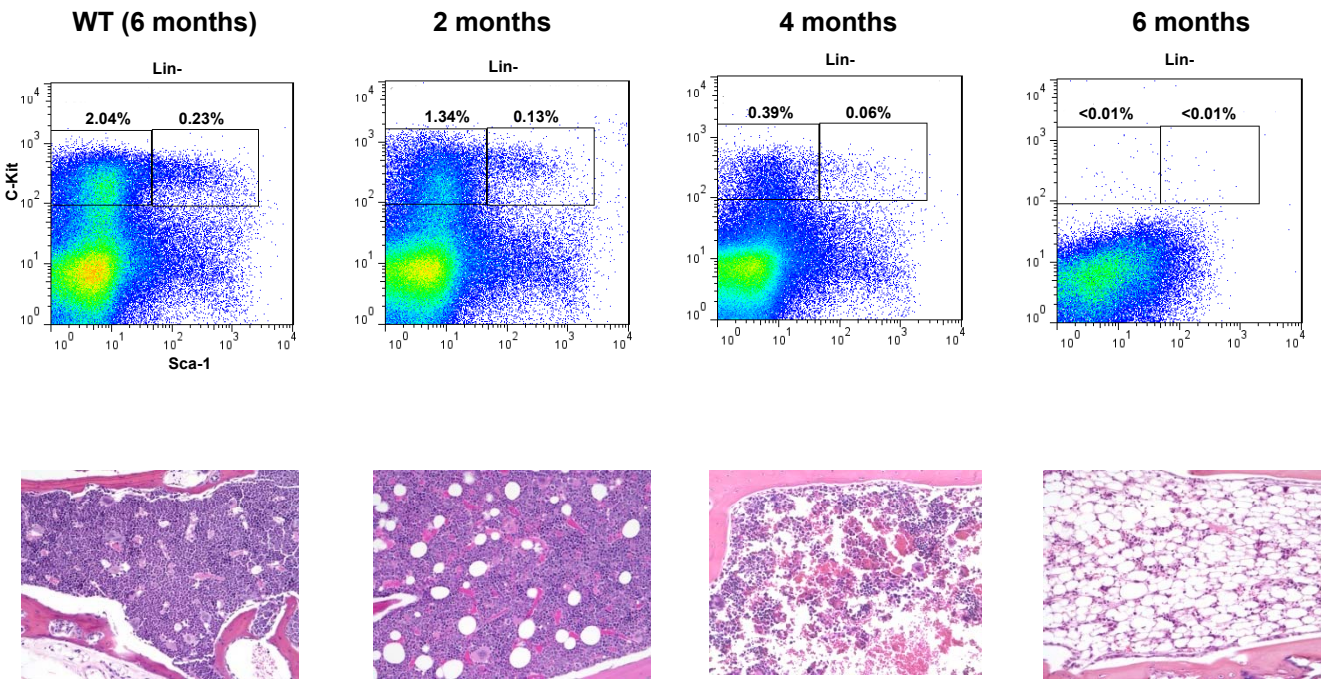


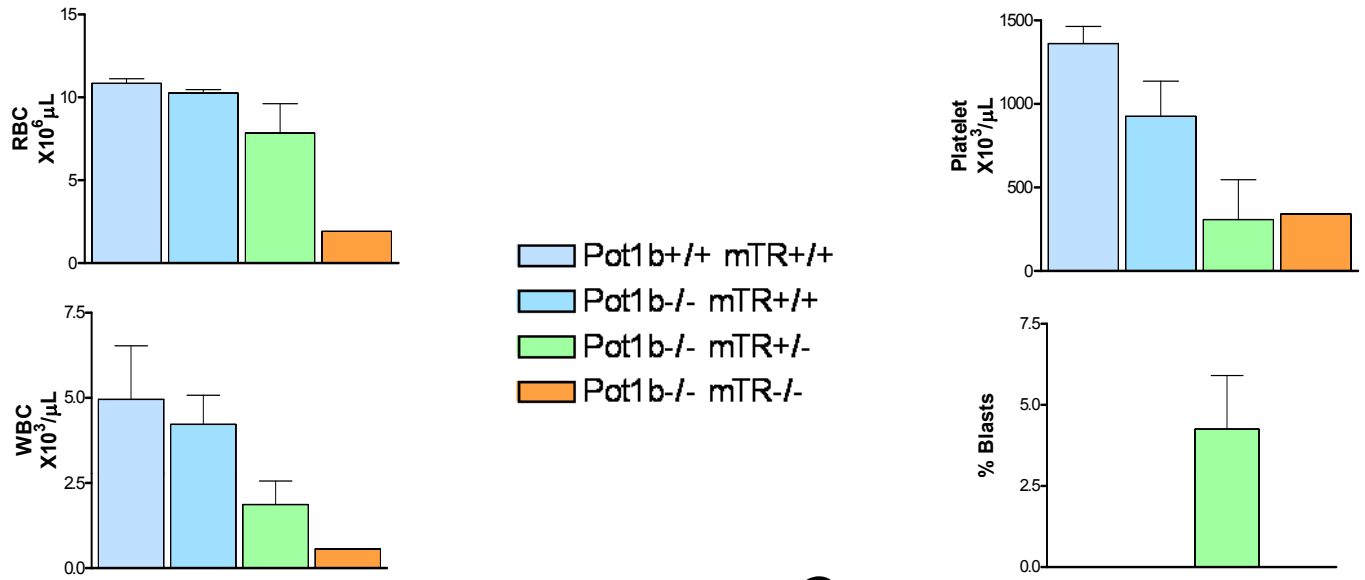
Depletion of hematopoietic progenitor/stem cells in Pot1b$^{-/-}$ mTR$^{+/-}$ bone marrow



Eric Wang

Peripheral blood defects in Pot1b$^{-/-}$ mTR$^{+/-}$ mice

A



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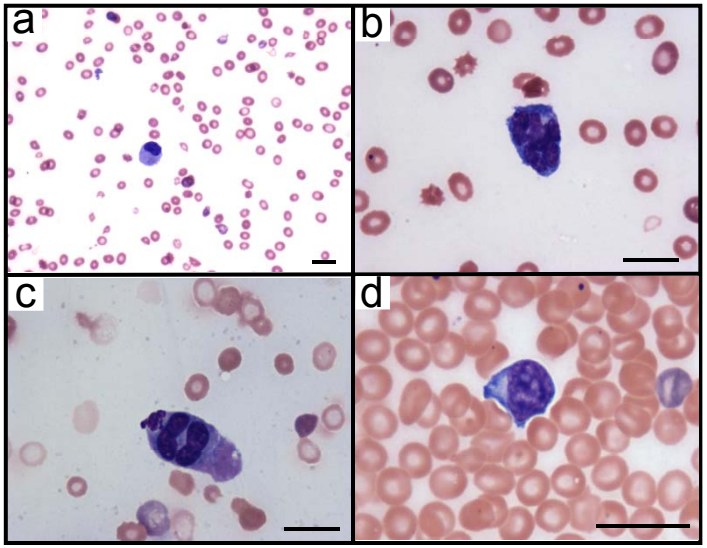

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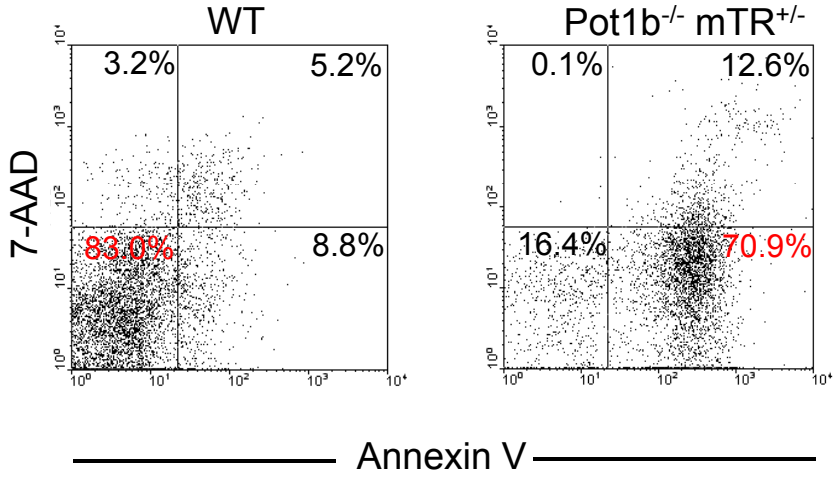
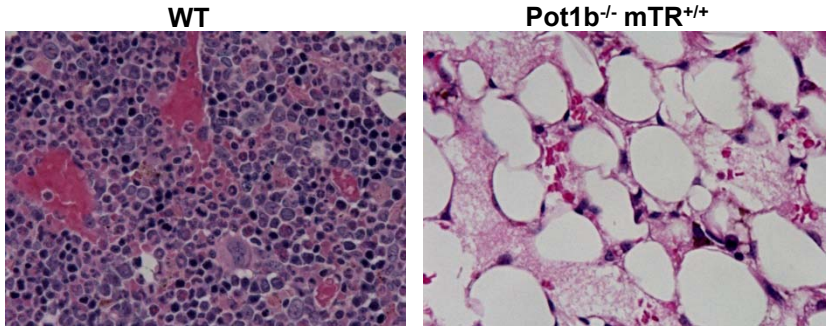


Pot1b$^{-/-}$ mTR $^{+/-}$ mice displays phenotypes resembling Dyskeratosis congenita (DC)

- abnormal skin pigmentation

- nail dystrophy

- BM failure



Dyskeratosis congenita

Clinical characteristics of dyskeratosis congenita

Expert Reviews in Molecular Medicine ©2004 Cambridge University Press

Source: Lichtman MA, Shafer MS, Felgar RE, Wang N:
Lichtman's Atlas of Hematology: http://www.accessmedicine.com

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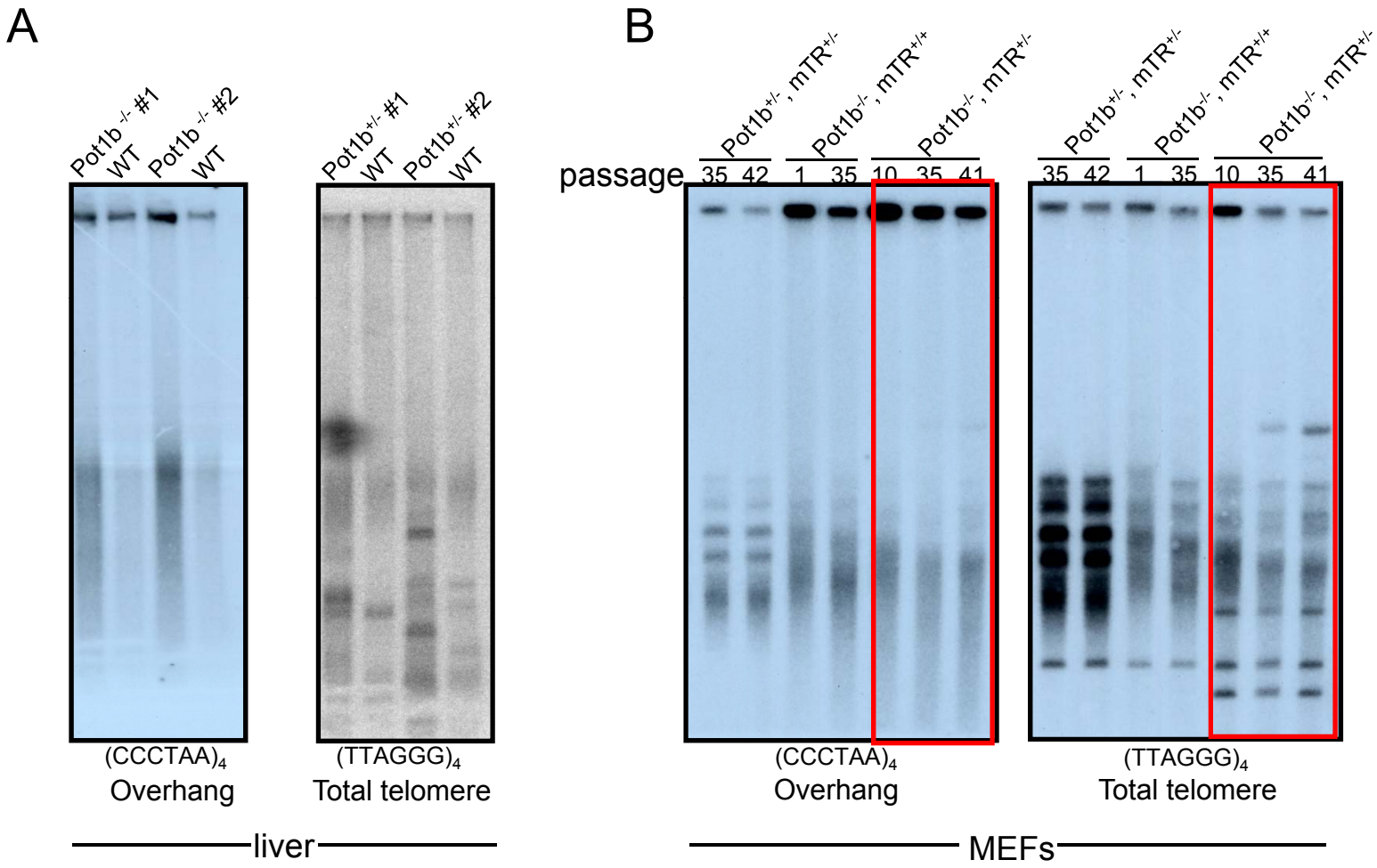
Dyskeratosis congenita is a telomere disease

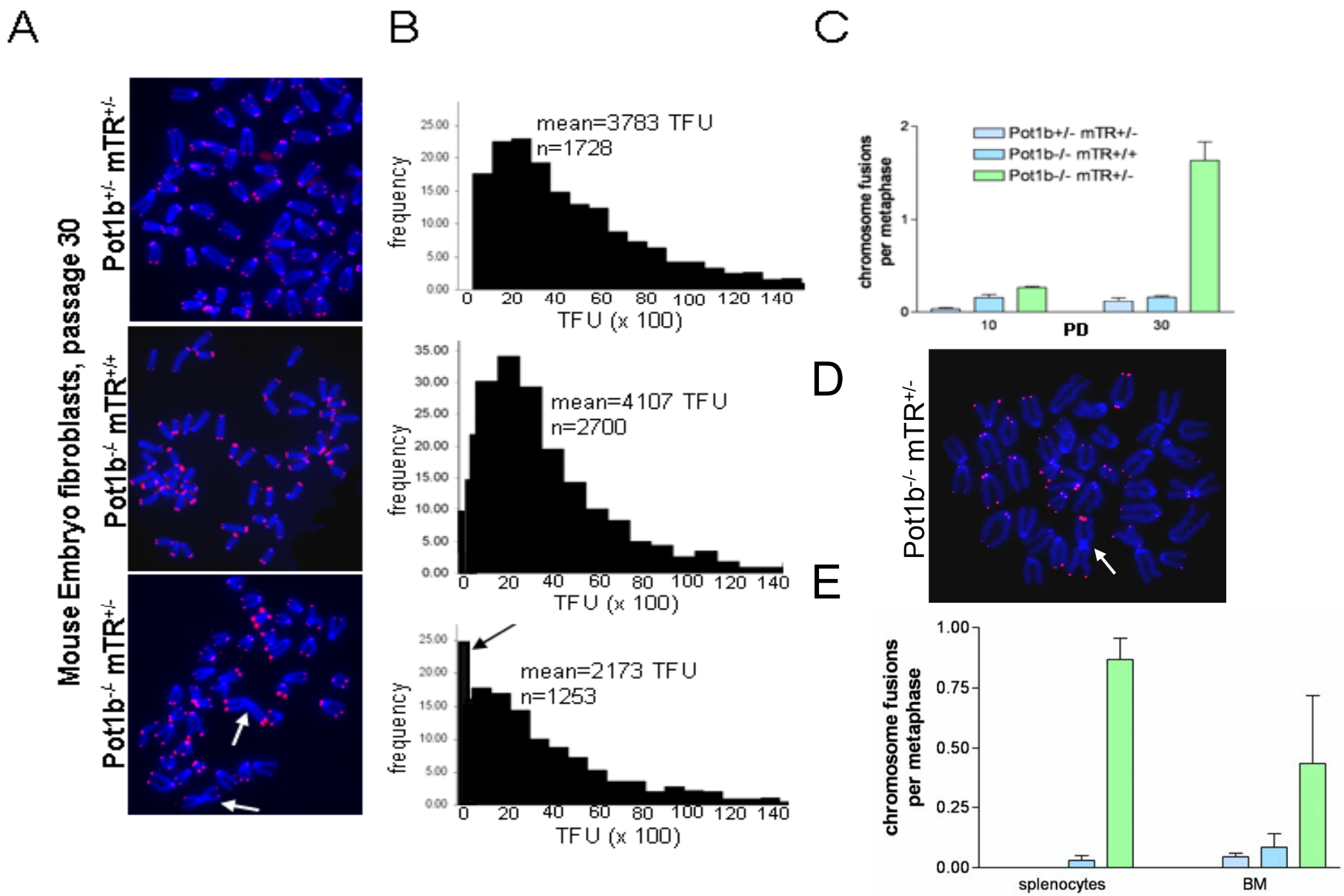
Dyskerin is a pseudouridine synthase; modifies rRNA, spliceosomal RNA, using a H/ACA small guide RNAs

