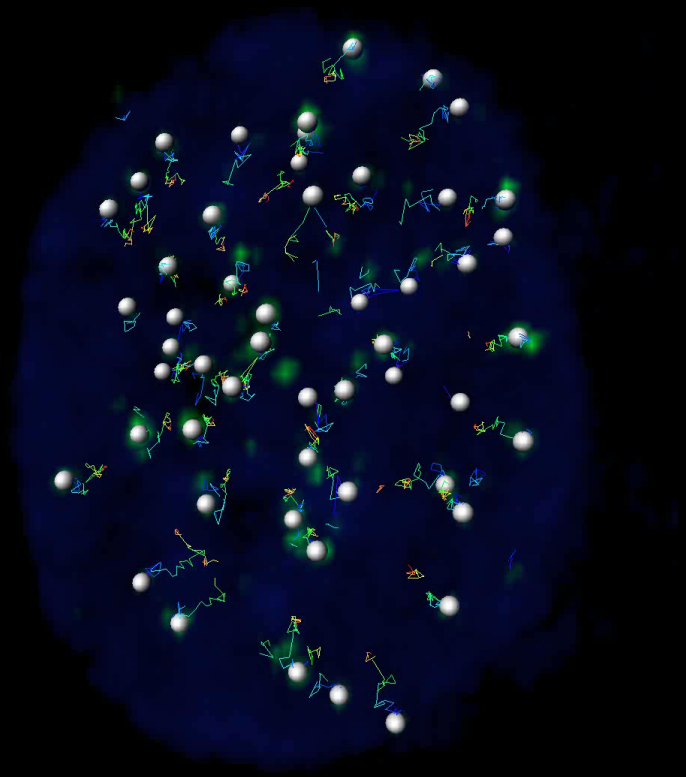
GETTING TO THE END BY ANY MEANS NECESSARY:

LESSONS LEARNED FROM PROTEOMICS OF HUMAN TELOMERES



Roderick J O'Sullivan
University of Pittsburgh Cancer Institute

Telomere length regulates proliferation

Telomere Shortening is a tumour suppression mechanism

NORMAL CELLS

Cell Divisions

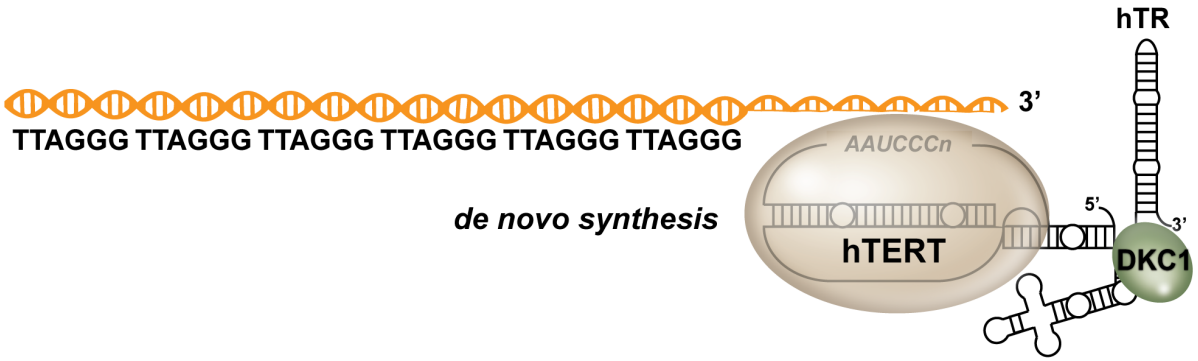
~12kb

Hayflick & Olovnikov
Shay & Wright

Hijacking telomere length mechanisms

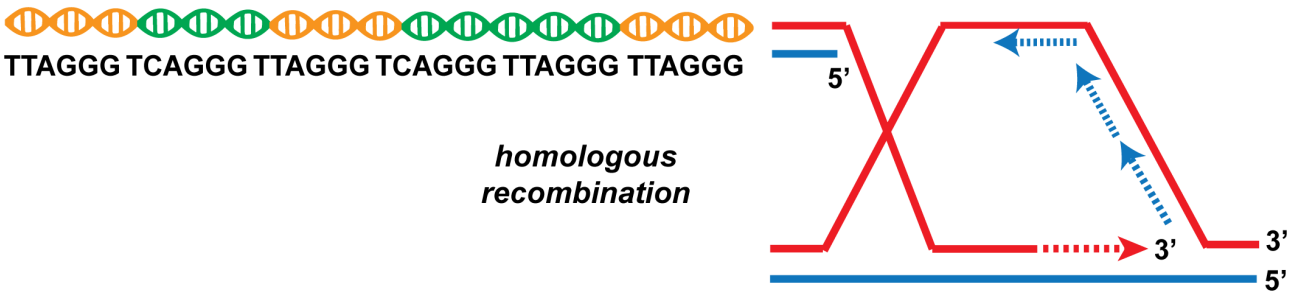
(i) Telomerase mediated Telomere Elongation

~85% Cancers



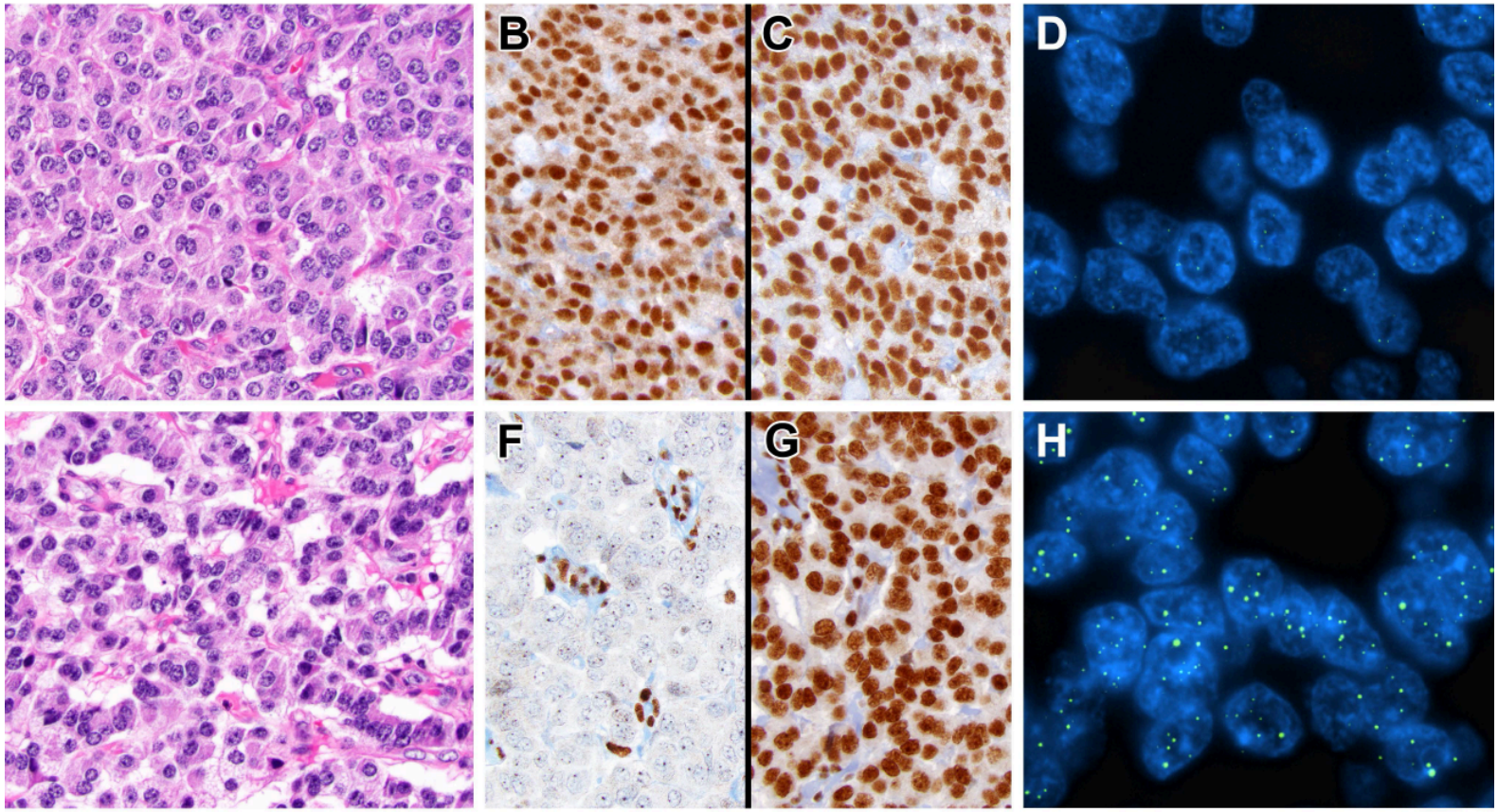
(ii) Alternative Lengthening of Telomeres (ALT)

~15% Cancers



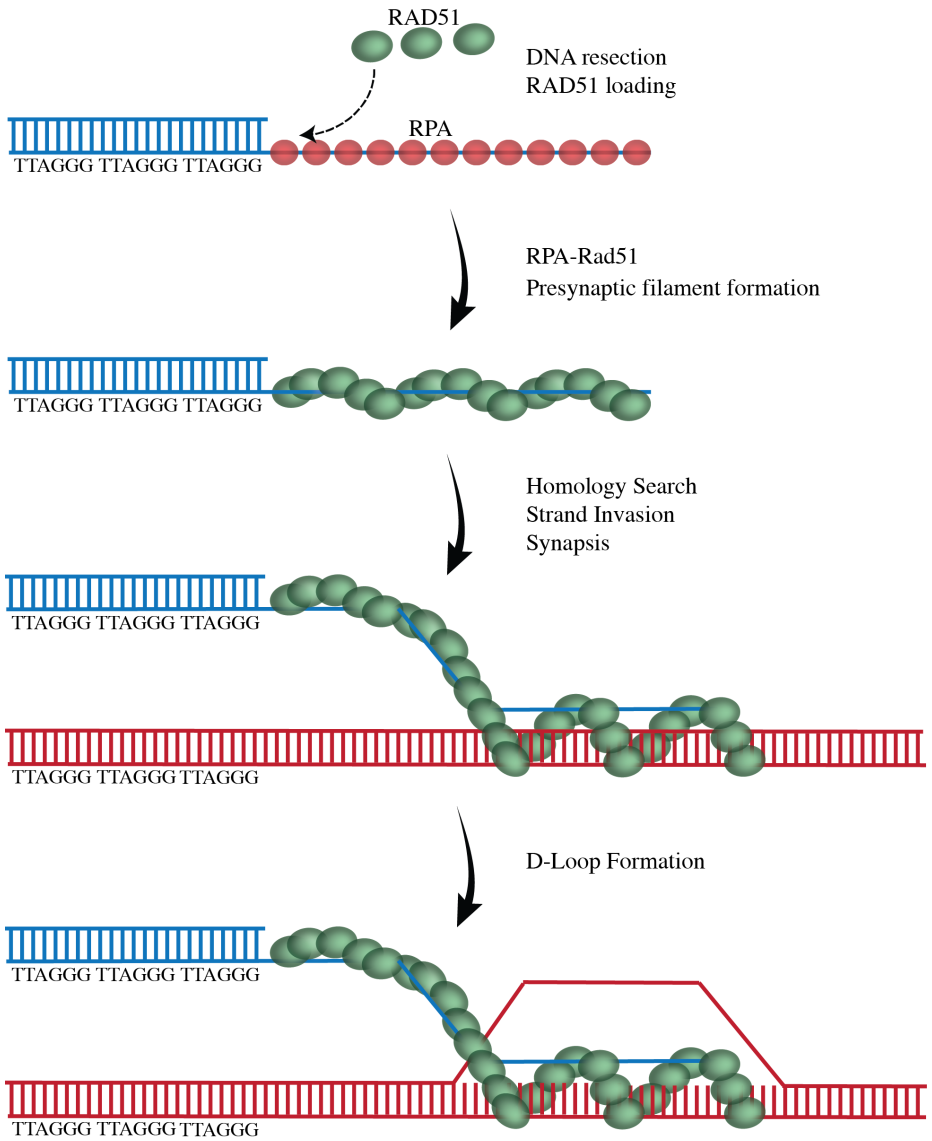
Loss of ATRX correlates with ALT

Pancreatic Neuroendocrine Tumors



Heaphy et al., Science (2012)
Singhi et al., CCR (2016)