- Mutated in X-linked dyskeratosis congenita (Collins)
  - aplastic anemia
  - pulmonary fibrosis, hyperpigmentation, oral leukoplakia, nail dystrophy
  - very short telomeres
- TERC or TERT are mutated in autosomal dominant DC (Dokal, Greider)
- NOP10, a small ribonucleoprotein, is mutated in autosomal recessive DC (Dokal)
- Heterozygous mutation in TINF2 recently observed in DC patients (Savage, Dokal)

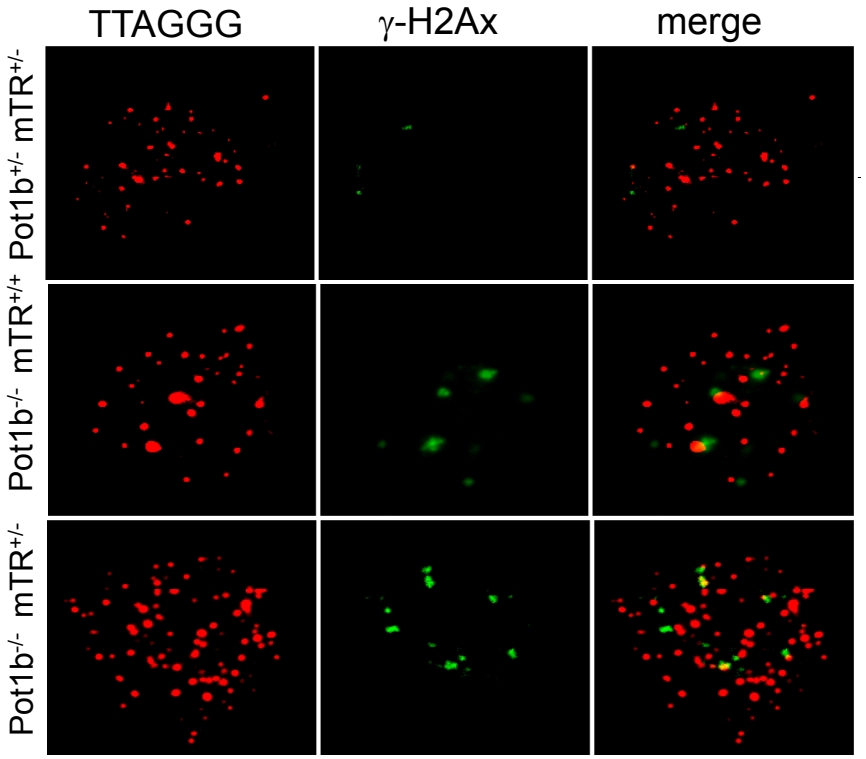
Increased G-strand overhang and progressive telomere shortening in the absence of Pot1b

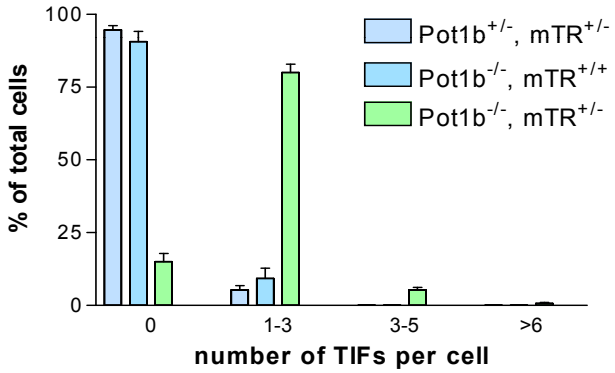


Increased chromosome fusion and telomere shortening in Pot1b$^{-/-}$ mTR$^{+/-}$ cells

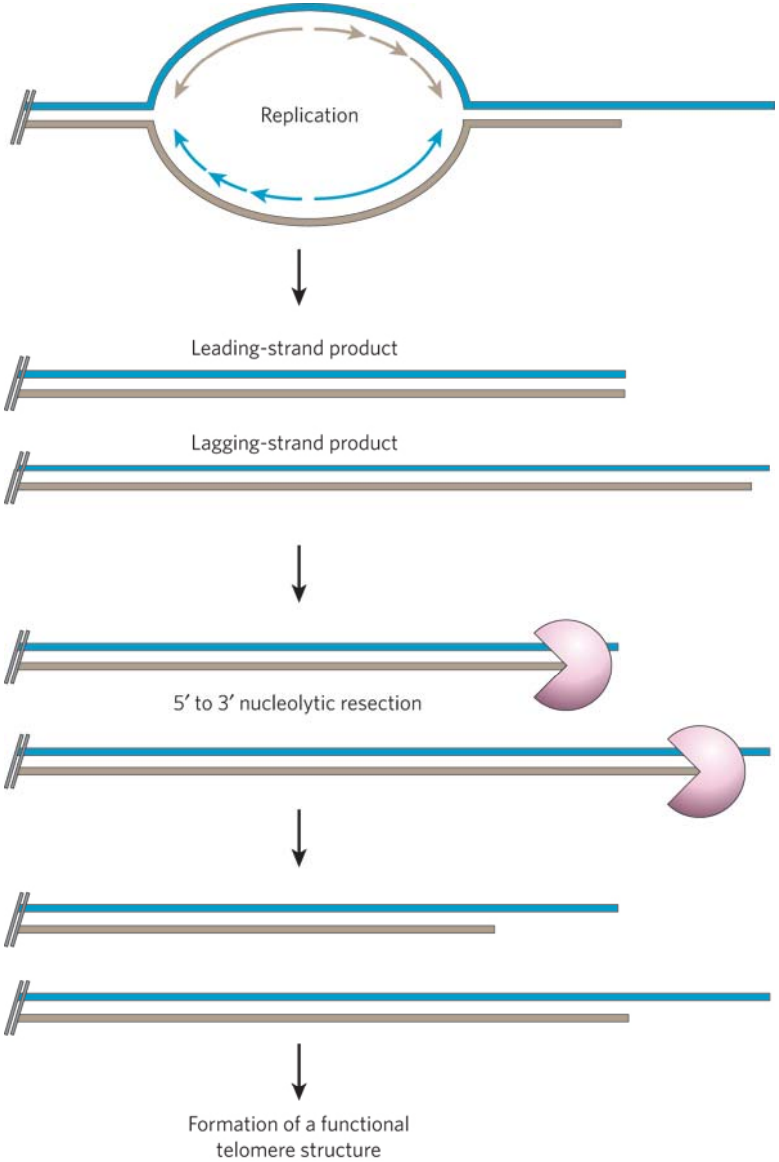


Pot1b$^{-/-}$ mTR$^{+/-}$ MEFs initiate a DNA damage response at telomeres

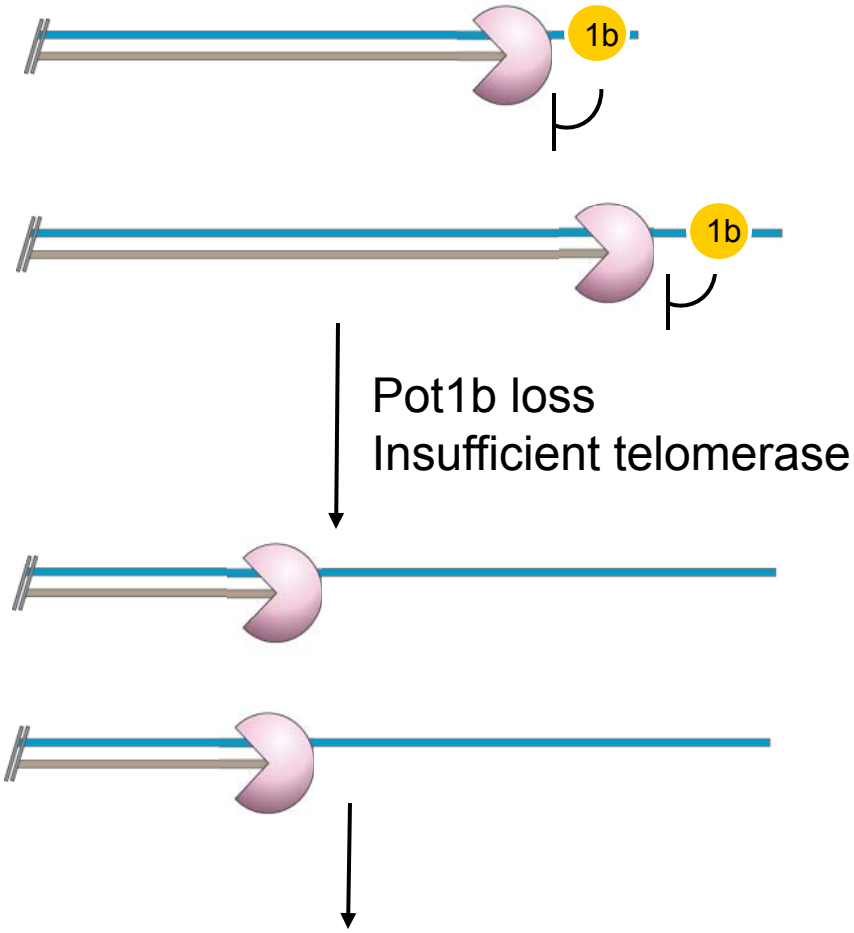




Pot1b is required to modulate ss overhang formation

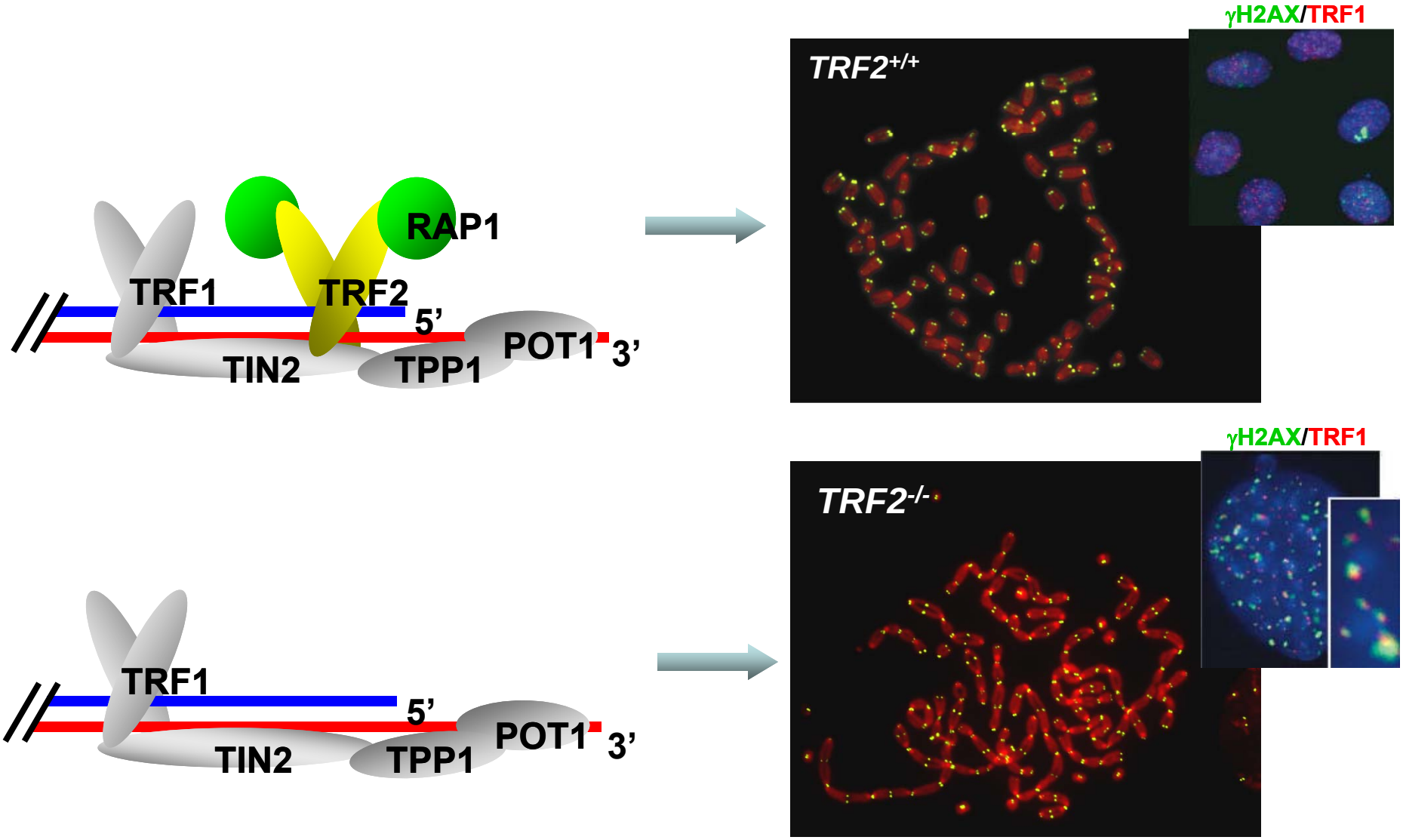


Adapted from Ramiro and Karlseder, Nature 2007



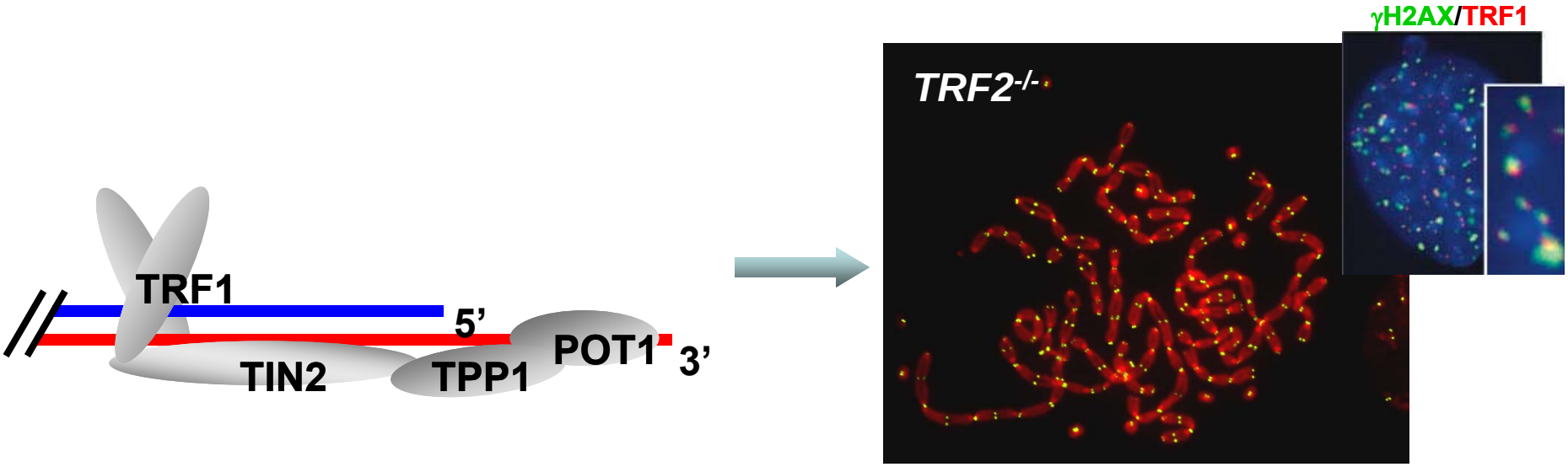
Increased overhang generation
Accelerated telomere shortening
Increased DDR at telomeres
p53-dependent apoptosis

Role of repressor activator protein 1 (RAP1) in telomere end protection



Celli, GB et al., (2006) Nature Cell Biology

Removal of TRF2 from telomeres results in DDR at telomeres and massive chromosome end fusions

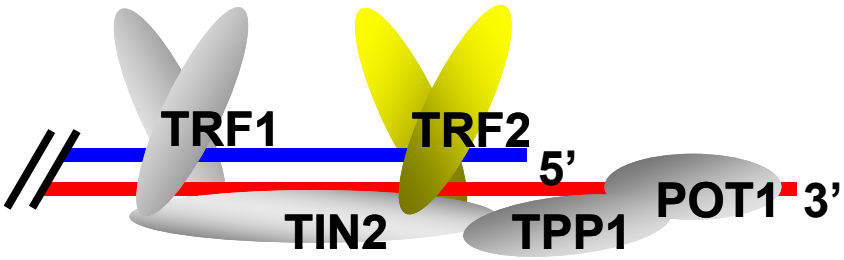


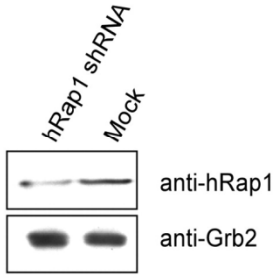
Removal of TRF2 results in rapid disappearance of Rap1 from telomeres.