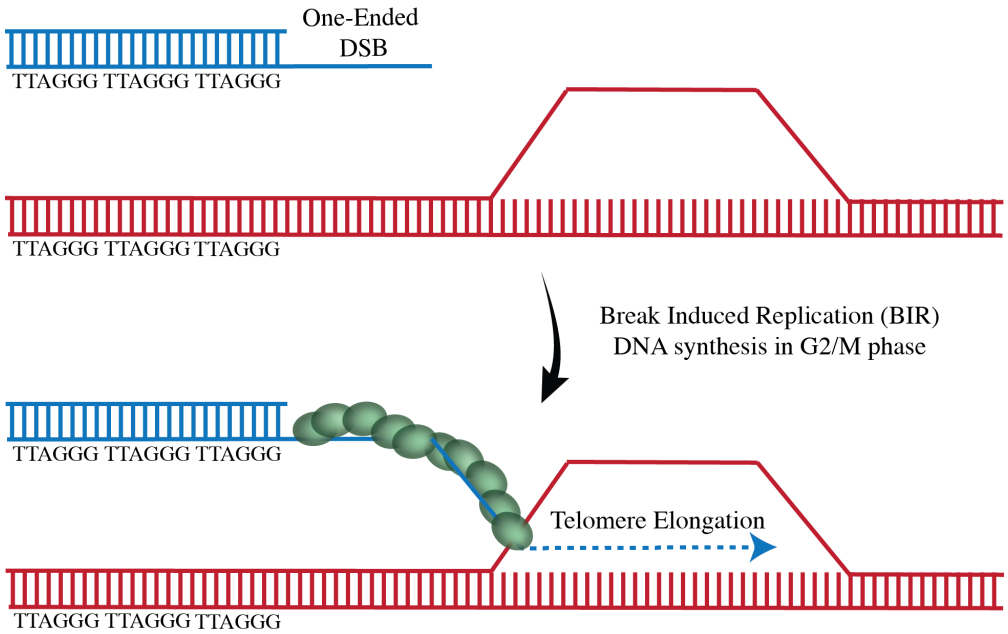
The ALT Mechanism



Roger Reddel, Sydney
Roger Greenberg, Penn

Break Induced Replication

Late Replication Intermediates



McEachern & Haber
Ann. Review Genetics
(2002

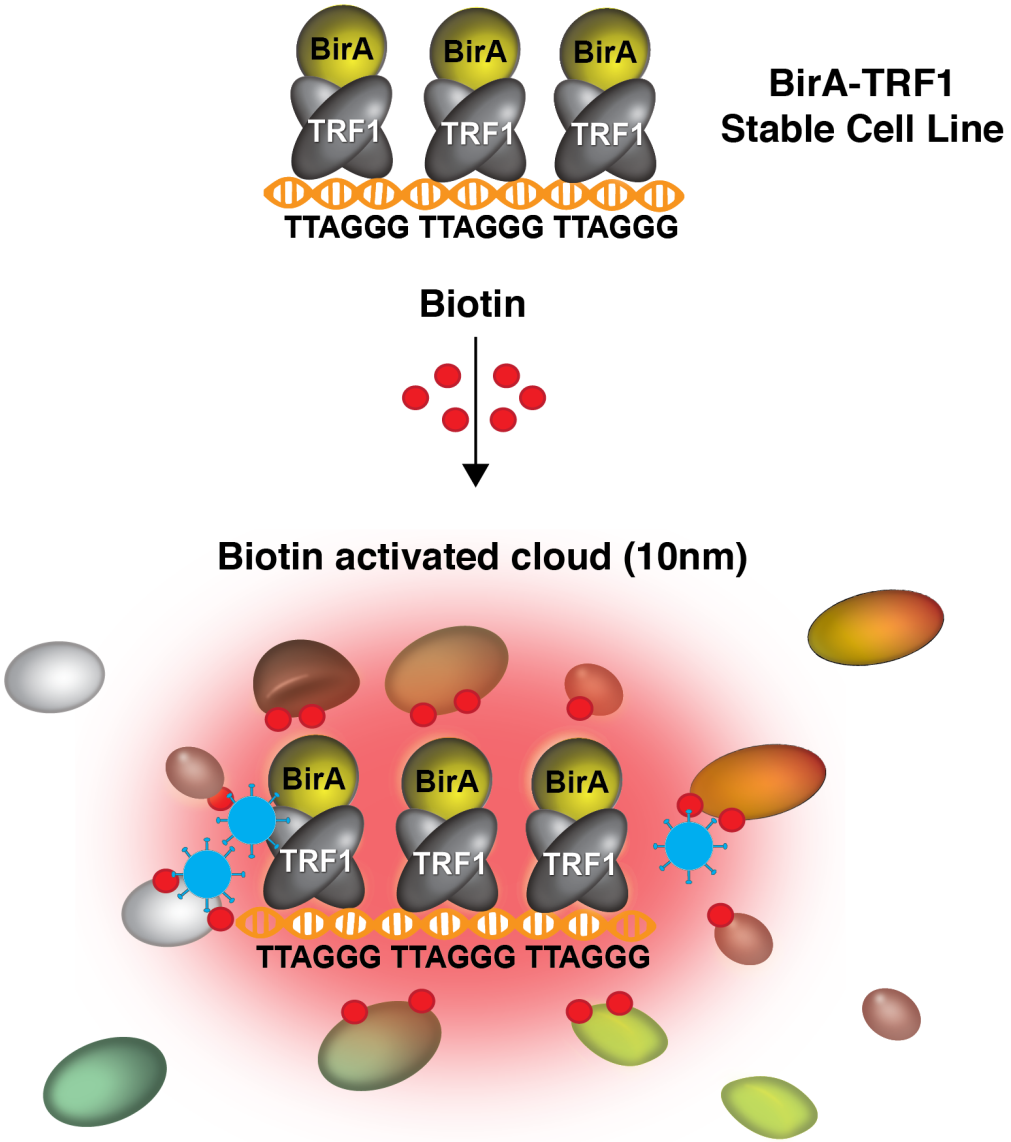
Identification of apical regulators of the ALT mechanism

Proximity dependent biotinylation (Bio-ID)

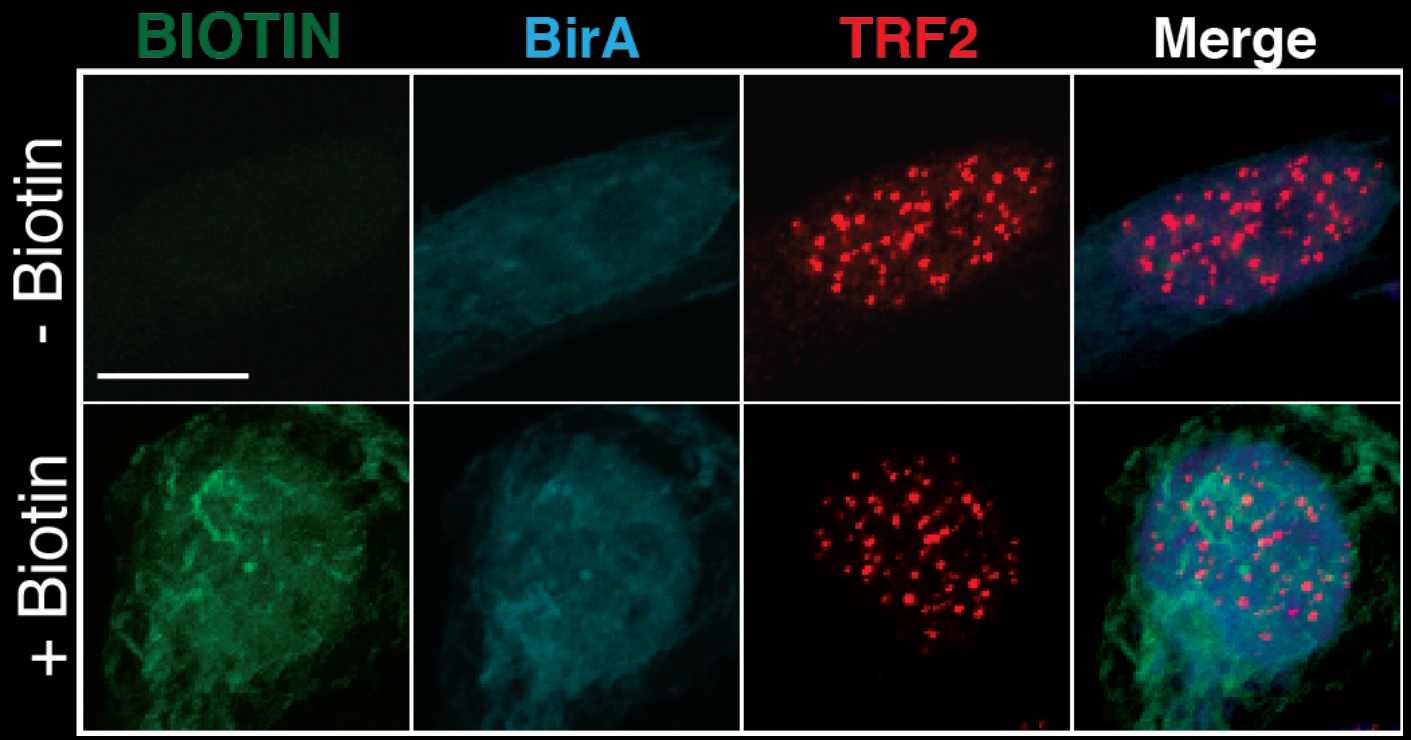
- **Developed by Kyle Roux (Sanford Burnham, Sioux Falls)**

- **Tagging of protein with prokaryotic Biotin Ligase (BirA*)**
  Roux et al., JCB (2012)
- **BirA-R118G (BirA*) is a more promiscuous biotin ligase**
  Kwon et al., MCB (2002)
- **BirA* has a labeling radius of ~10 nanometers**
  Kim et al., PNAS (2014)