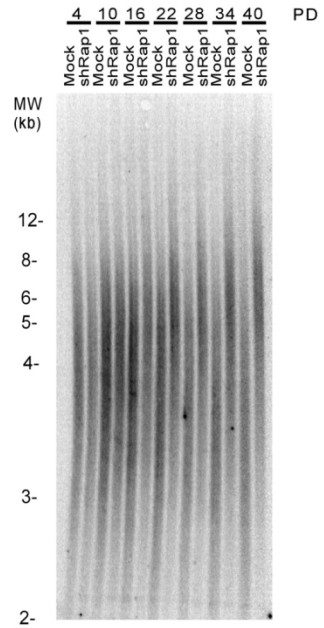
In yeast, Rap1 is involved in protecting telomeres from NHEJ independent from TRF2-like homologs.

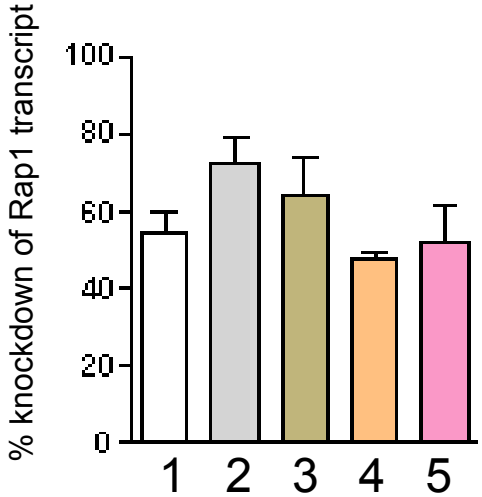
What are the roles for mammalian Rap1 in telomere end protection?

Knockdown of Rap1 by shRNA does not work well

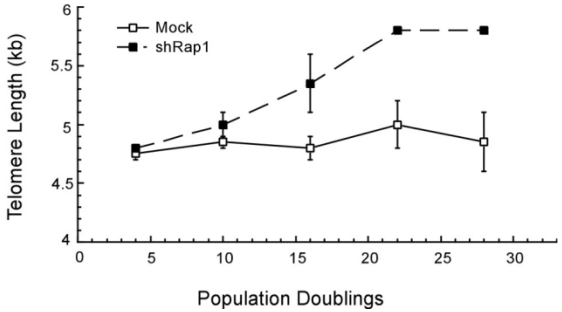






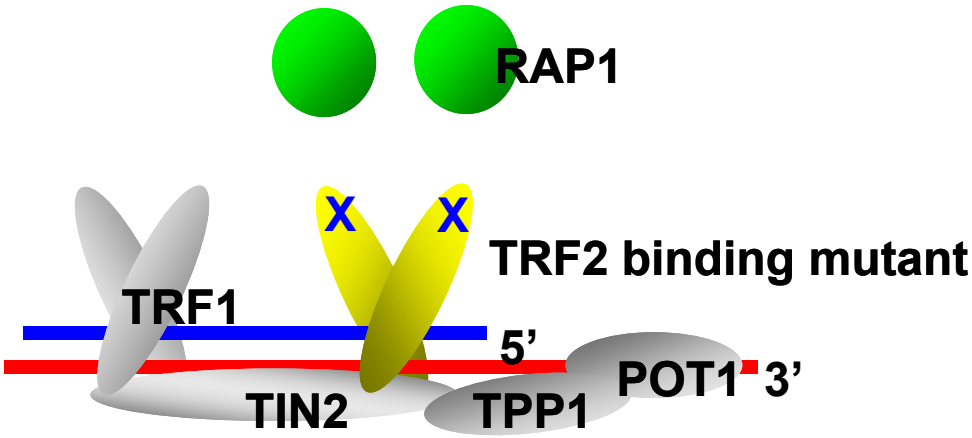


Knockdown of RAP1 transcripts using 5 shRAP1 constructs.



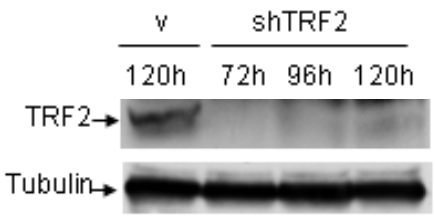
O'Connor , *JBC* 2004

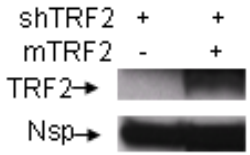
Strategy to study the *in vivo* function of Rap1