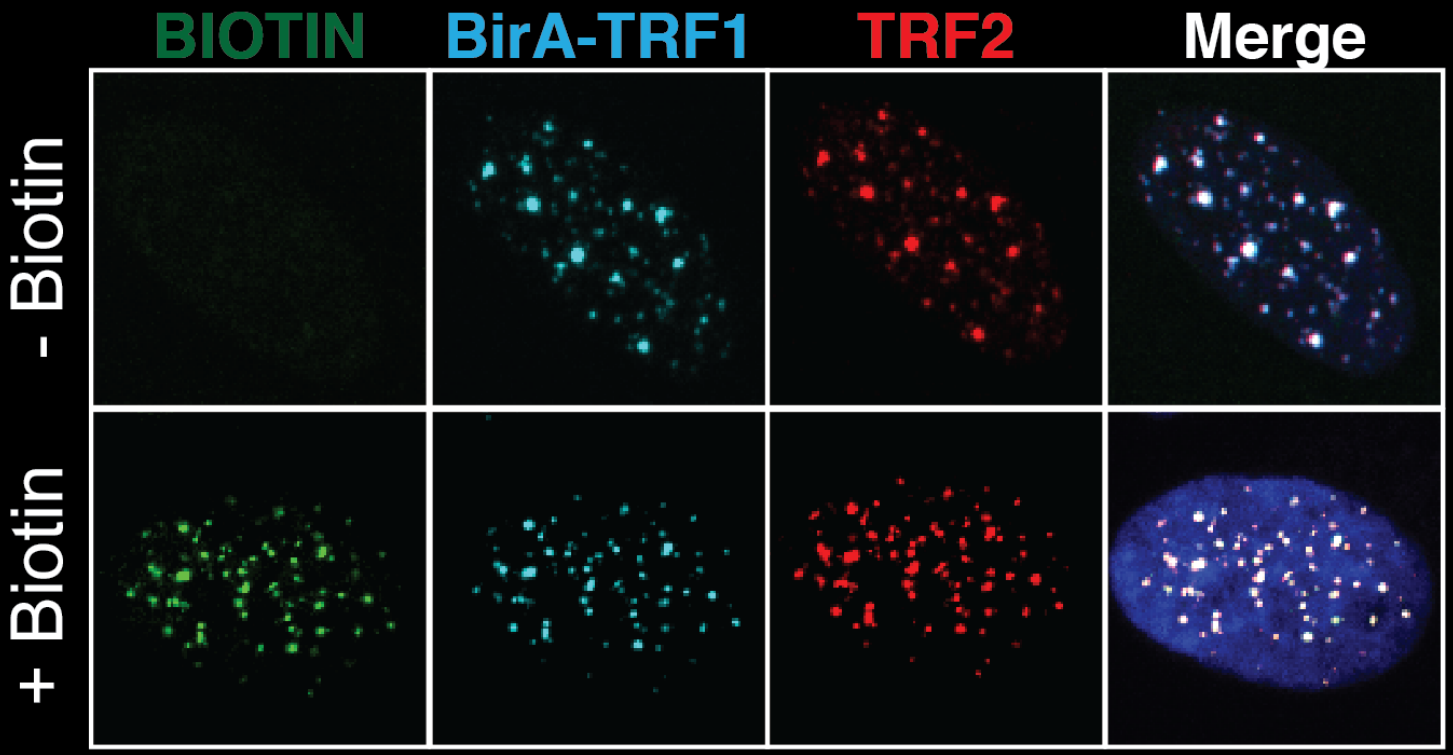
Bio-ID of the telomeric proteome



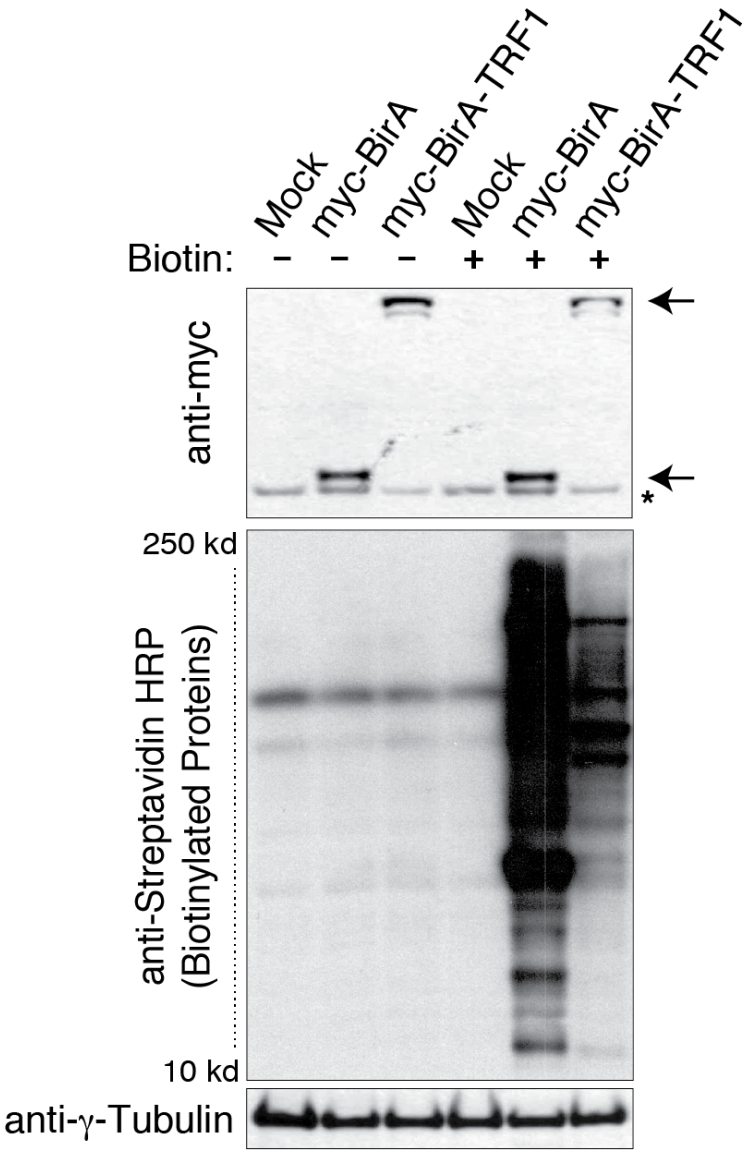
Precise tethering of BirA to telomeres

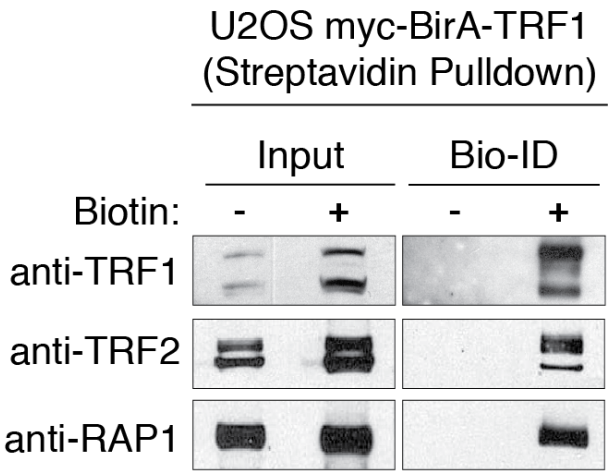


Precise tethering of BirA to telomeres

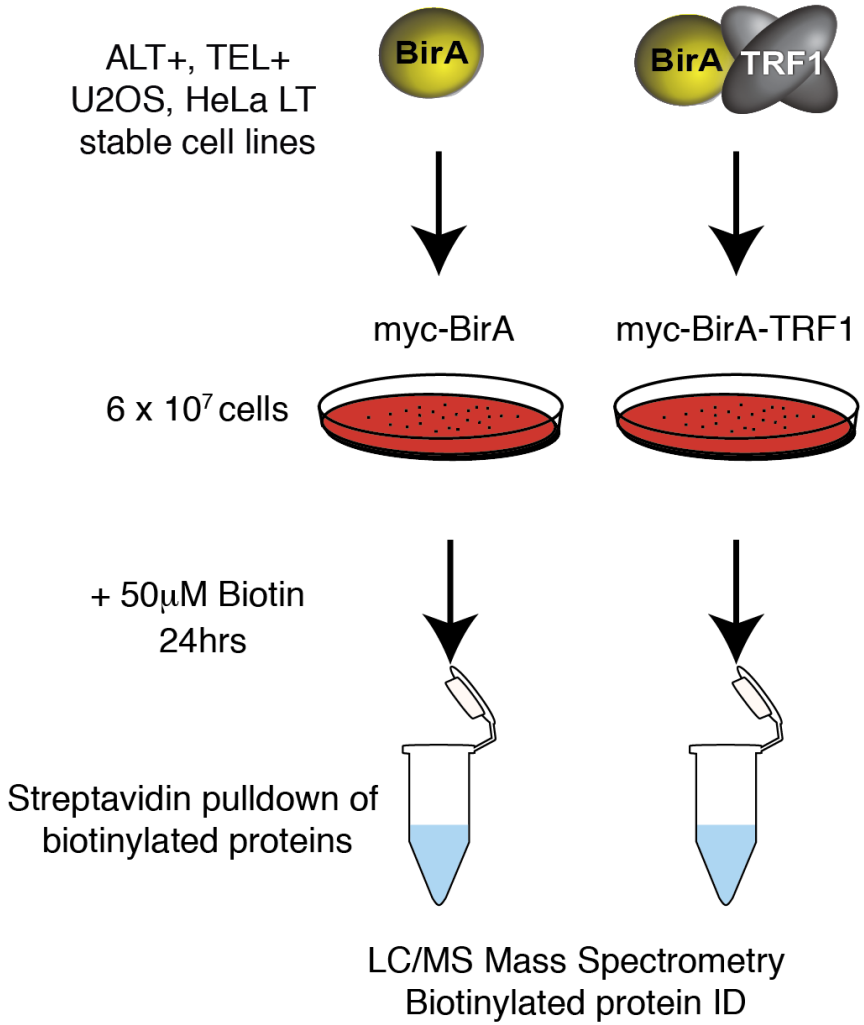


Validation of telomeric Bio-ID

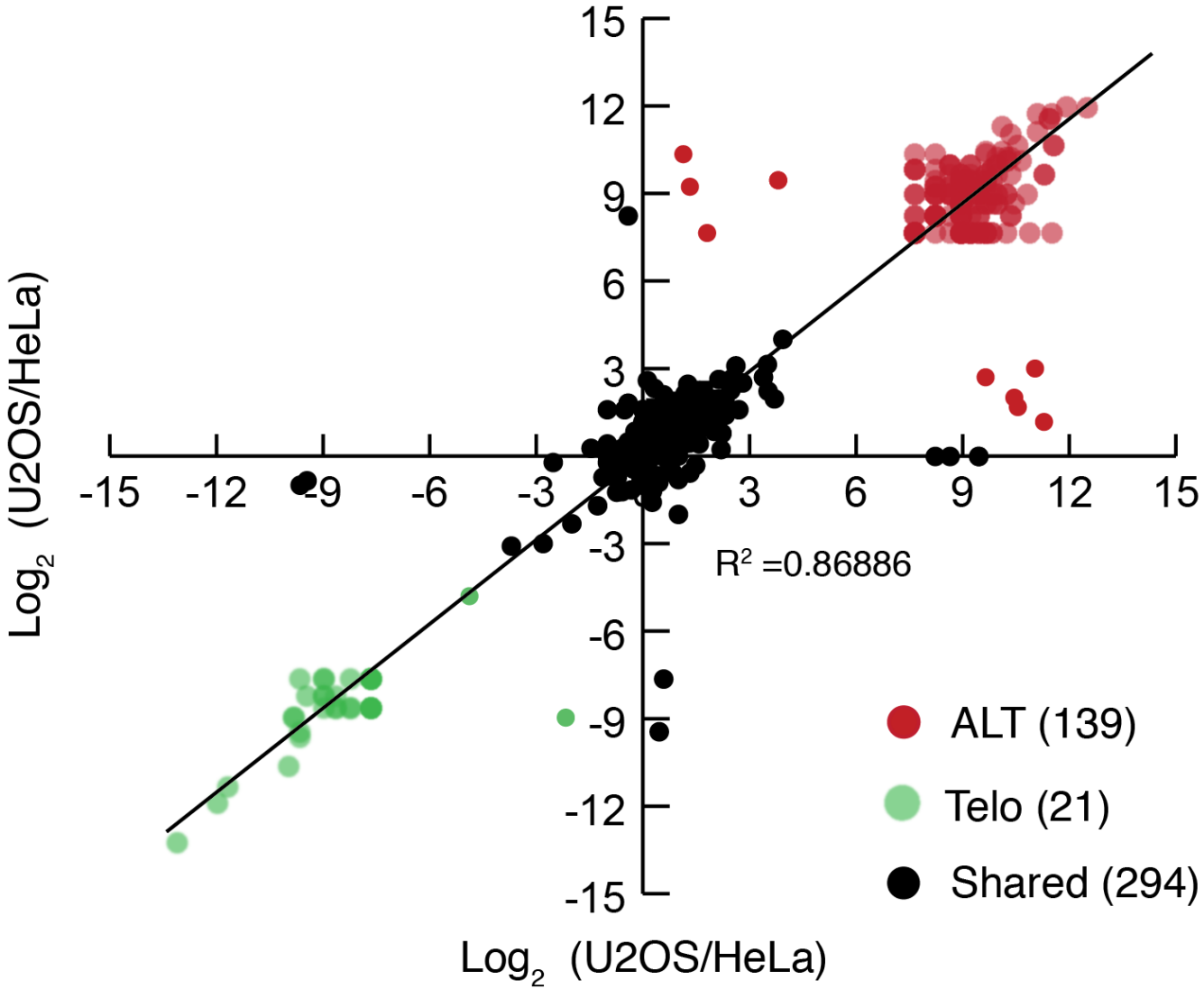