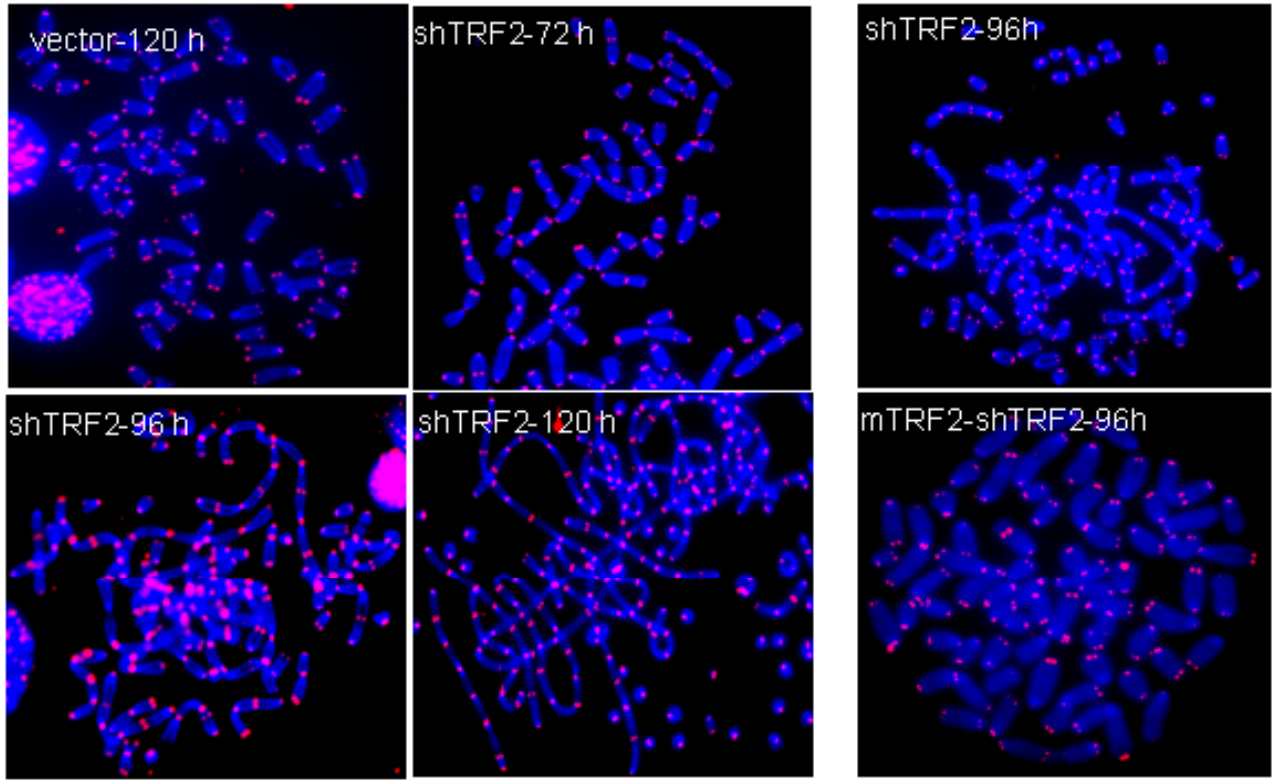


Replacement of endogenous TRF2 with Rap1 binding deficient mutant

shRNA mediated knockdown of TRF2 results in massive telomere fusions

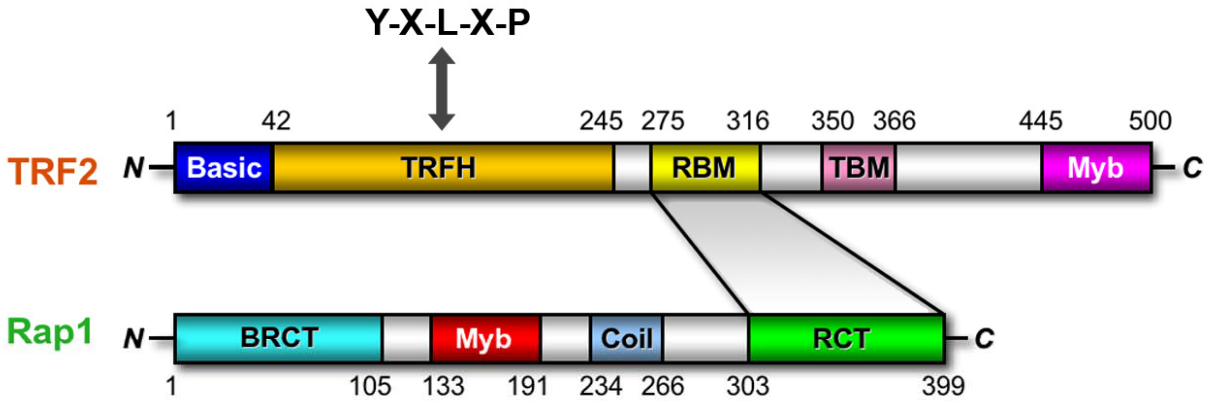


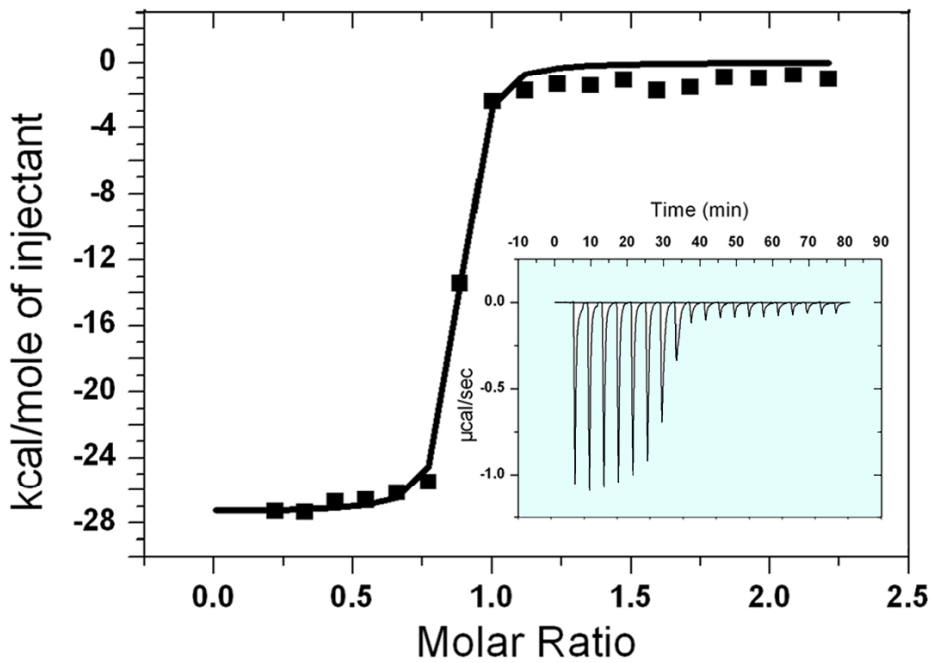




Deng et. al., Nature 2009

Characterization of the TRF2-Rap1 interaction



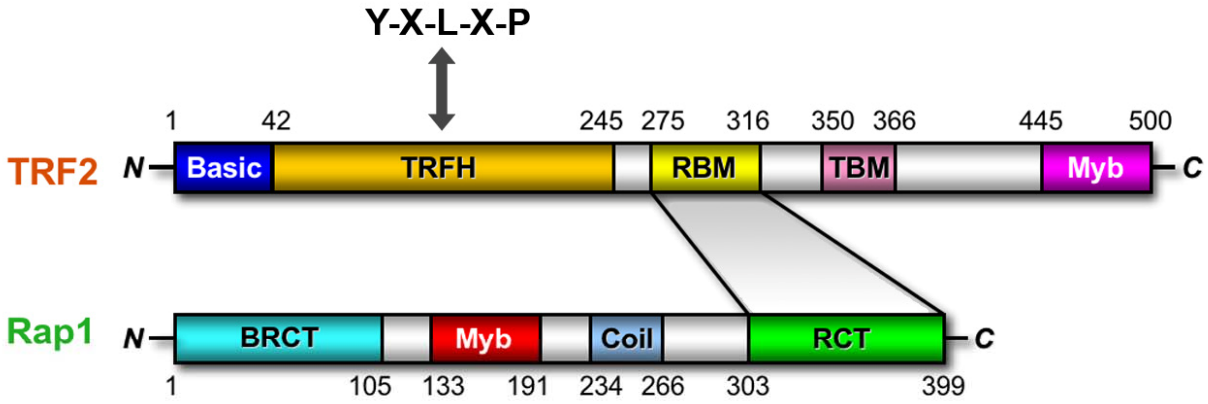


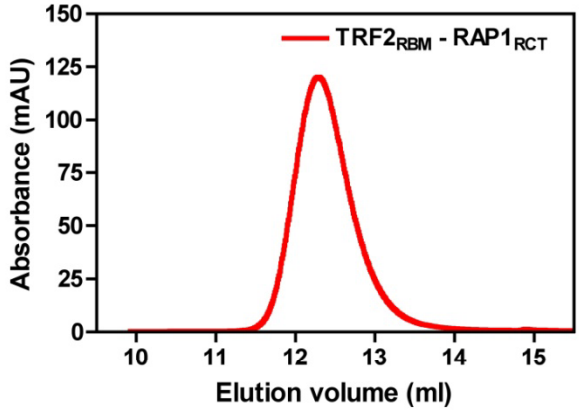
Isothermal titration Calorimetry

K_d=16.5 nM

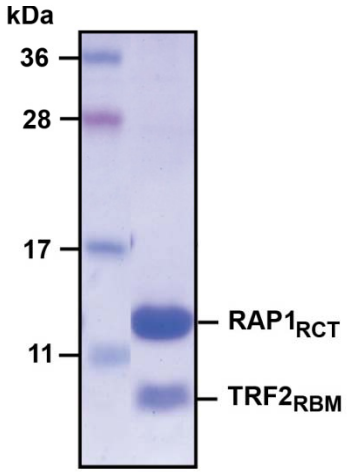
Ming Lei, U of Michigan

Purification and crystallization of the $TRF2_{RBM}$ and $Rap1_{RCT}$ complex





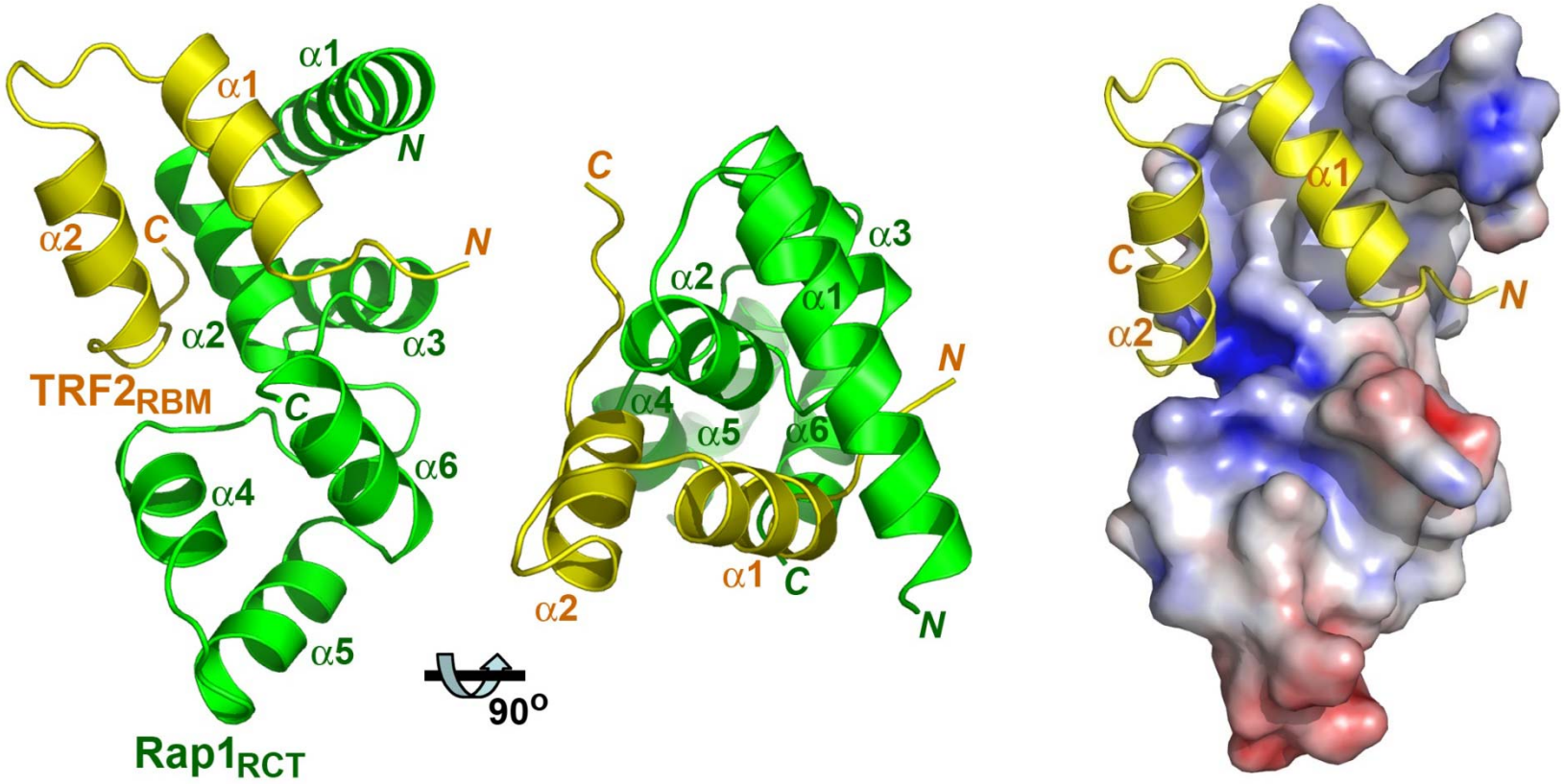
gel-filtration



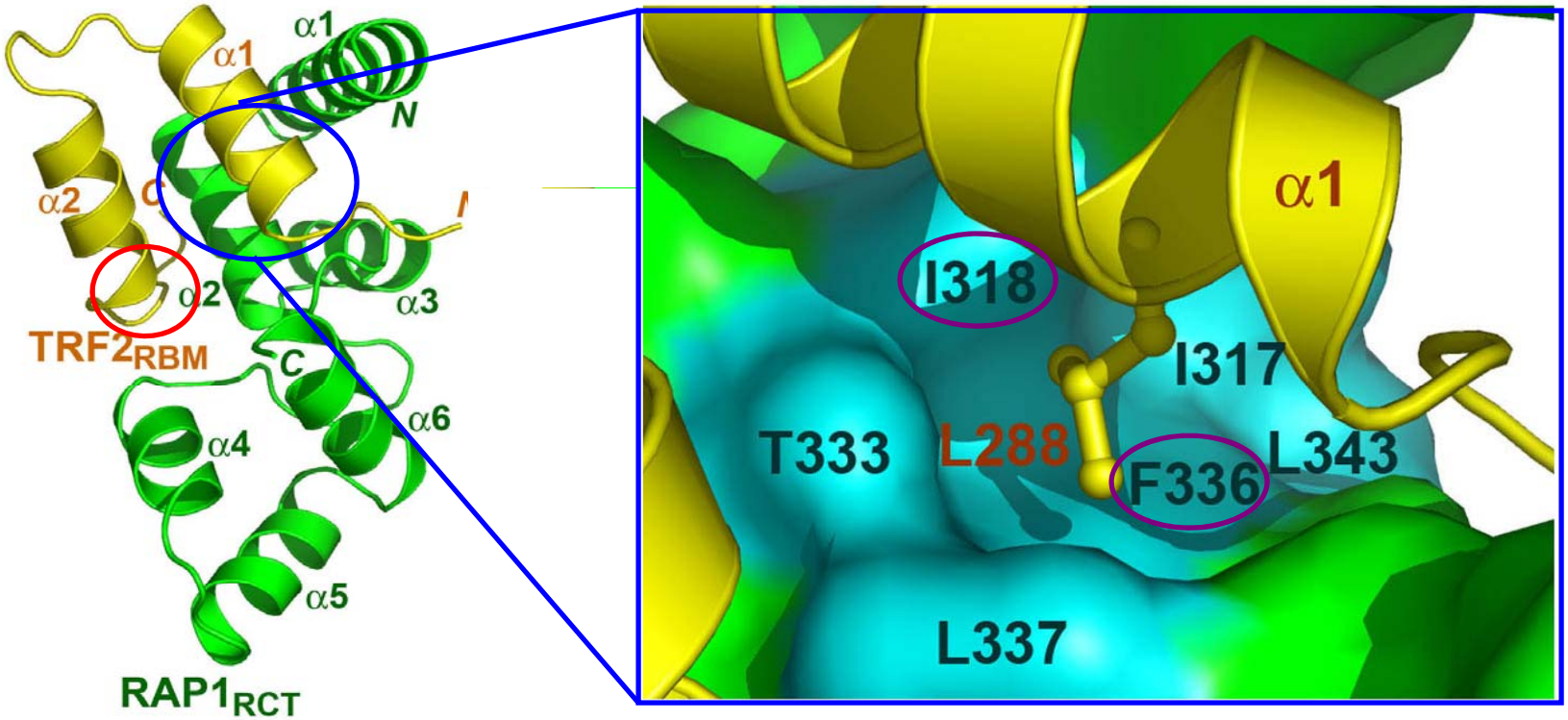
SDS-PAGE

Crystal of the $TRF2_{RBM}$-$Rap1_{RCT}$ complex

Crystal Structure of the $TRF2_{RBM}$-$Rap1_{RCT}$ Complex at 1.95 A resolution

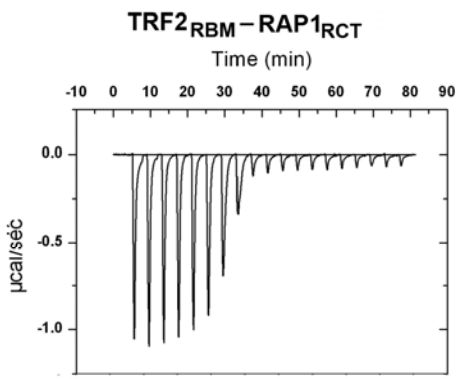


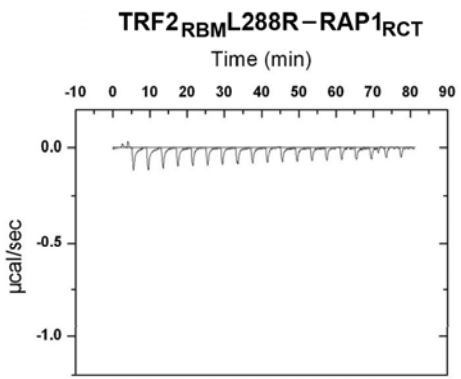
The $TRF2_{RBM}$-$Rap1_{RCT}$ interface



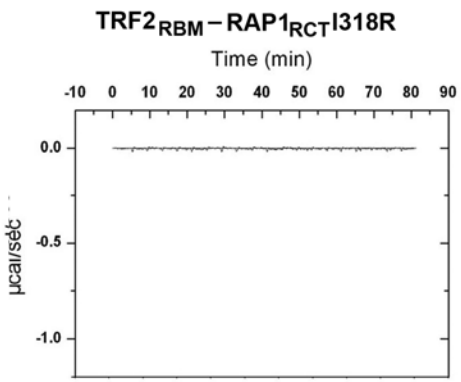
TRF2: L288 Rap1: I318, F336

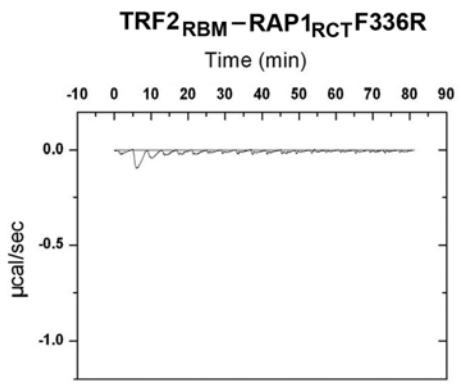
ITC analysis of the $TRF2_{RBM}$-$Rap1_{RCT}$ interface

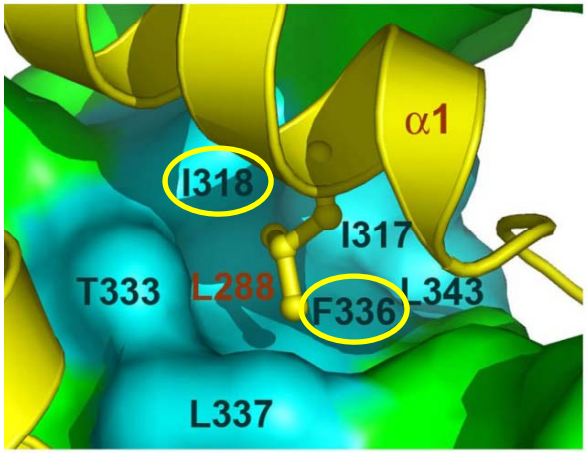




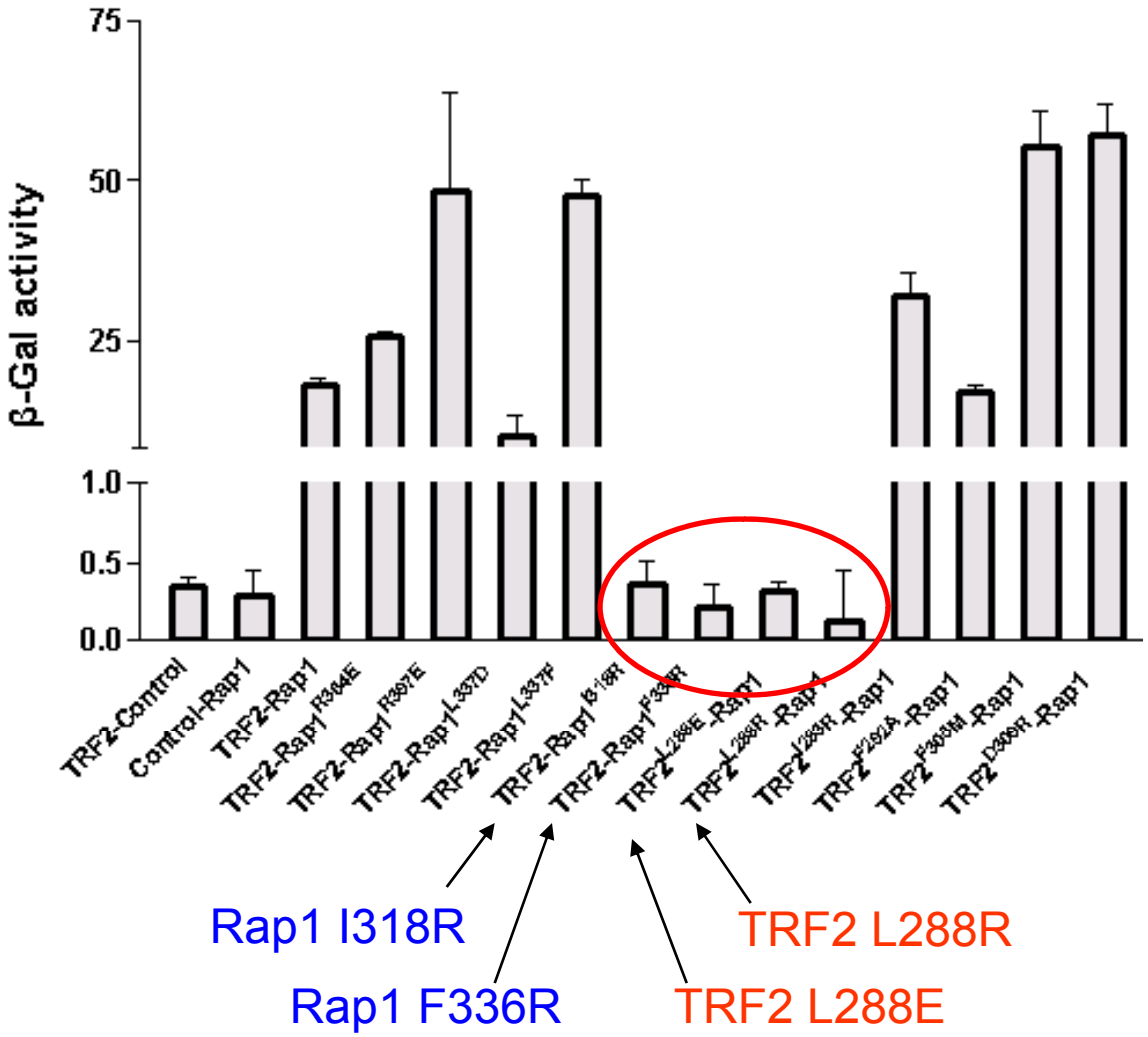
TRF2	RAP1	K_d (nM)
$TRF2_{RBM}$	$RAP1_{RCT}$	16.5
TRF2	$RAP1_{RCT}$	23.9
$TRF2_{RBM}$	$RAP1_{RCT}$I318R	nd
$TRF2_{RBM}$	$RAP1_{RCT}$F336R	nd
$TRF2_{RBM}$L288R	$RAP1_{RCT}$	nd

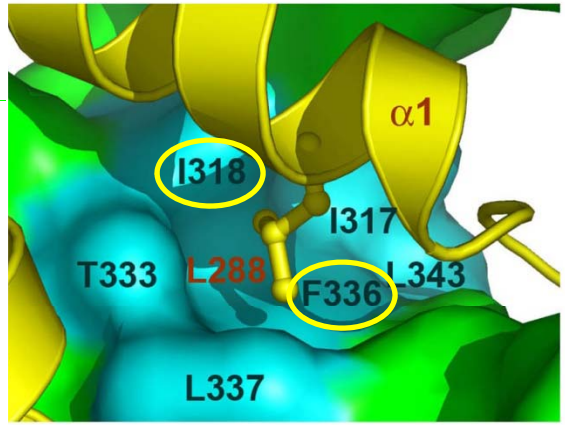






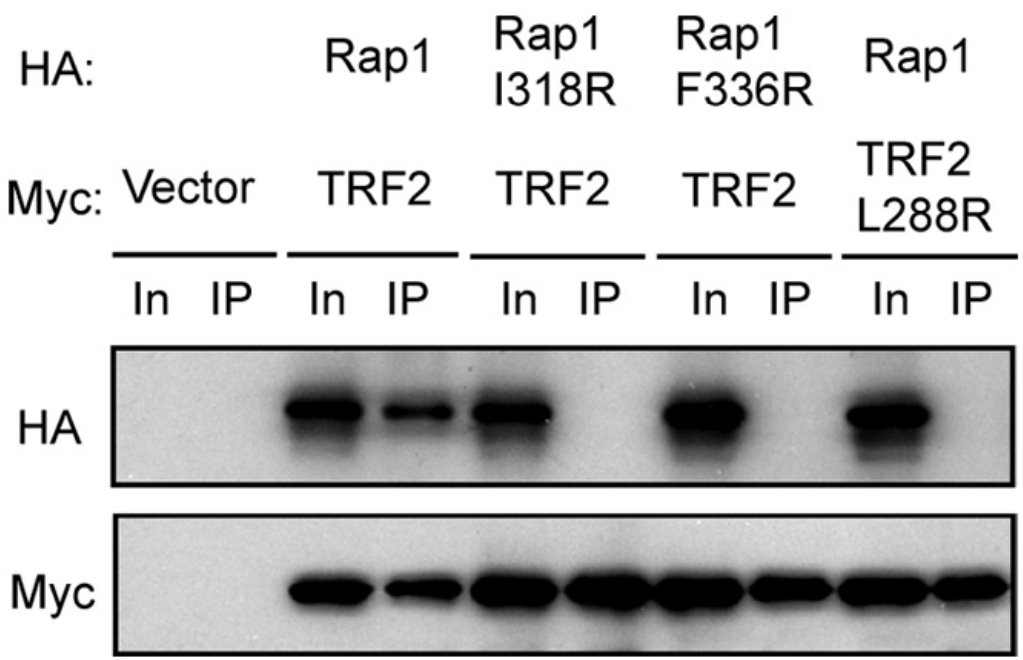
Yeast two-hybrid analysis of the $TRF2_{RBM}$-$Rap1_{RCT}$ interface

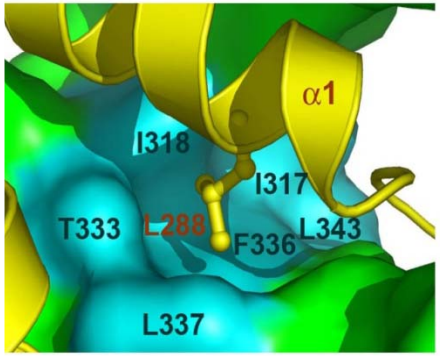




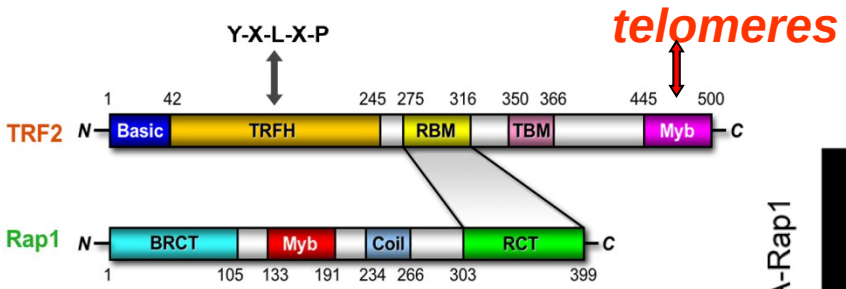
A single point mutation inside the hydrophobic pocket is sufficient to disrupt the ability of TRF2 to bind Rap1.

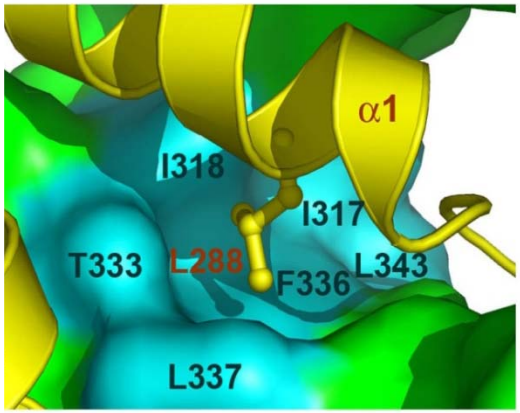
Co-IP analysis the TRF2-Rap1 Interface in mammalian cells

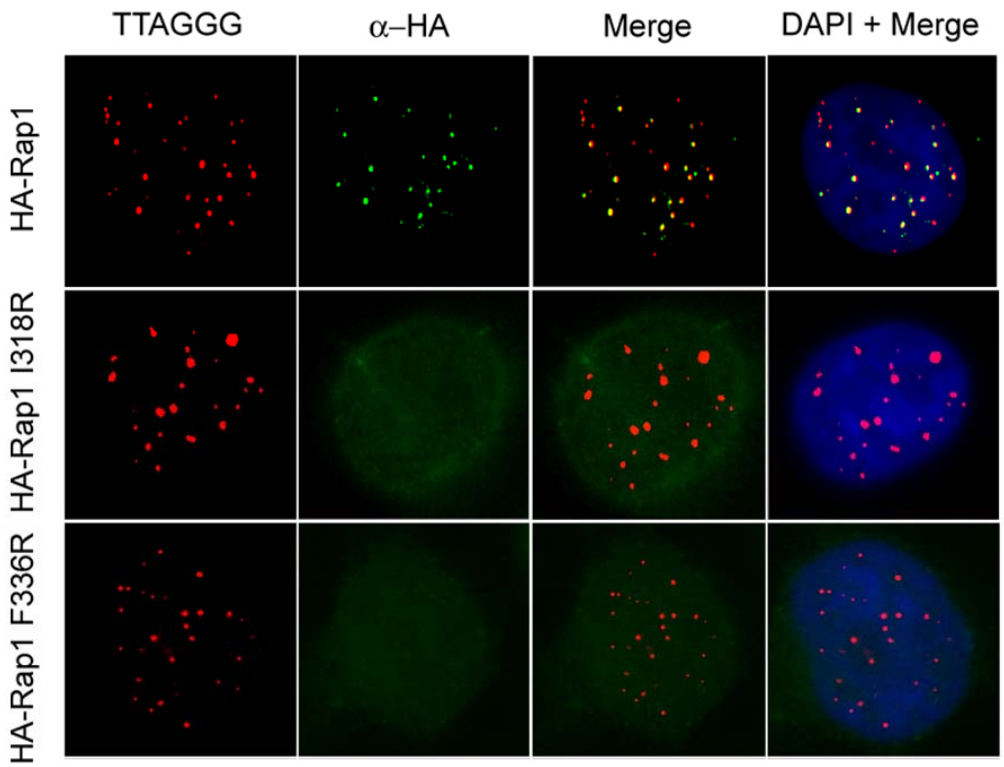




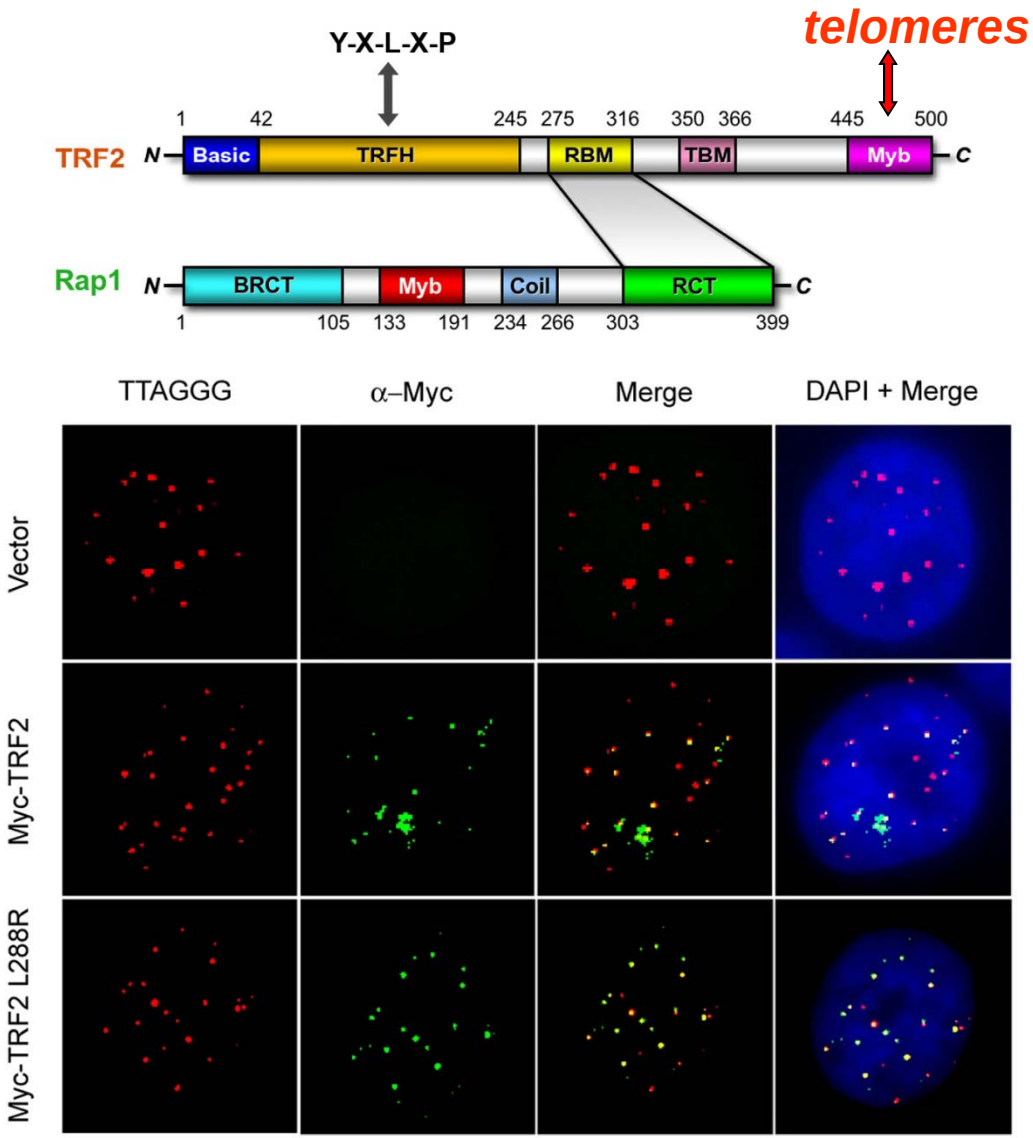
TRF2 is essential for Rap1 localization to telomeres



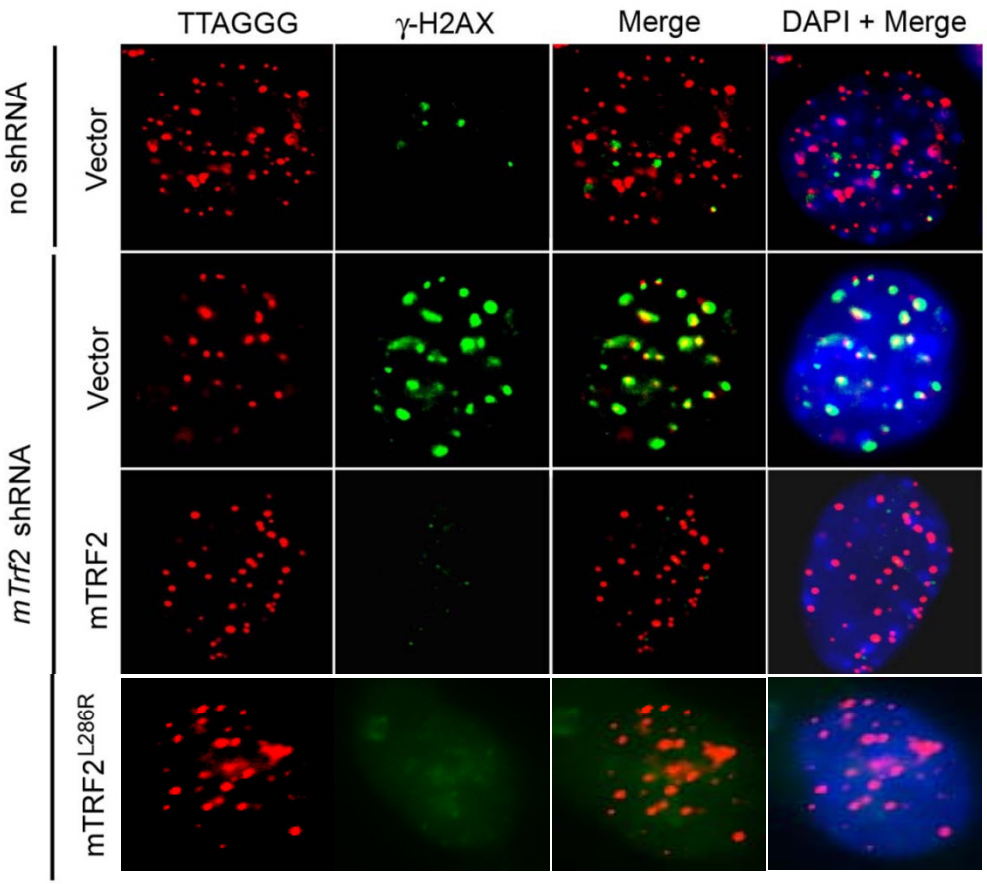


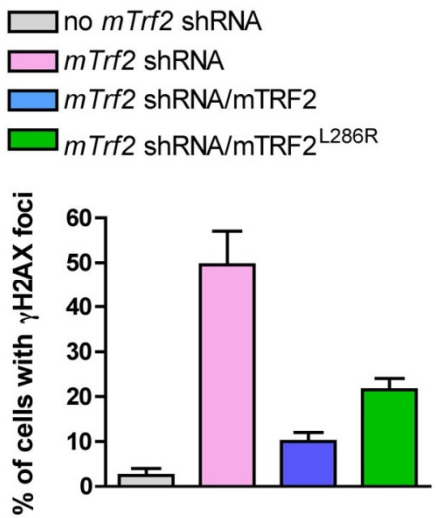


The TRF2-Rap1 interaction not required for TRF2 telomere localization



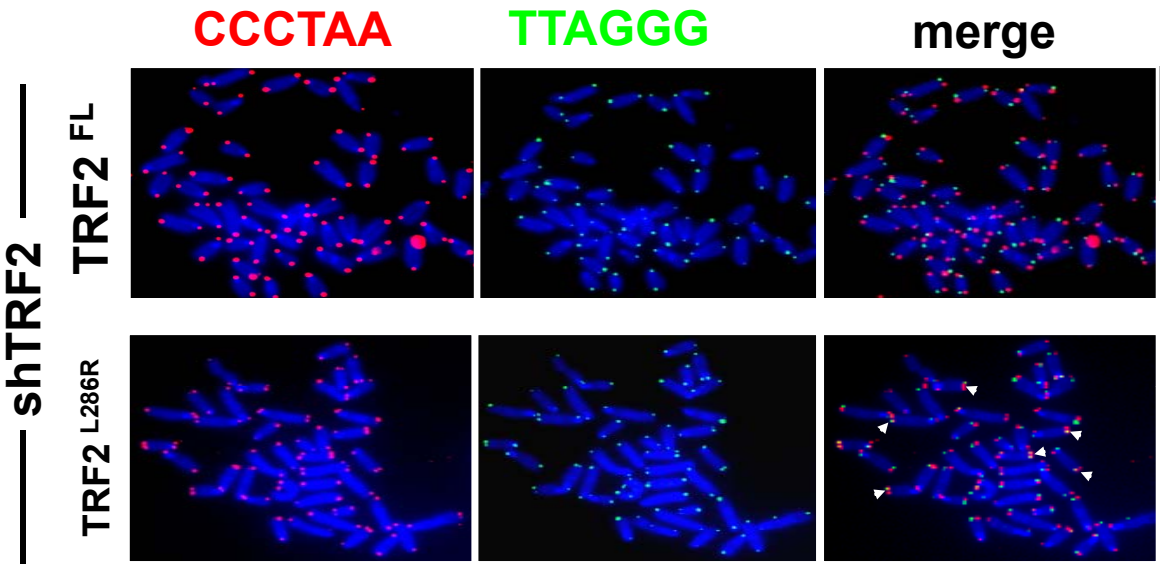
Minimal DNA damage response at telomeres in the absence of Rap1

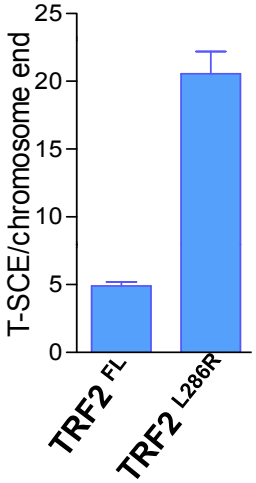


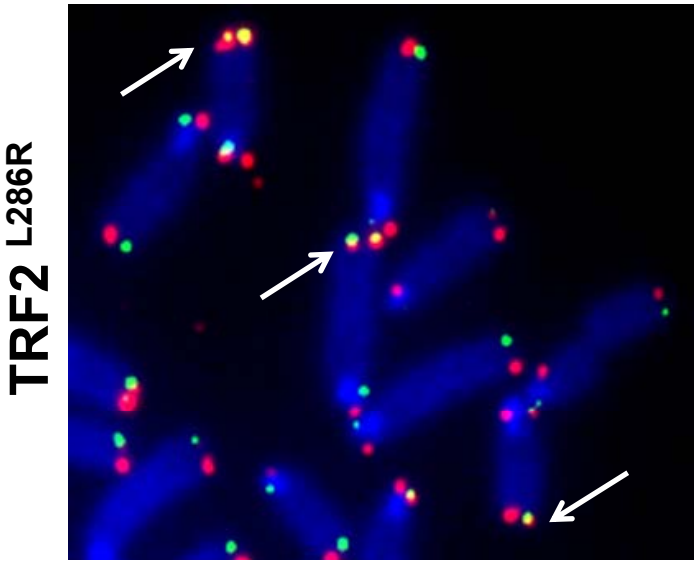


TRF2, not Rap1, is required to repress the DDR at telomeres.

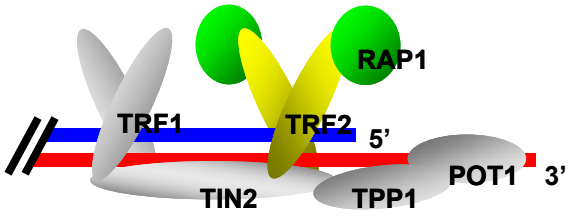
Rap1 is required to repress telomere HR

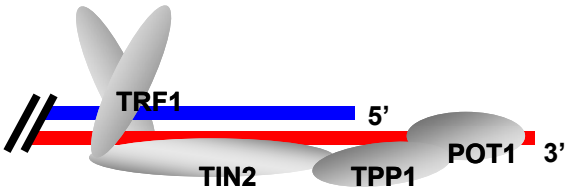


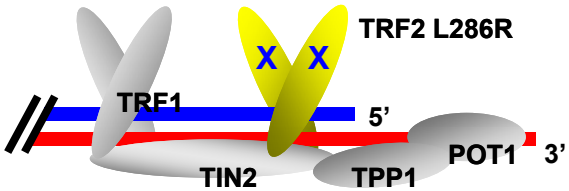




Distinct roles for Rap1 and TRF2 in telomere end protection



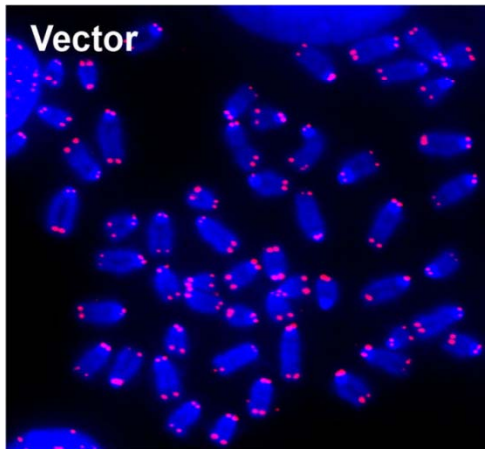


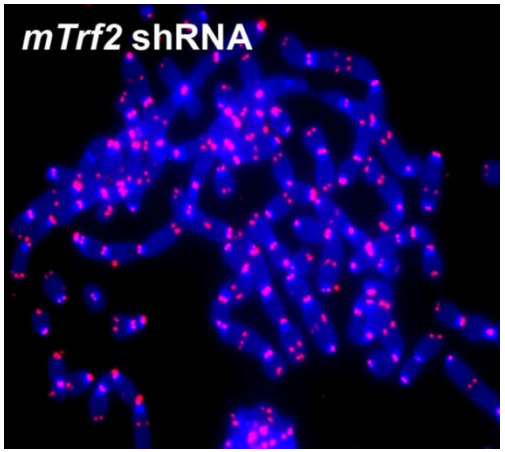


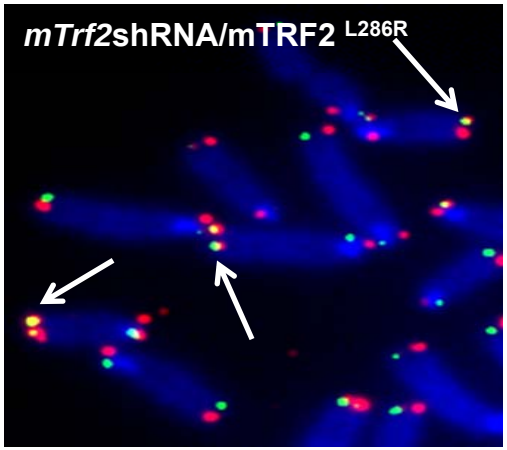
no DDR
NHEJ repression

massive DDR
rampant chrom fusions

minimal DDR
few chrom fusions
telomere HR







Rap1 is not required to repress DDR and NHEJ at telomeres
Rap1 repress HR at telomeres.