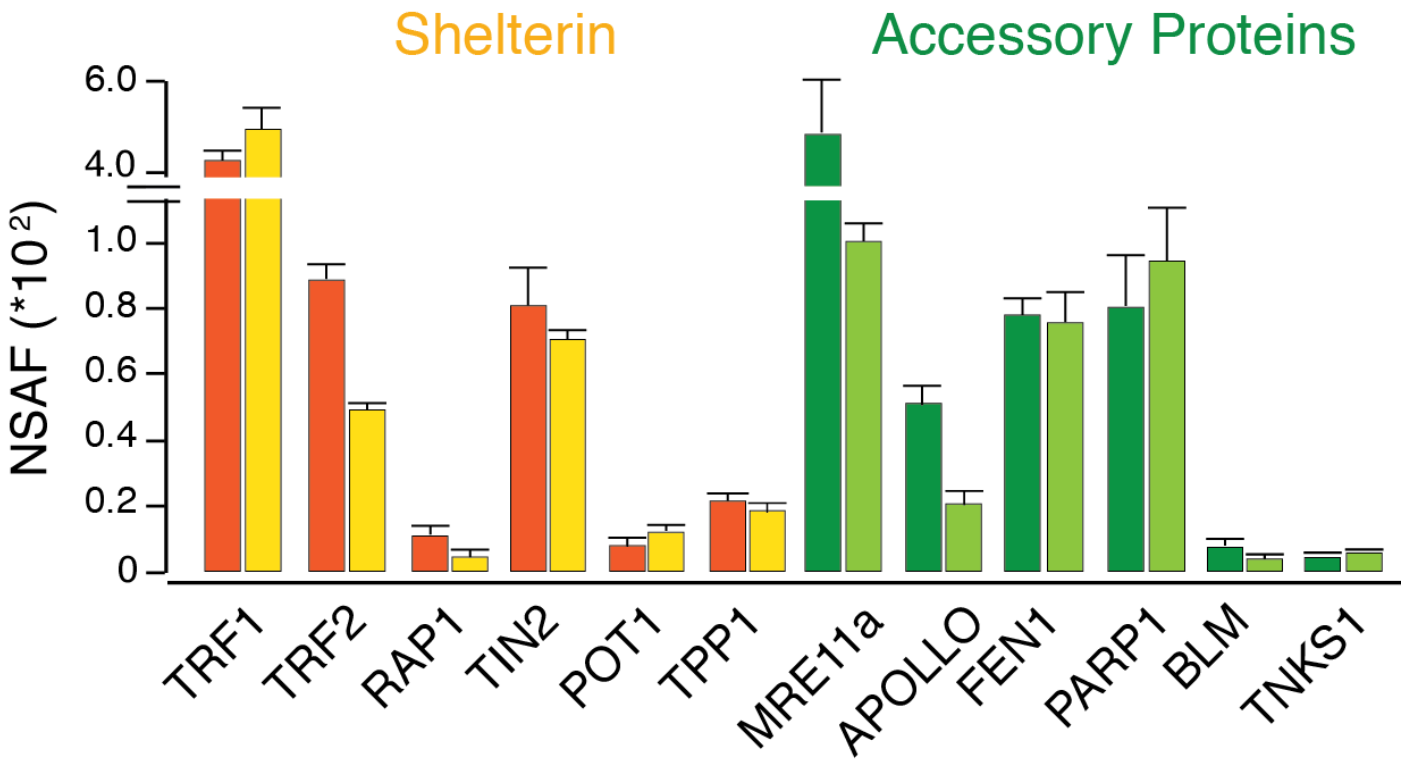
Proteomic profile of telomeres



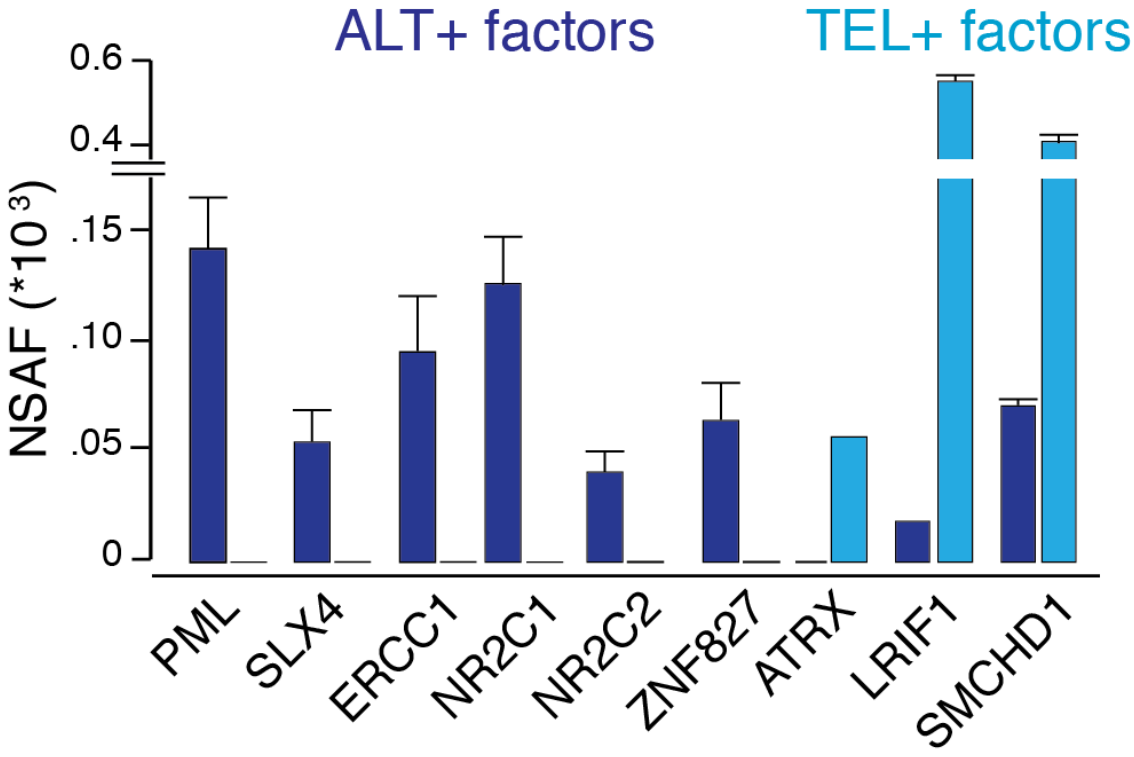
Proteomic composition of telomeres



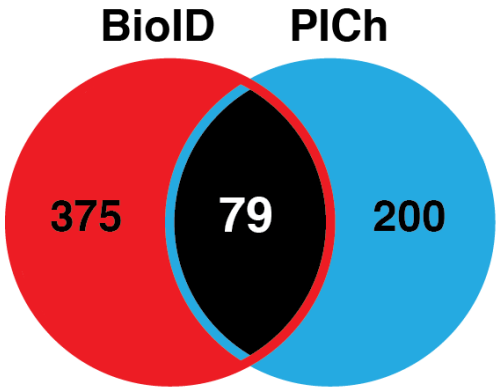
Efficient capture of telomeric proteins

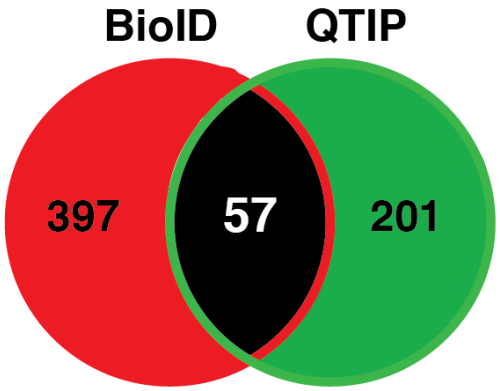


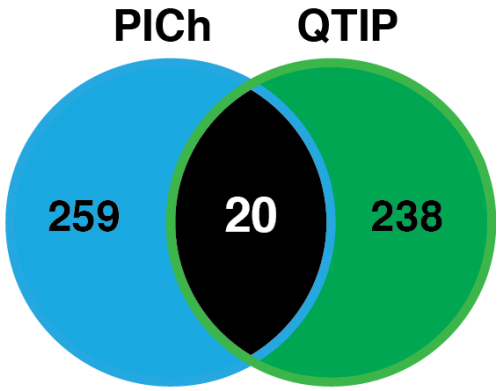
Efficient capture of ALT specific proteins



Comparison with other methods





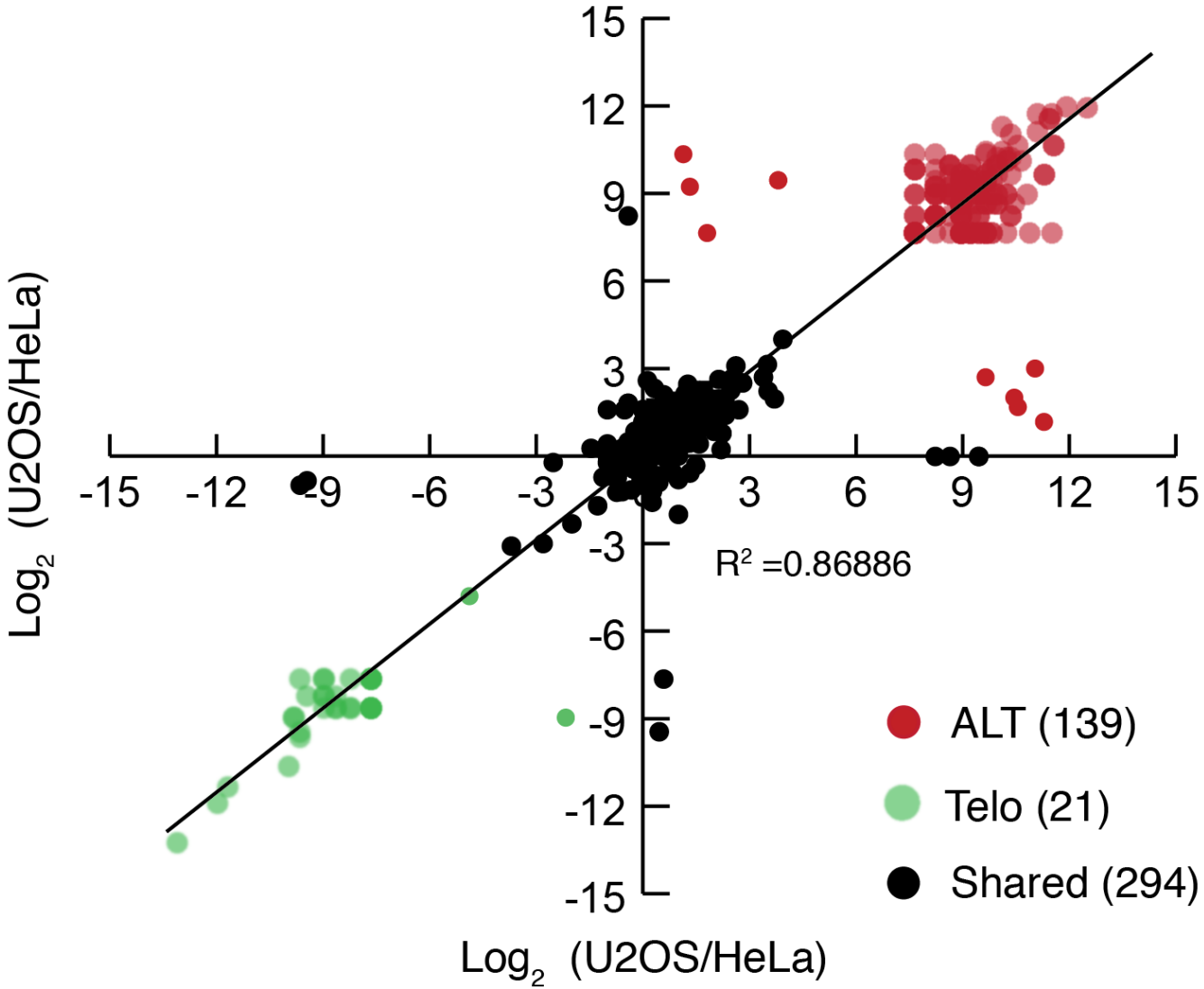


Proteins not detected by Bio-ID
*TERT (but DKC1 was identified)
*RAD51 (& paralogs)
*MUS81-EME1
*RTEL, WRN, PIF1
*ATM, ATR, RPA2
*ASF1a-ASF1b

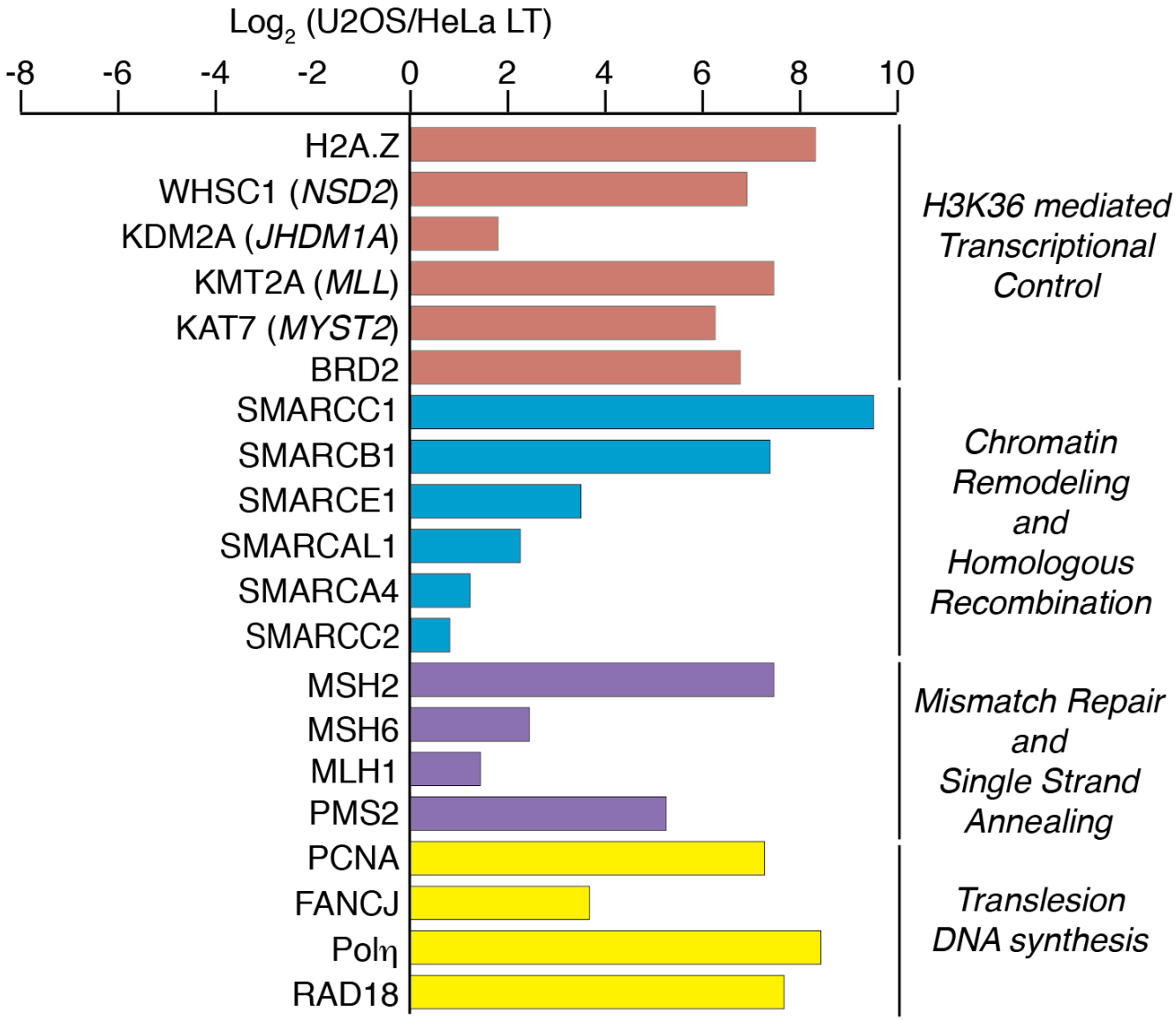
PICh: ***proteomics of isolated chromatin*** **- hybridization and capture with PNA oligos**

QTIP: ***quantitative telomeric isolation protocol*** **– IP/MS of shelterin complex associations**