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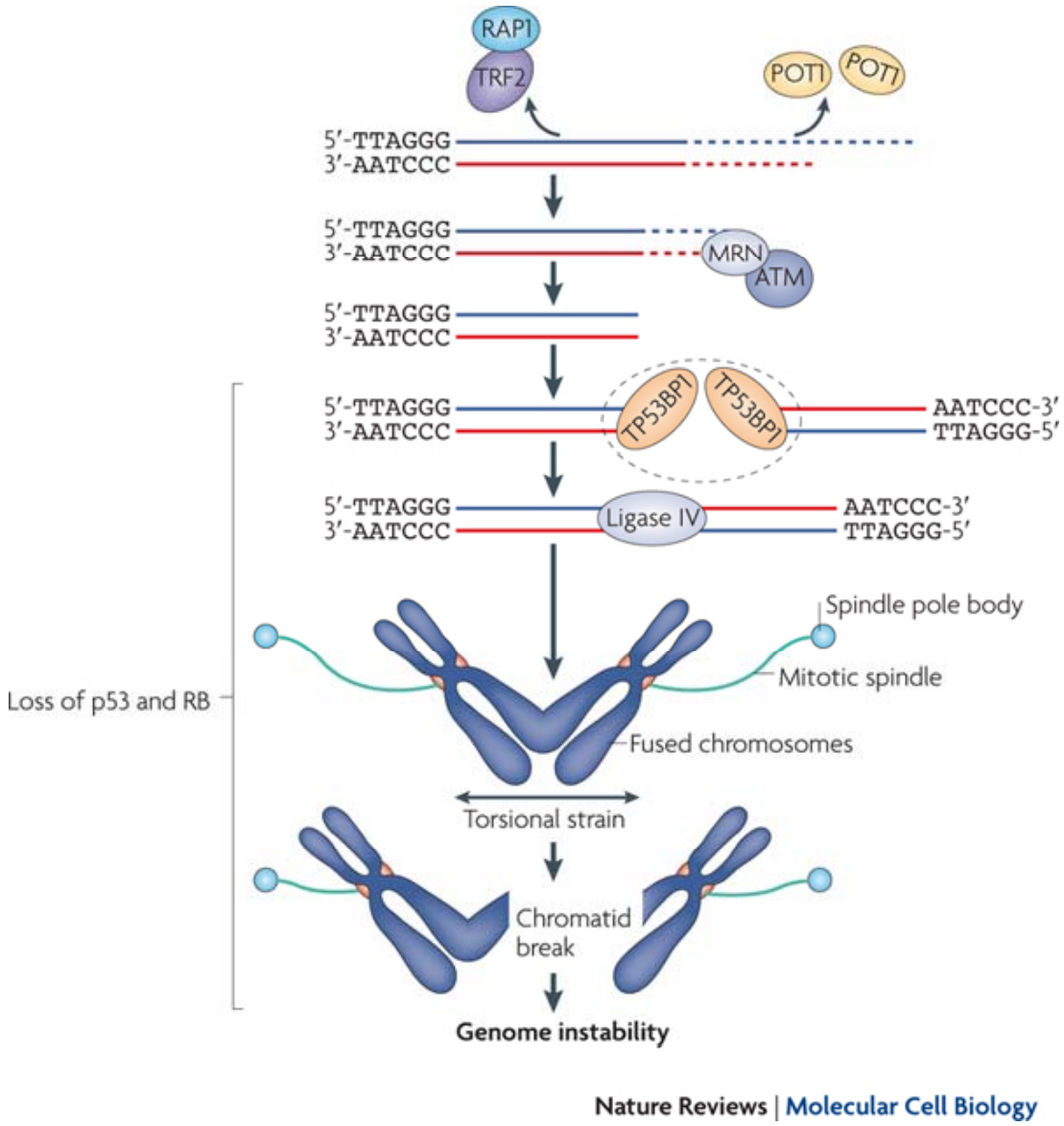
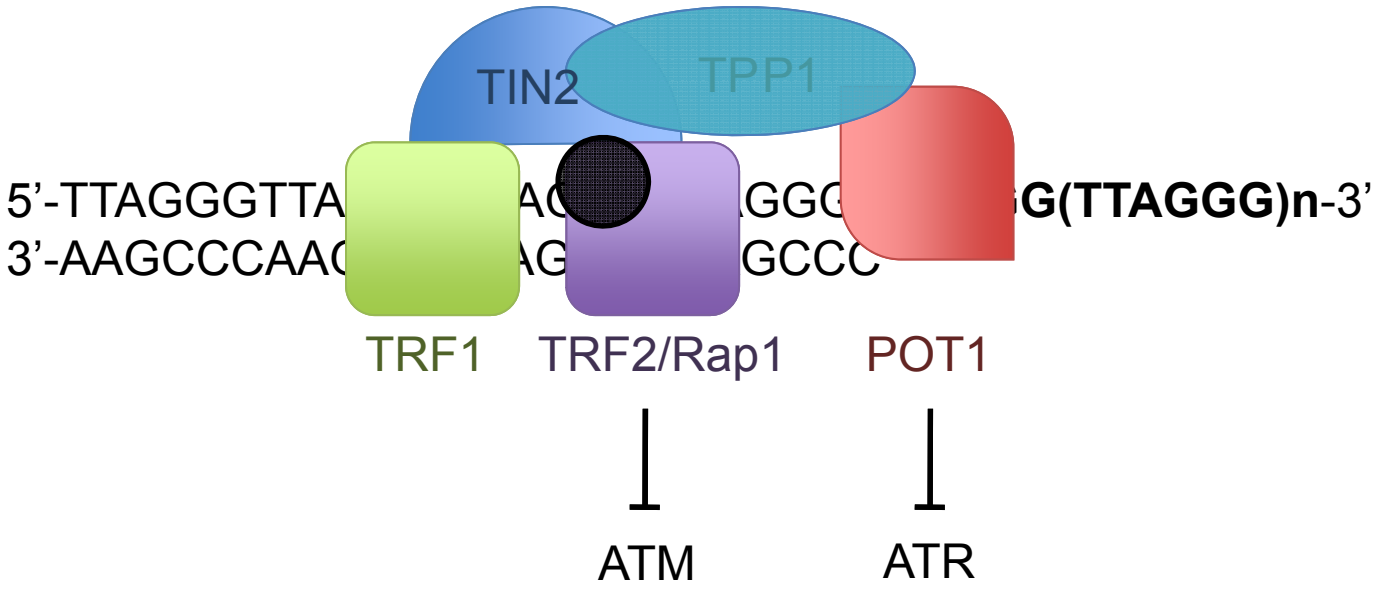
Repair of dysfunctional telomeres by the NHEJ pathway



O'Sullivan and Karlseder, Nature Reviews MCB 2010

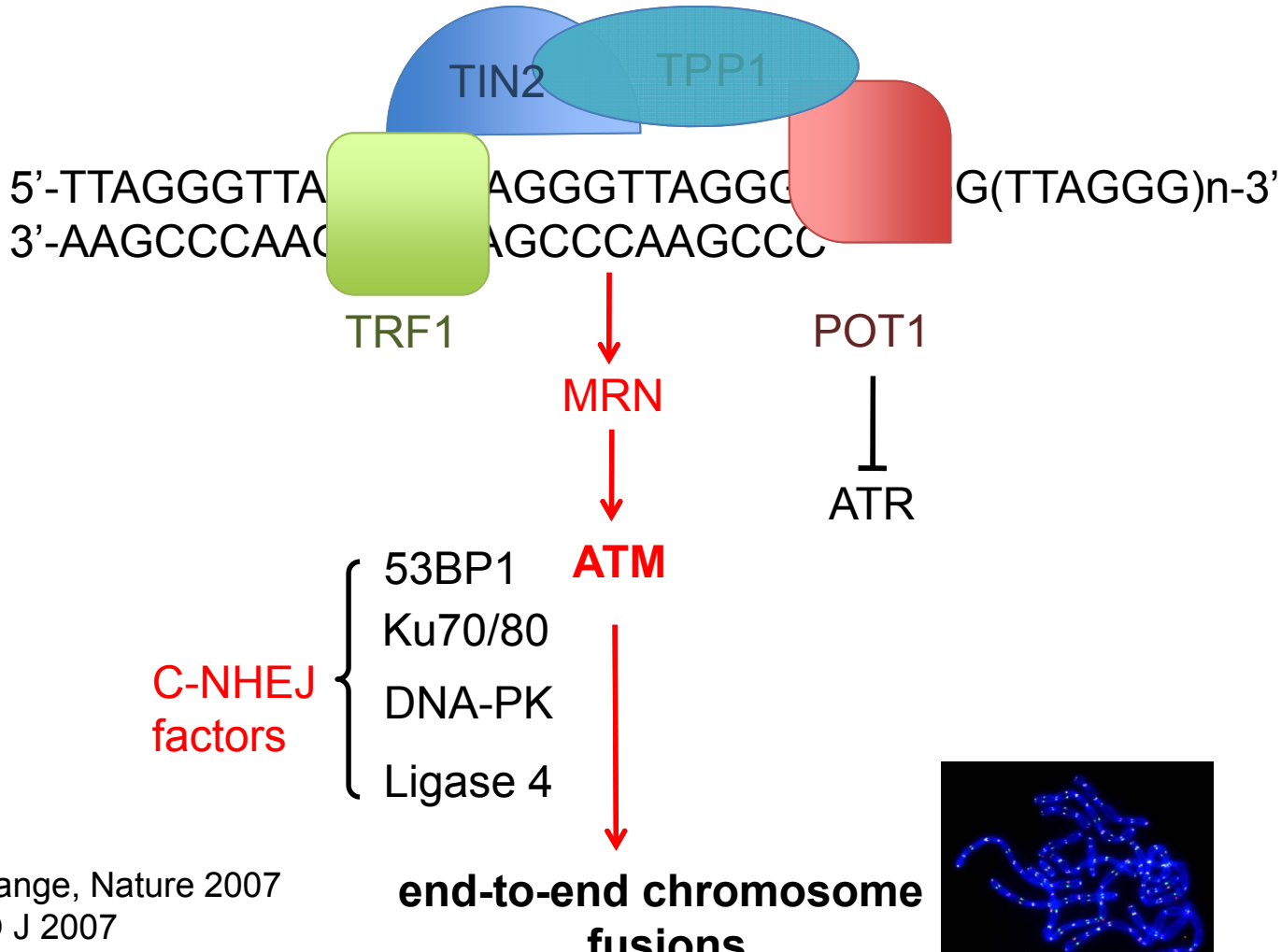
Trf2 and Pot1a repress distinct DNA damage signaling pathways



Possibility that TRF2 and POT1-TPP1 repress distinct DNA repair pathways at telomeres.

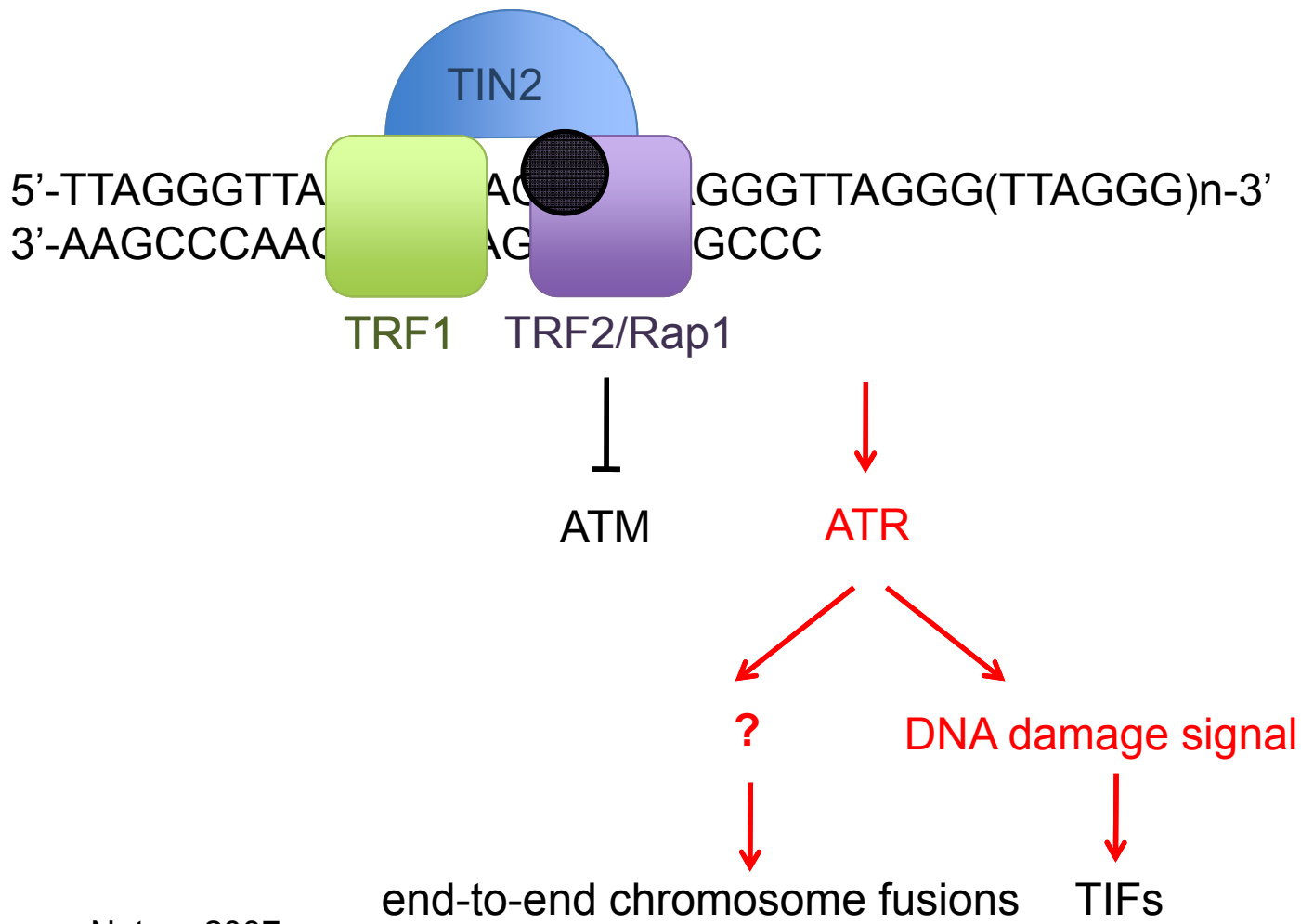
Denchi and de Lange, Nature 2007
Guo et al; EMBO J 2007

Disruption of TRF2 leads to ATM-dependent telomere fusions through C-NHEJ



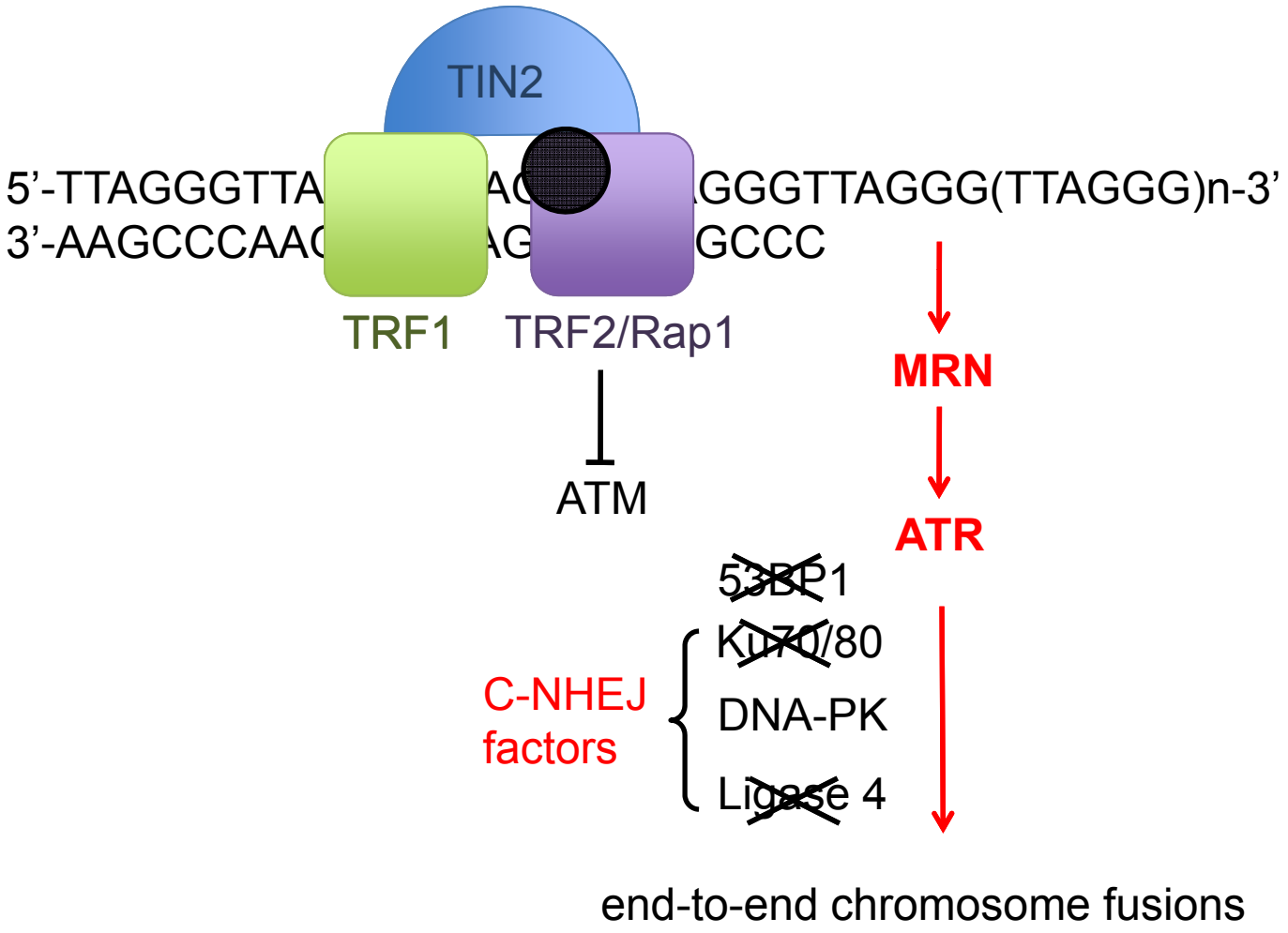
Denchi and de Lange, Nature 2007
Guo et al; EMBO J 2007
Deng et al., Nature 2009
Dimitrova et al., MCB 2009

Disruption of Pot1a-Tpp1 leads to ATR-dependent telomere fusions

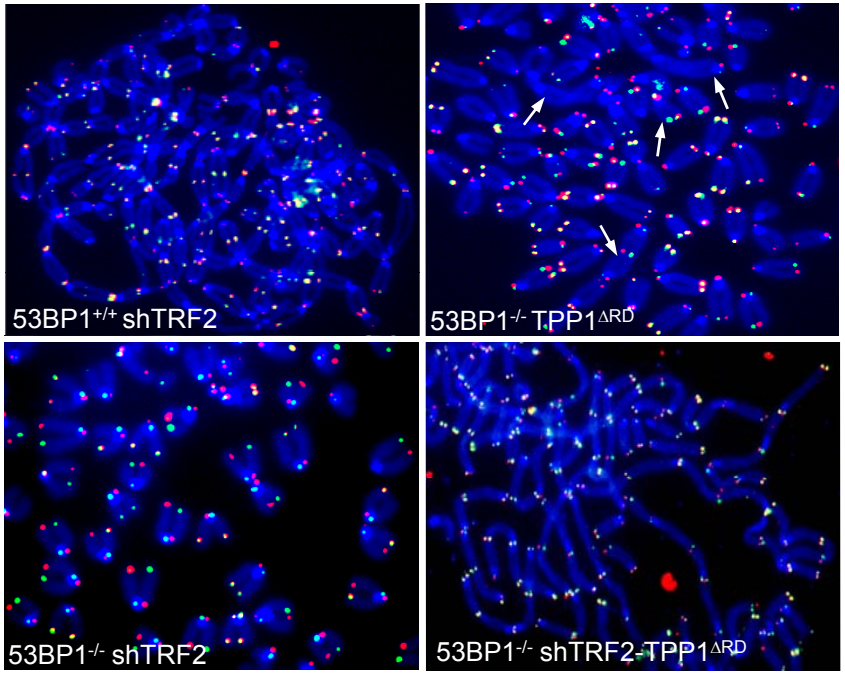


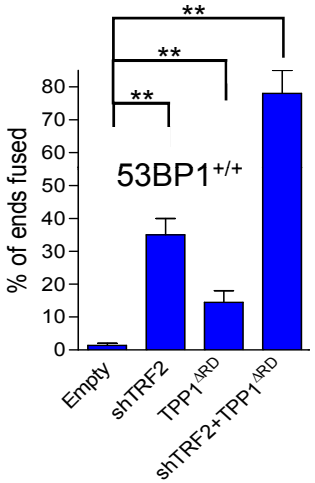
Denchi and de Lange, Nature 2007
Guo et al; EMBO J 2007

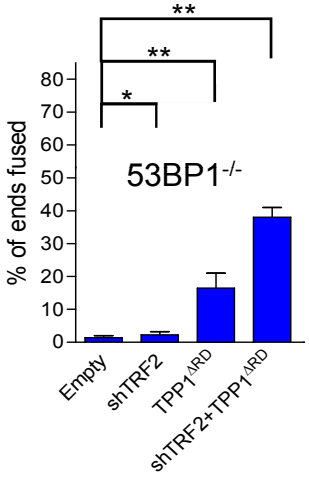
Disruption of Pot1a-Tpp1 leads to chromosome fusions independent of C-NHEJ



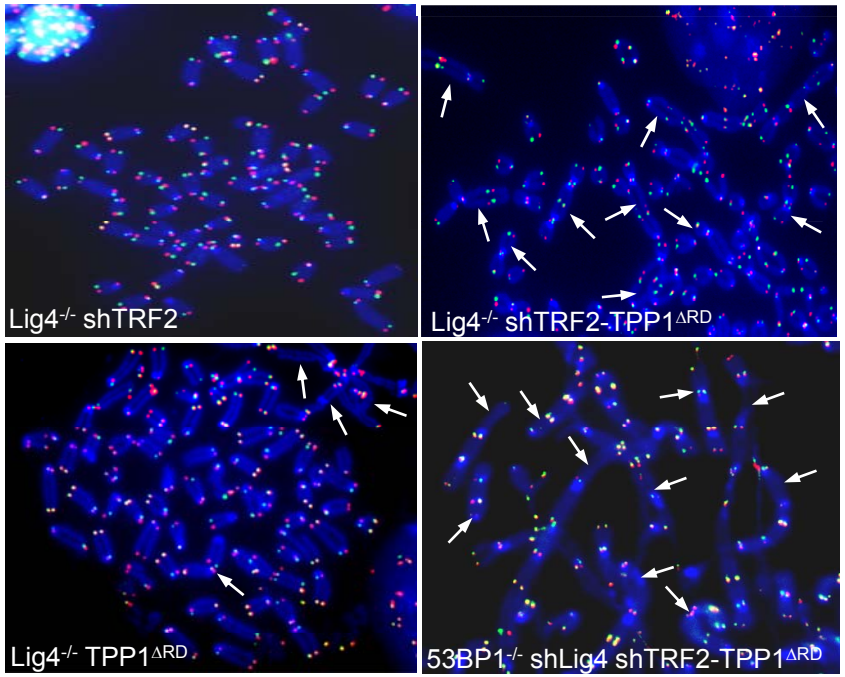
Chromosome fusions in the absence of Tpp1-Pot1a does not require 53BP1

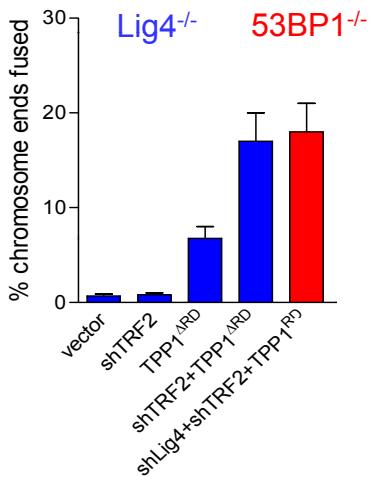




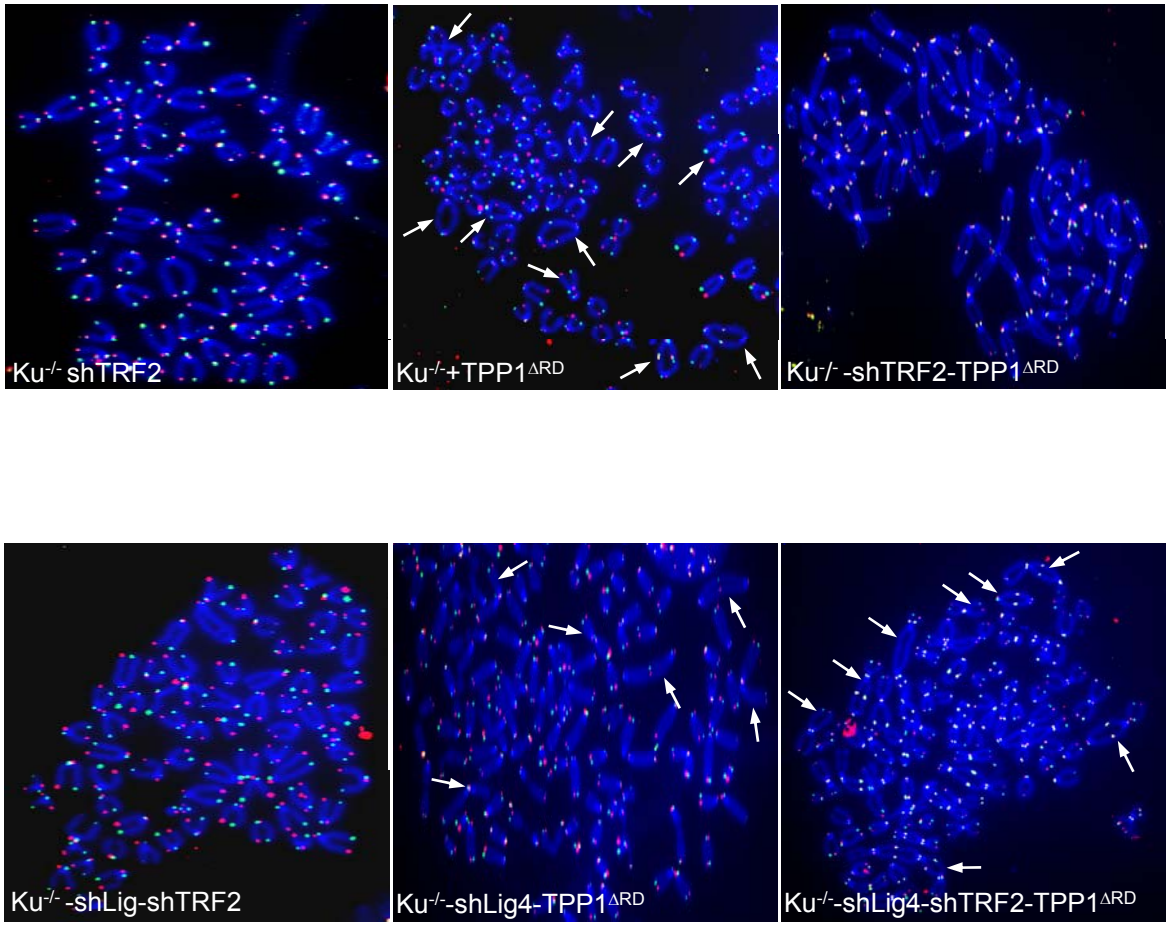


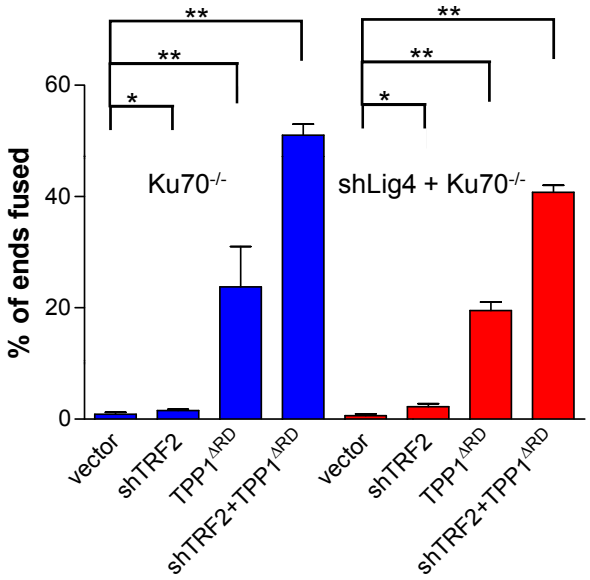
Chromosome fusions in the absence of Tpp1-Pot1a does not require Lig4