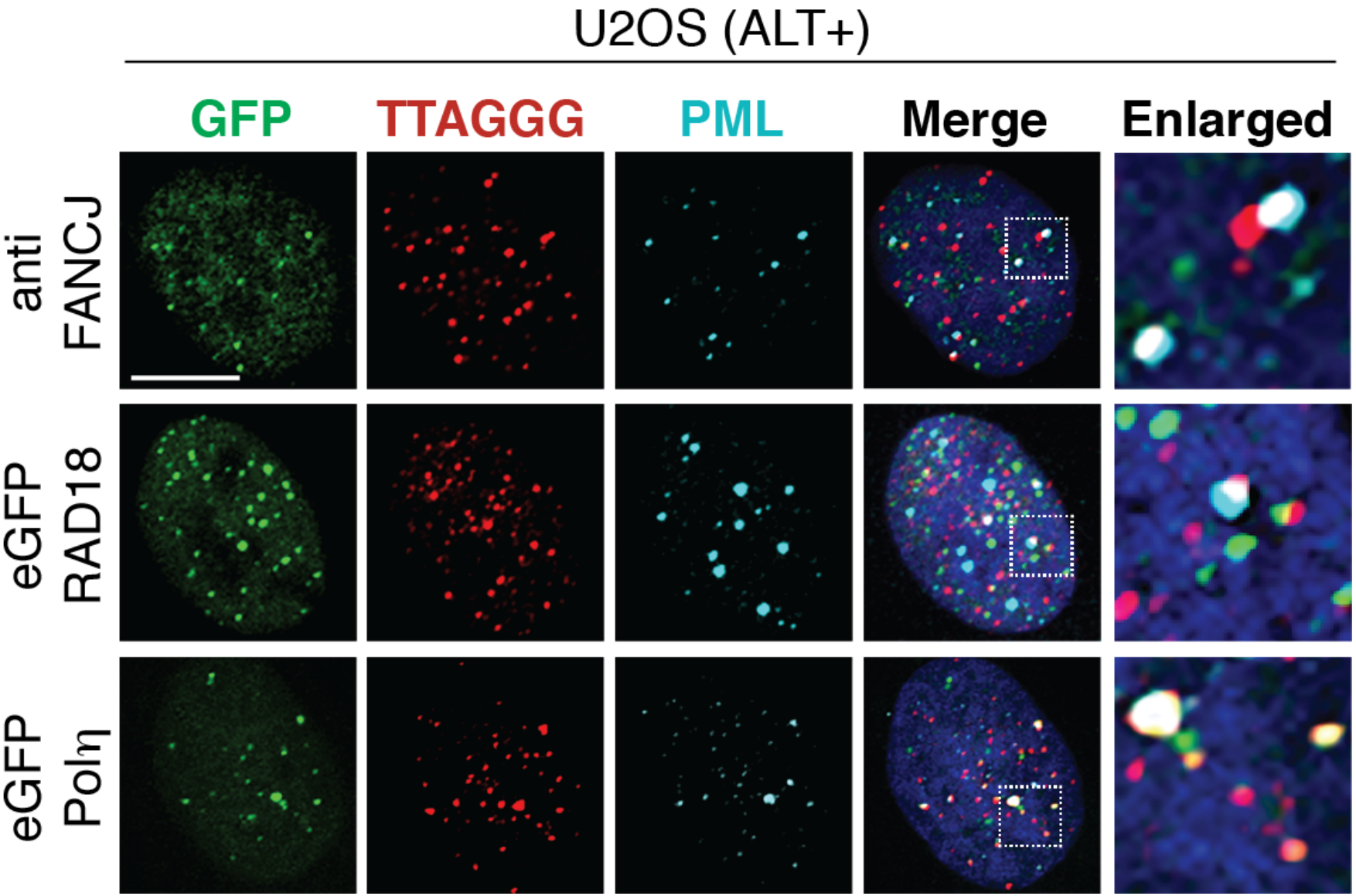
Proteomic composition of telomeres



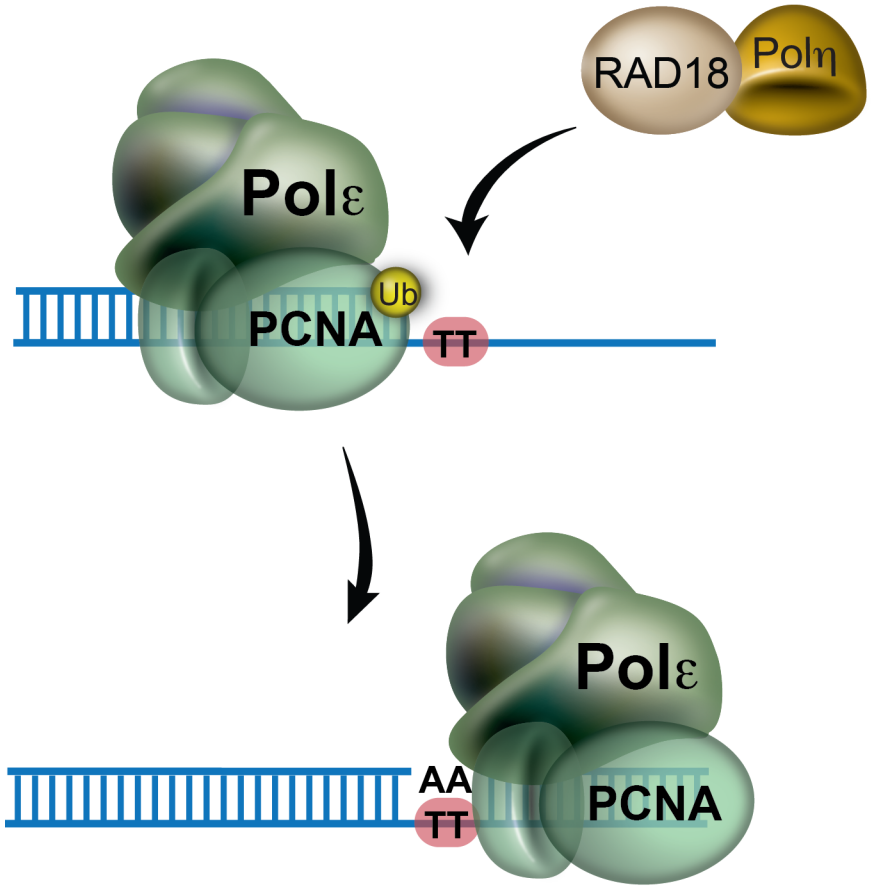
DNA repair networks at ALT telomeres



TLS proteins at telomeres in ALT cells

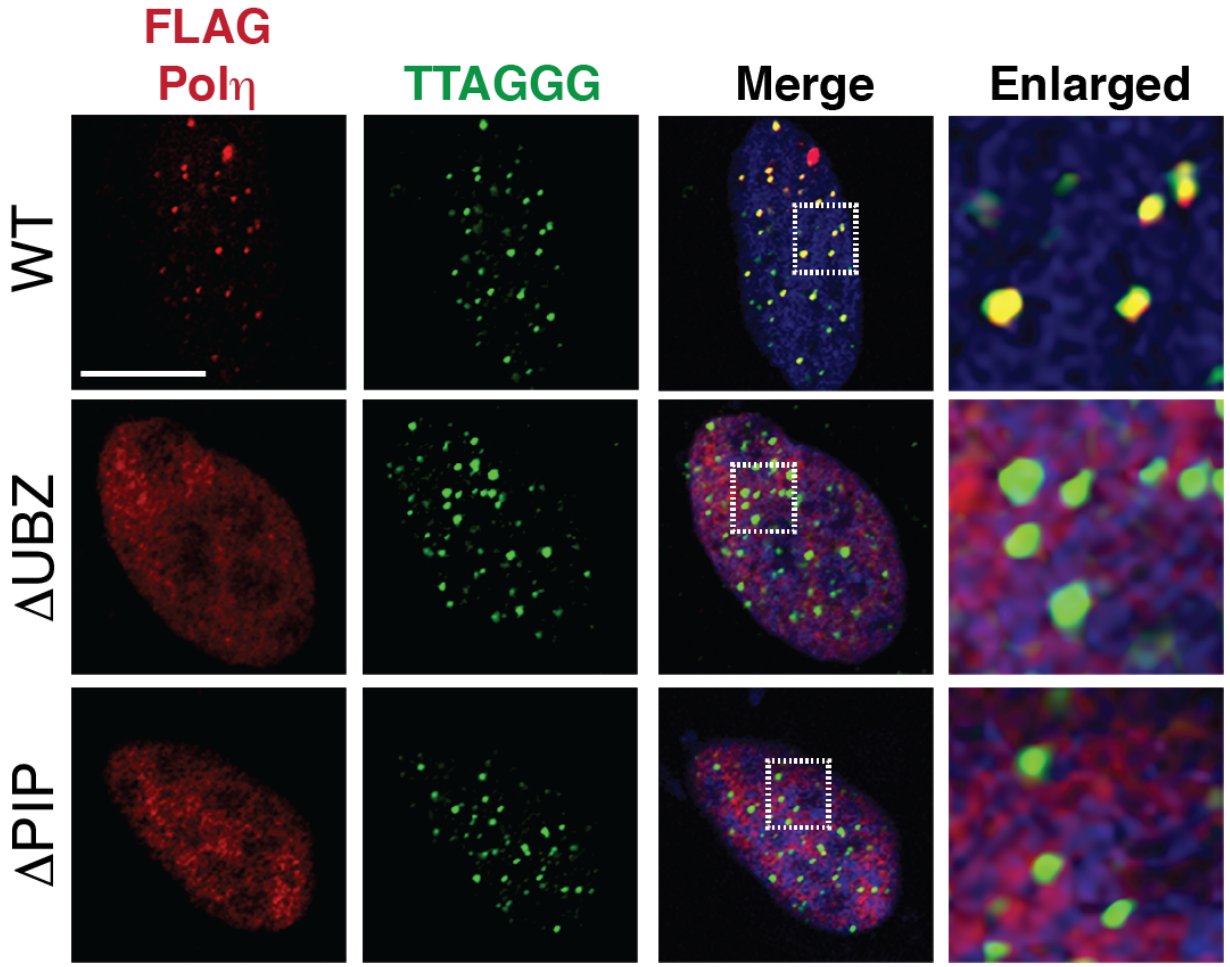


The role of Polη in translesion DNA synthesis



Adapted from Sale et al., 2010

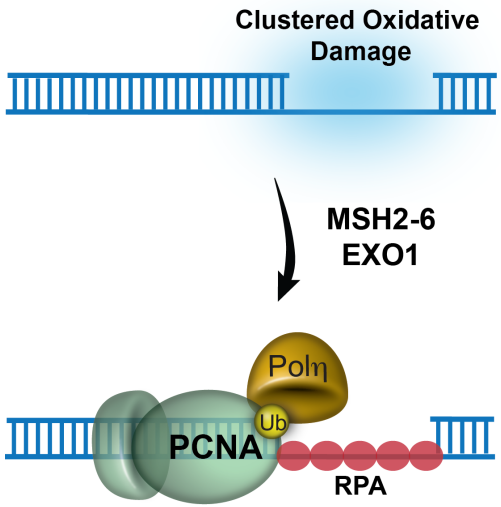
Localization of Polη to ALT telomeres is dependant on ^{Ub}-PCNA



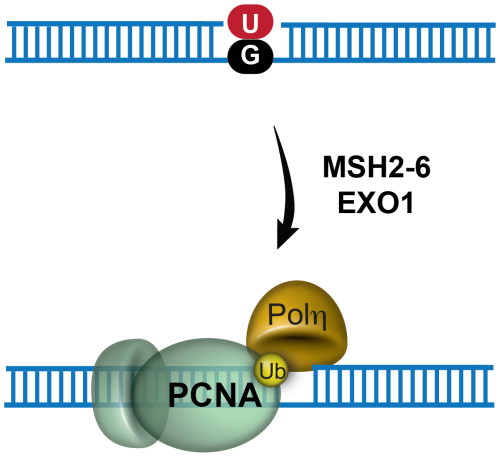
Thanks to: Ivan Dikic & Alan Lehmann

Non-canonical roles of Polη

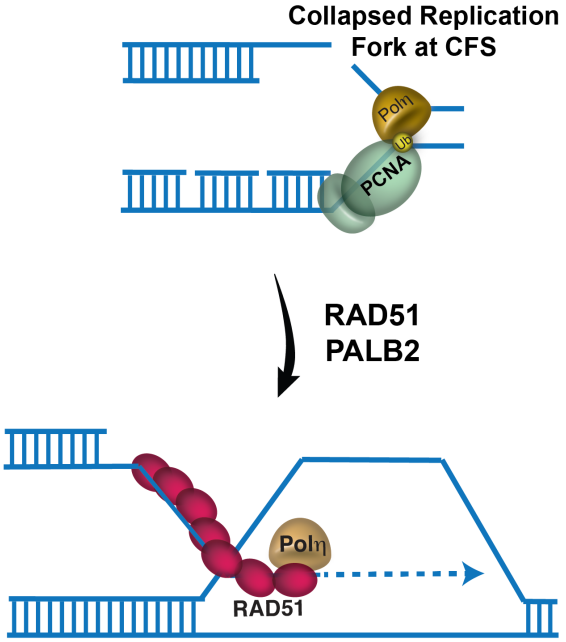
Repair of Oxidative Damage



Somatic Hypermutation

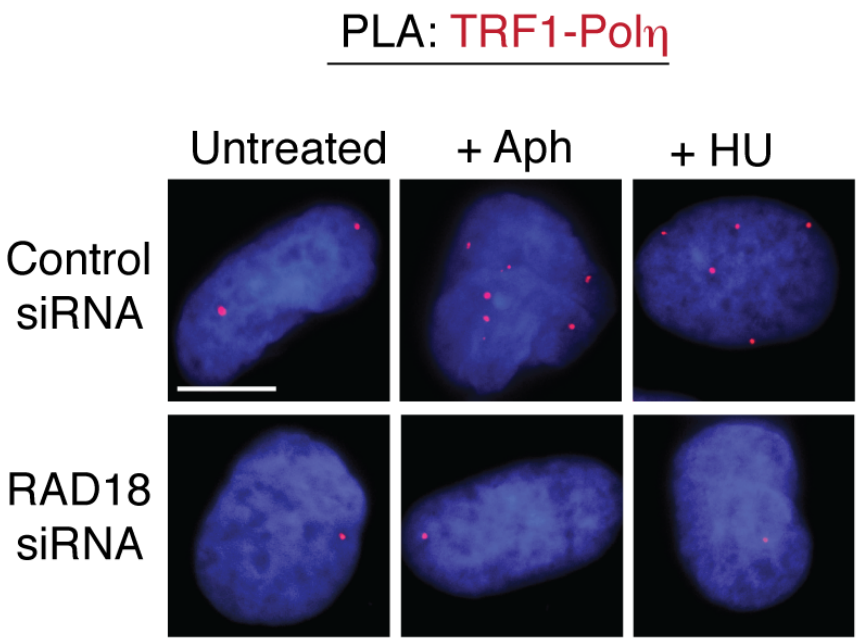


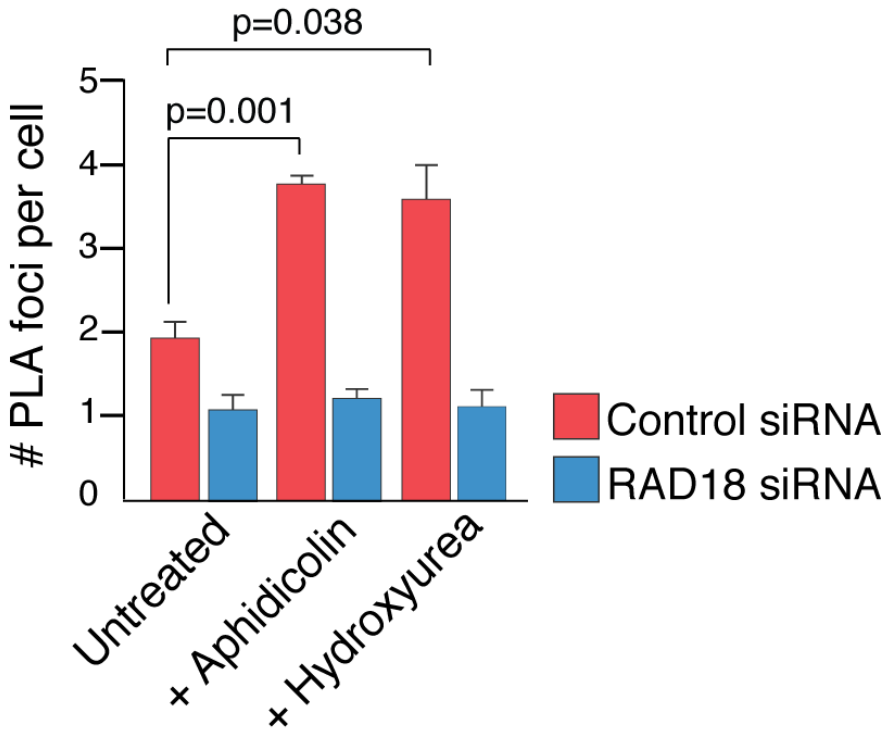
Homologous Recombination



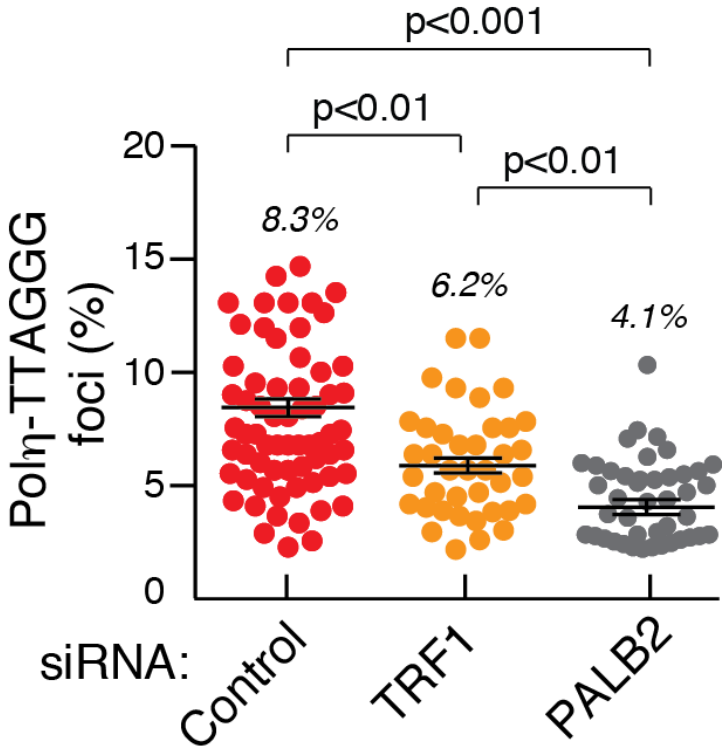
Adapted from Sale et al., 2010

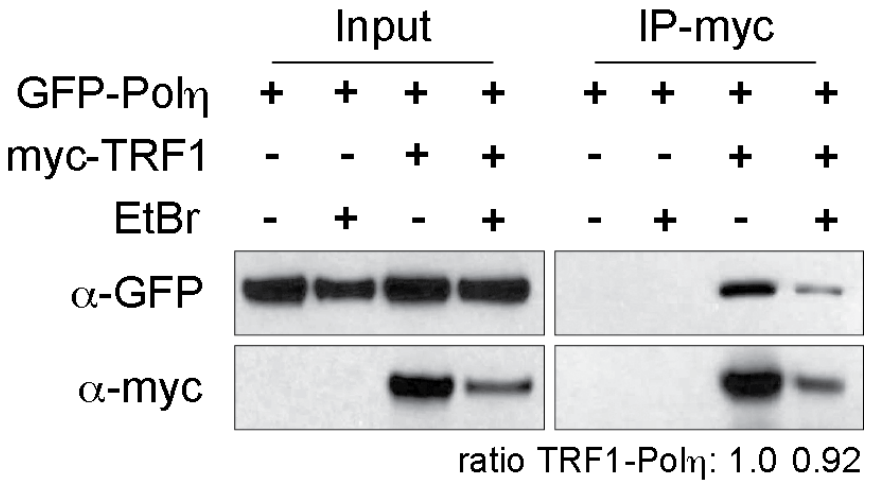
Accumulation of Polη at ALT telomeres due to replicative stress





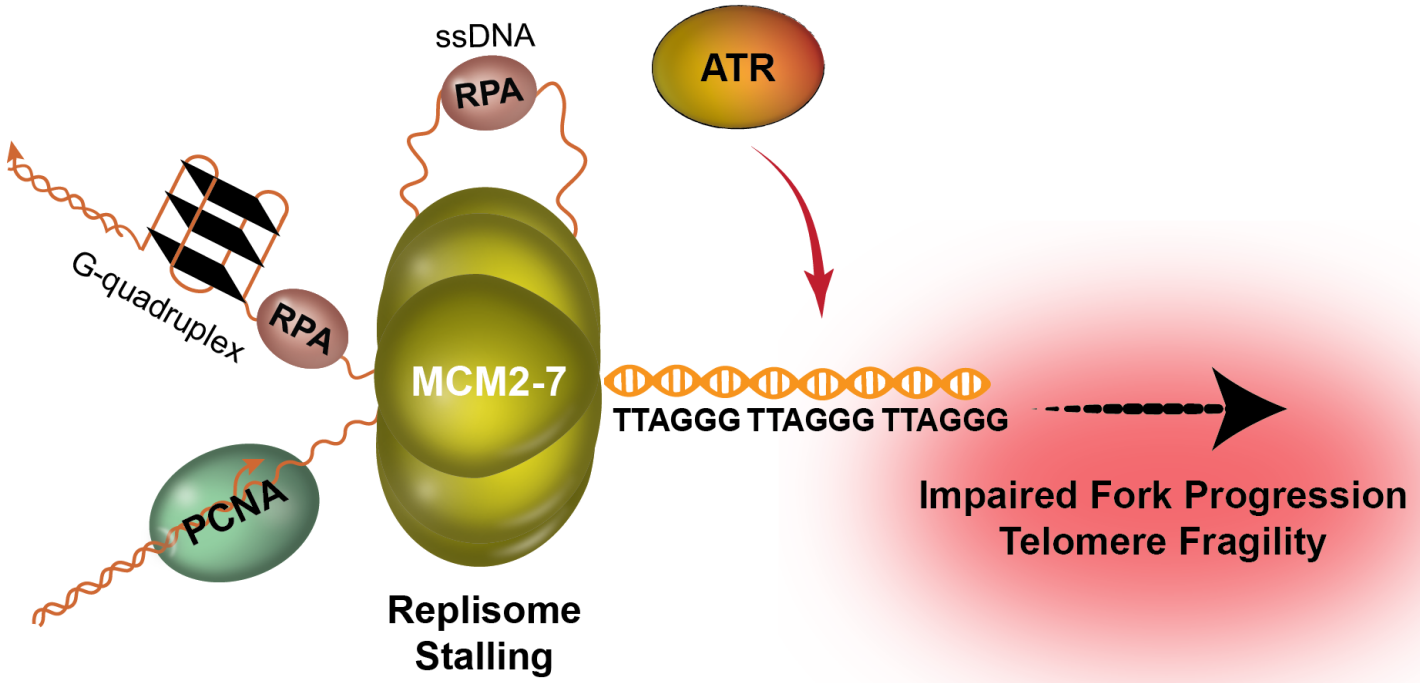
Association between Polη and TRF1





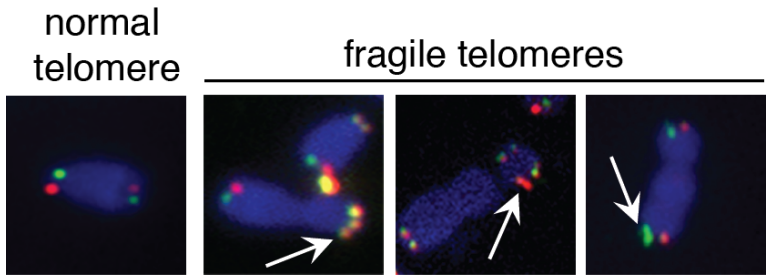
TRF1 regulates telomere replication

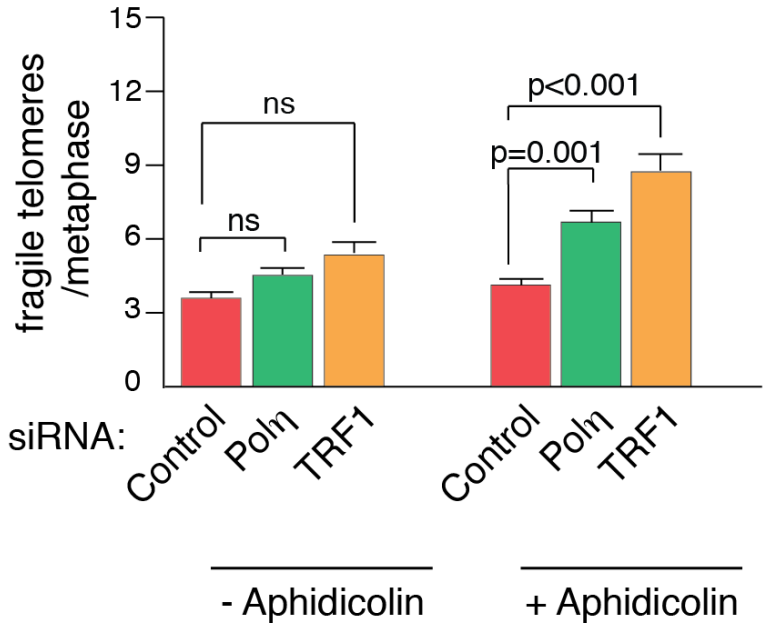
TRF1 KO + aphidicolin



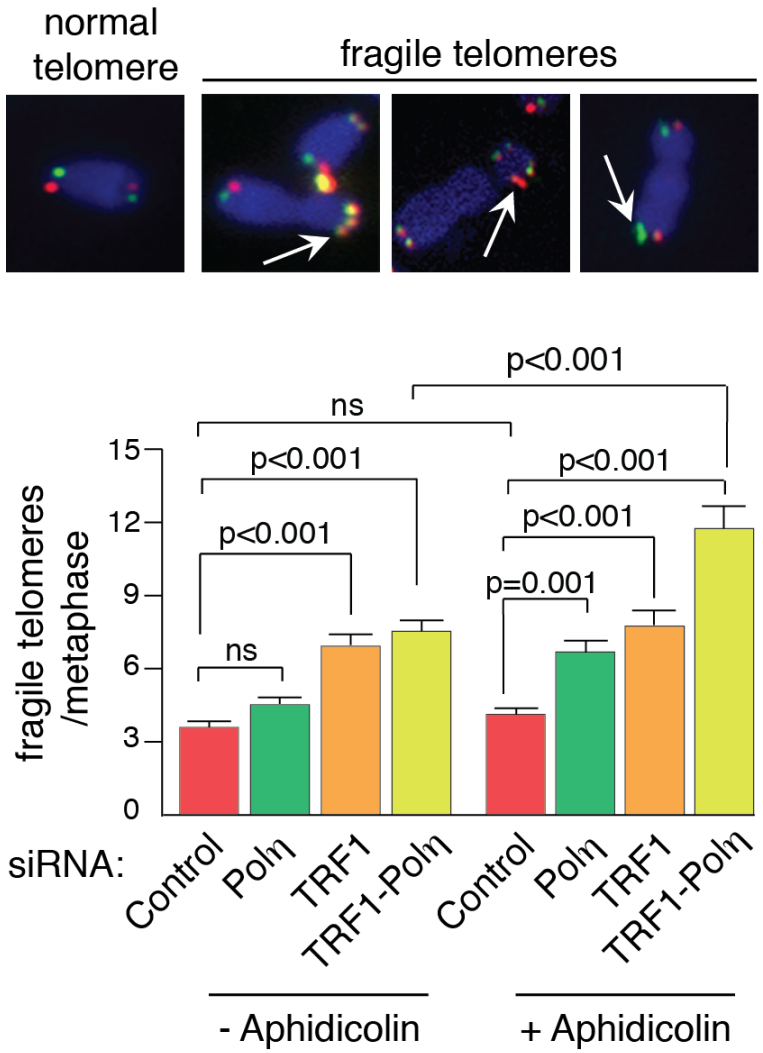
Agnel Sfeir, Cell (2009)