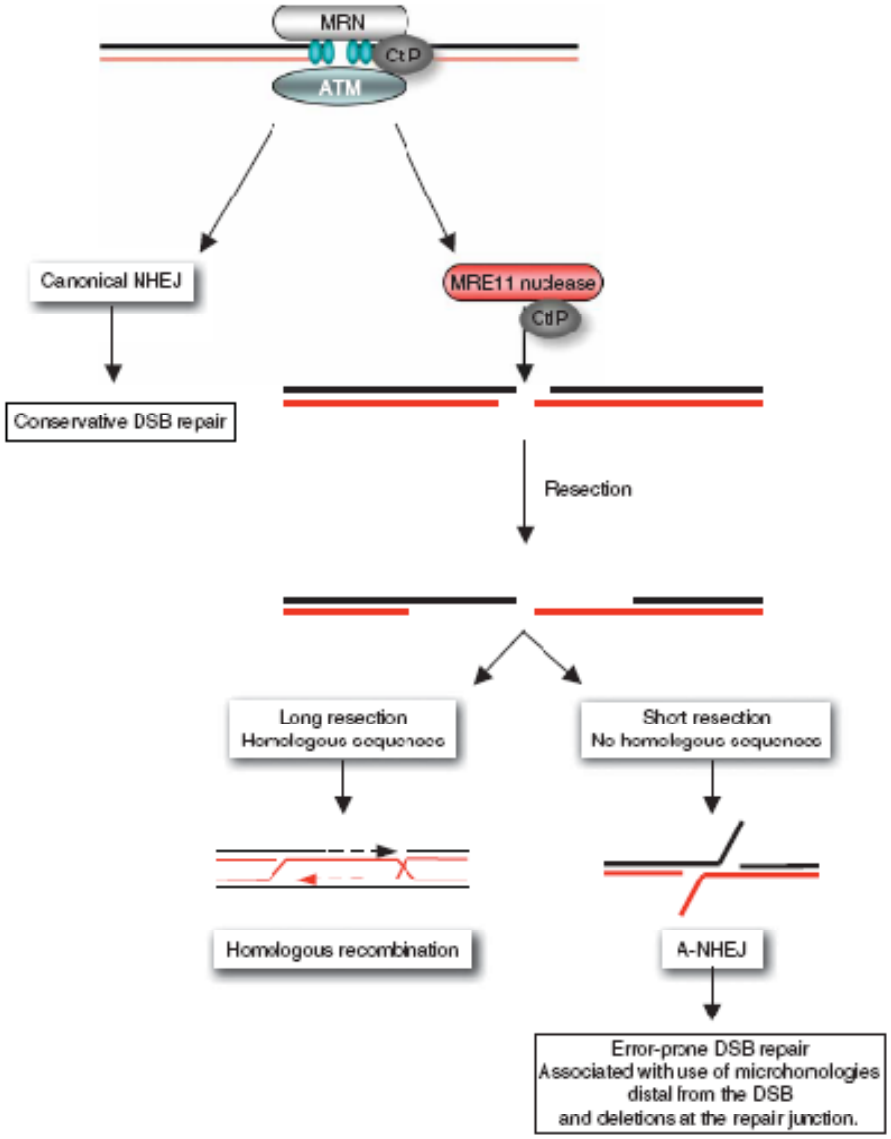


Chromosome fusions in the absence of Tpp1-Pot1a does not require Ku70



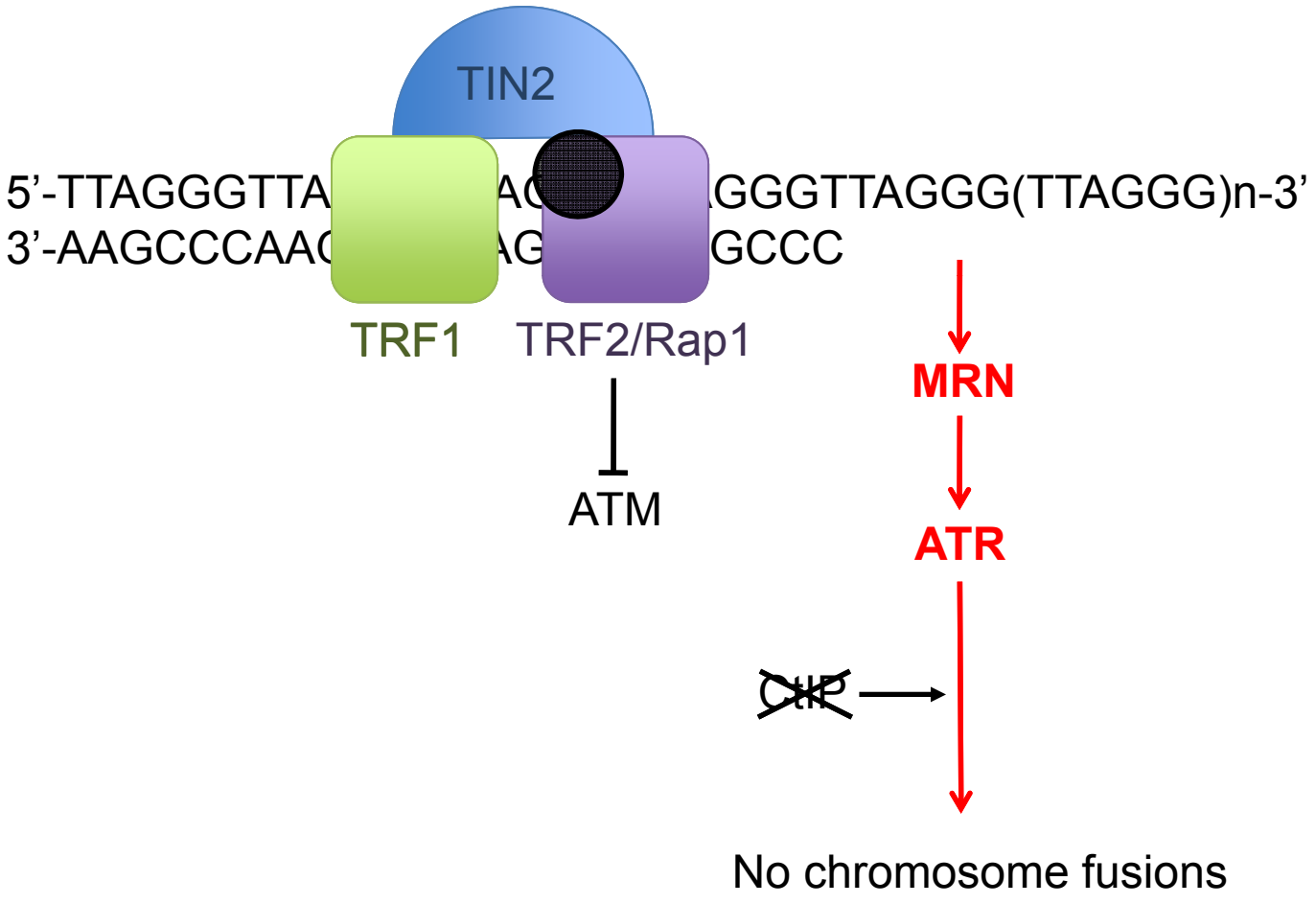


A-NHEJ mediated repair of DNA double strand breaks

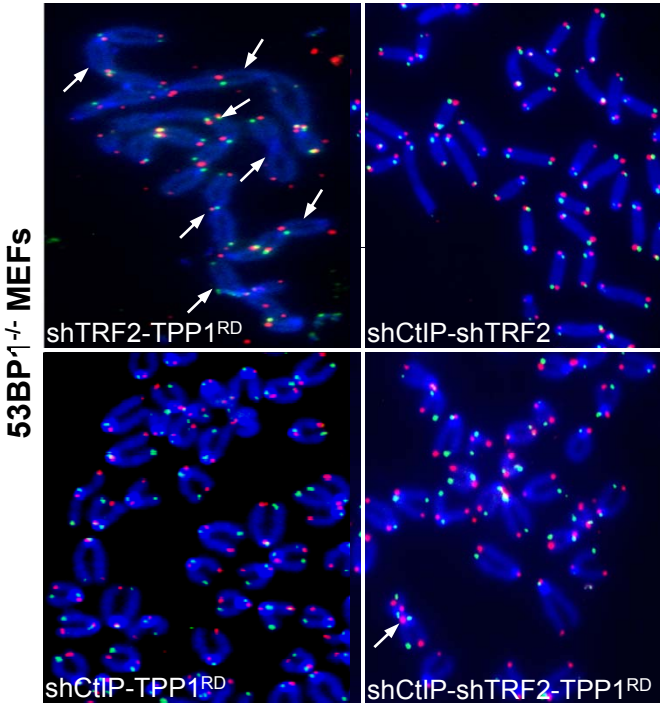


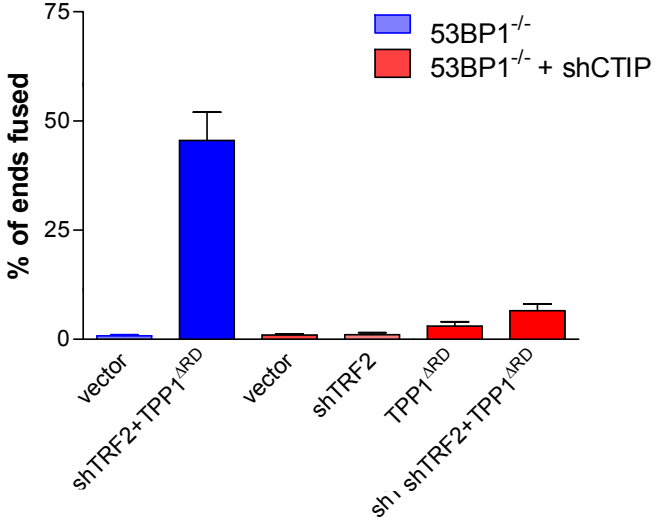
Rass et al., NSMB 2009

Disruption of Pot1a-Tpp1 leads to alternative NHEJ mediated chromosome fusions



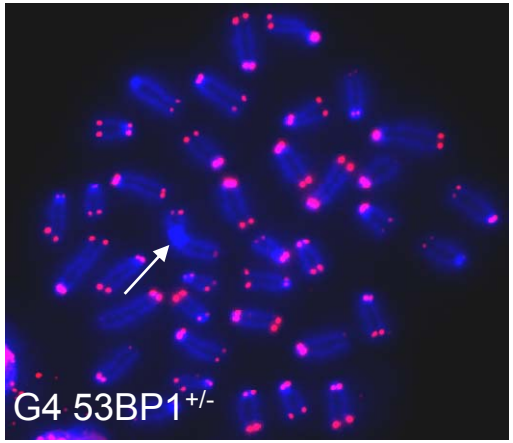
CtIP is required for A-NHEJ mediated repair of telomeres lacking Tpp1-Pot1a

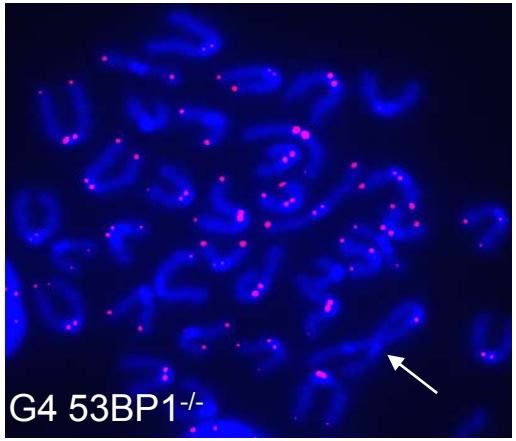




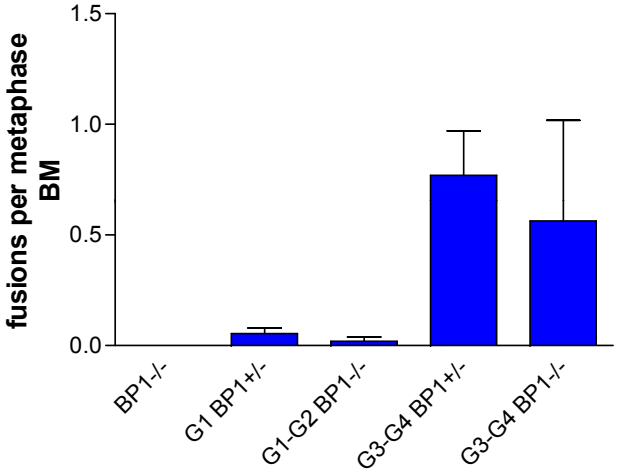
Naturally shortened telomeres are repaired independent of the C-NHEJ pathway

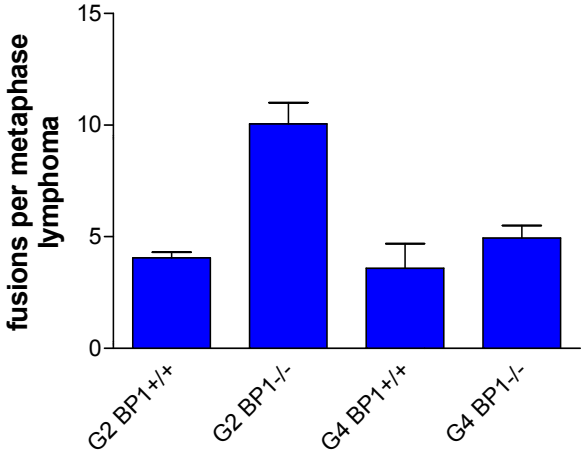
G4 mTerc$^{-/-}$ 53BP1$^{-/-}$ mice
G4 mTerc$^{-/-}$ 53BP1$^{+/-}$mice



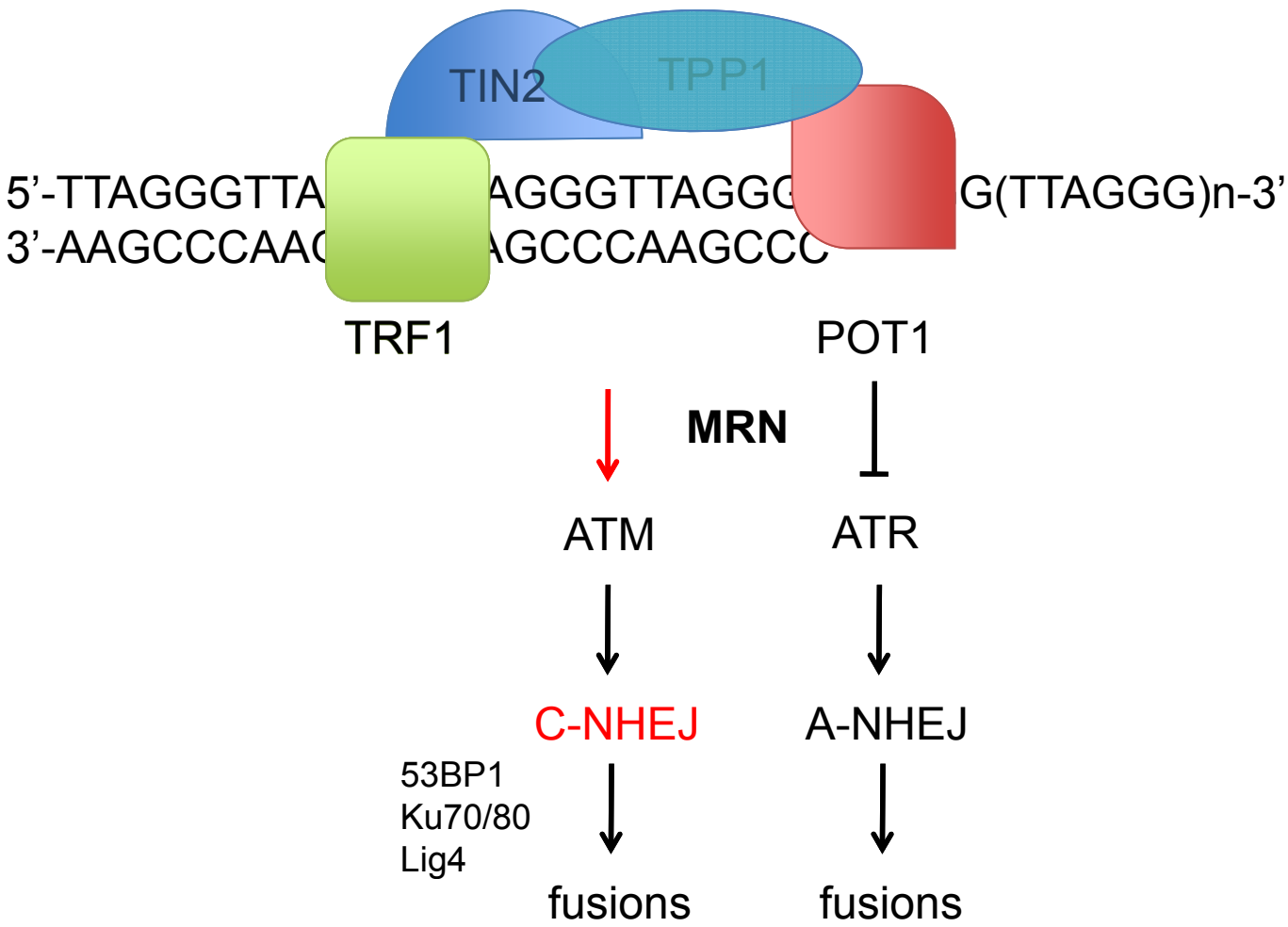


MEFs

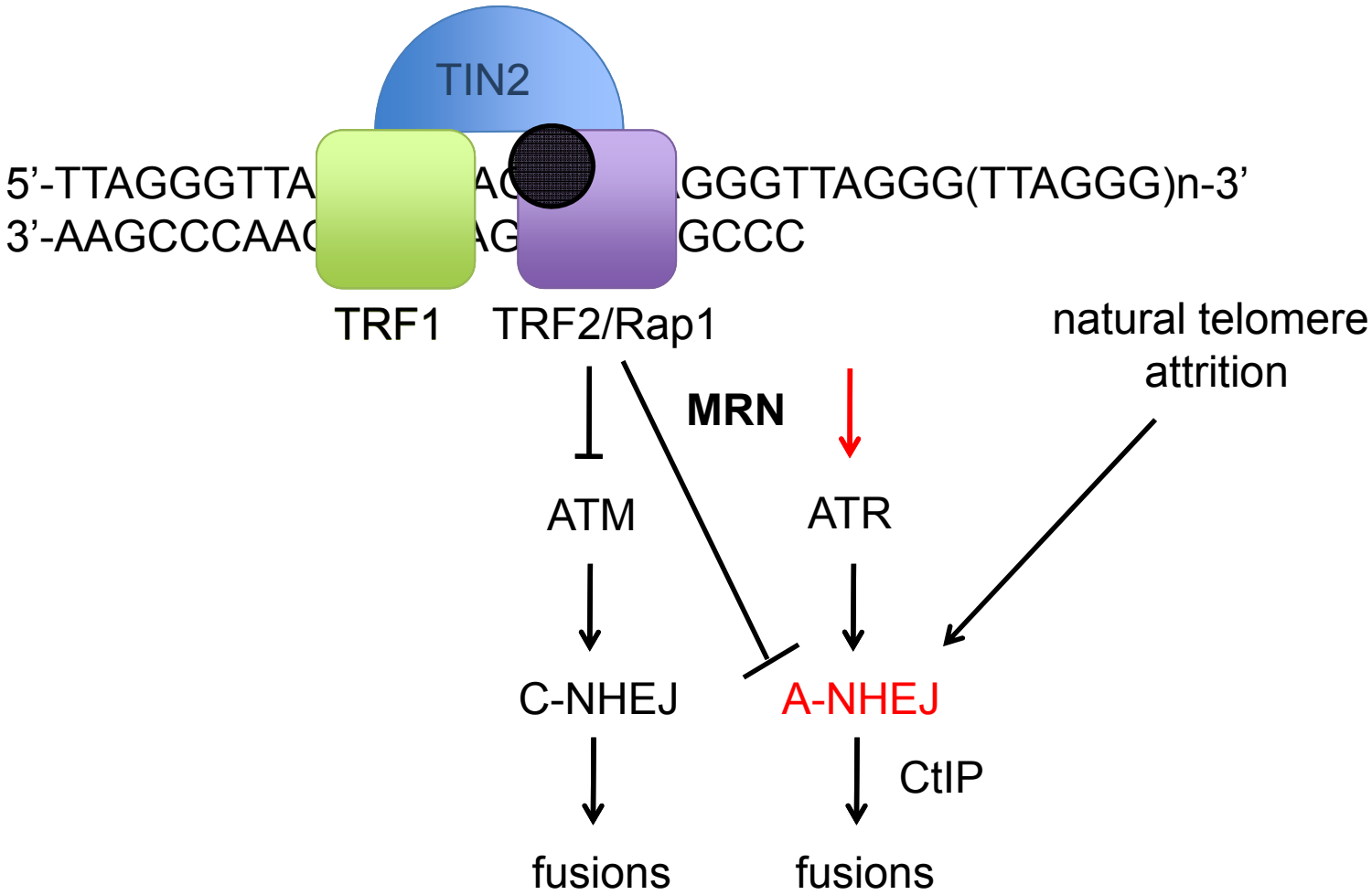




Distinct repair pathways depending on how telomeres are uncapped



Distinct repair pathways depending on how telomeres are uncapped



Acknowledgements

Pot1a
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Pot1b
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A-NHEJ
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Hong Zheng
Philip Carpenter, UT Medical School

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