Polη loss has similar effects as TRF1 loss



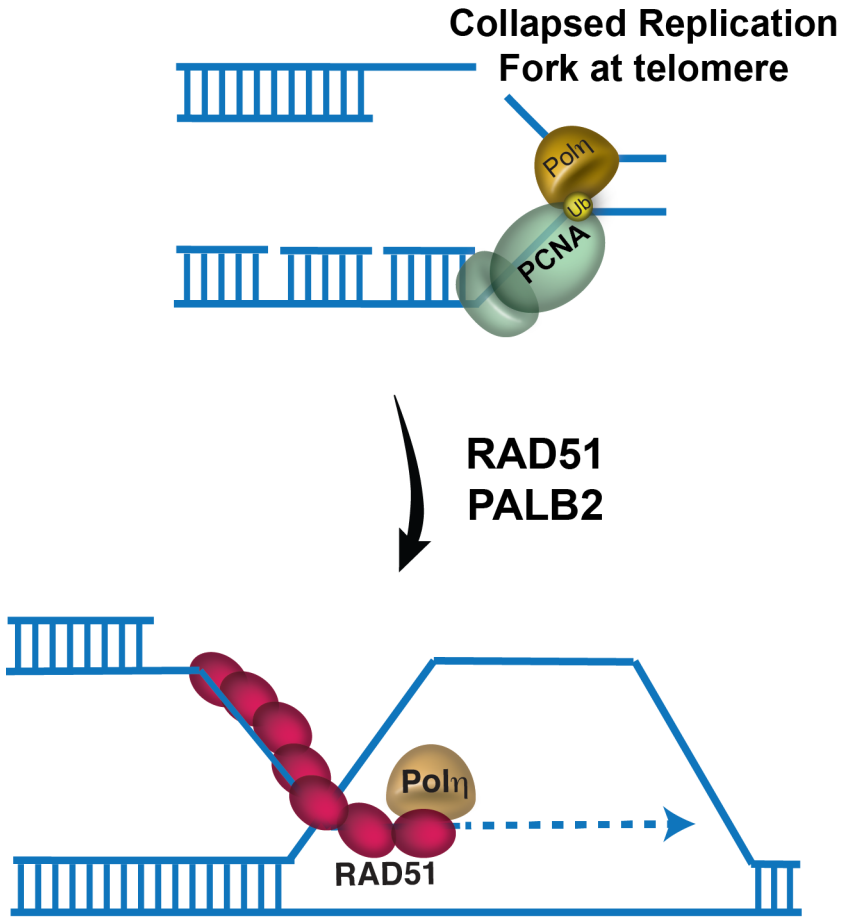


Polη and TRF1 might regulate the same pathway

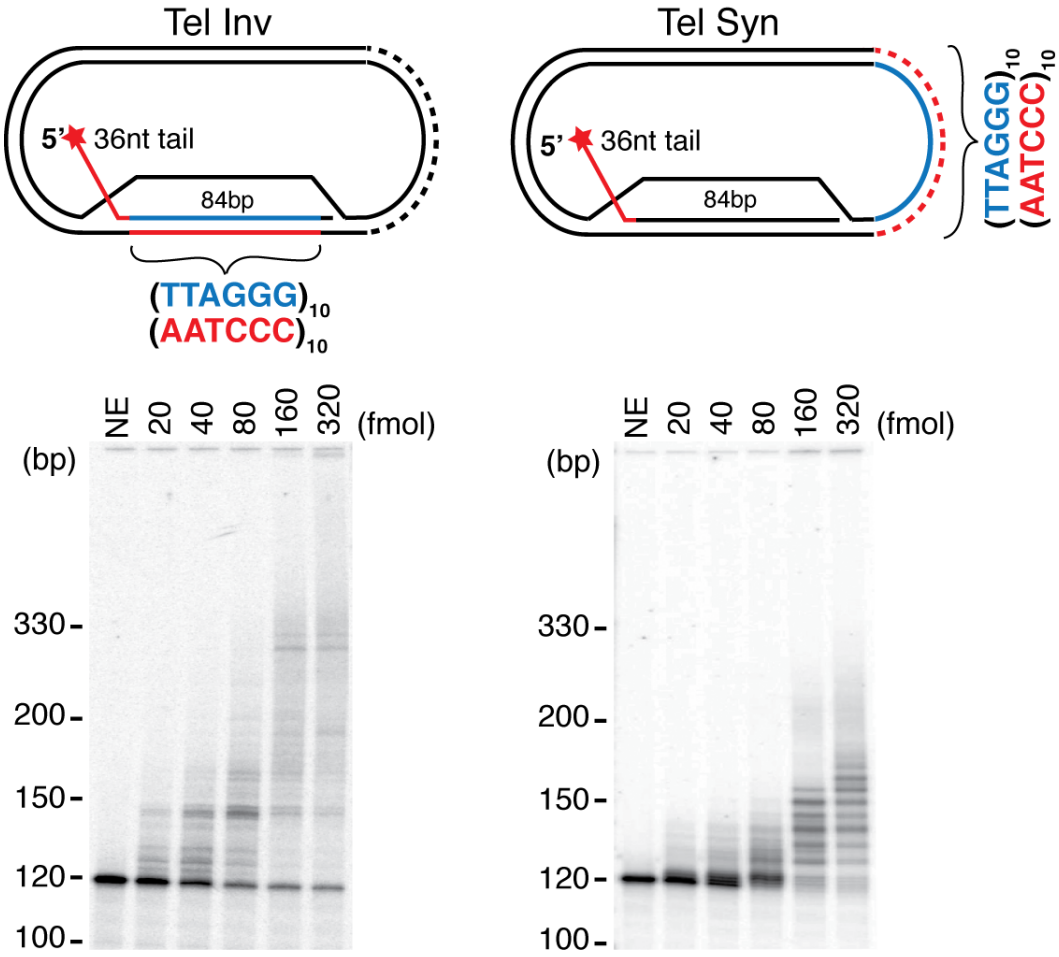


Working Model

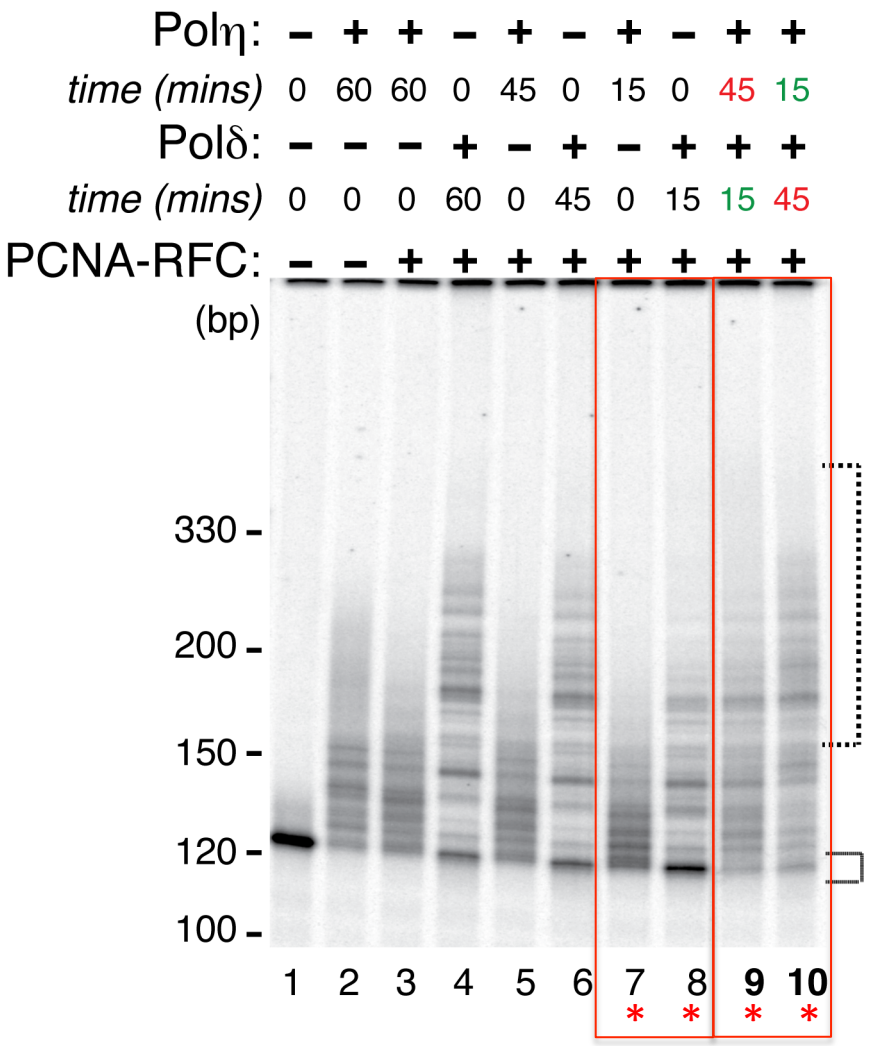
Homologous Recombination

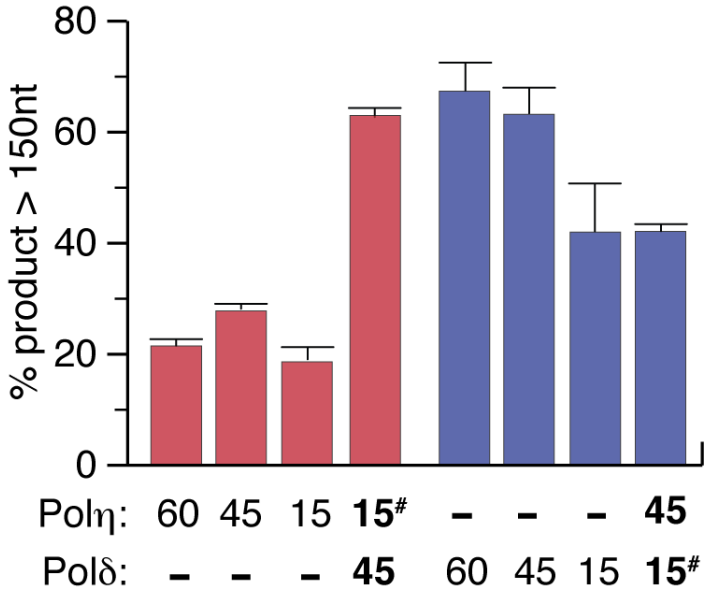


Polη initiates DNA synthesis from D-loops

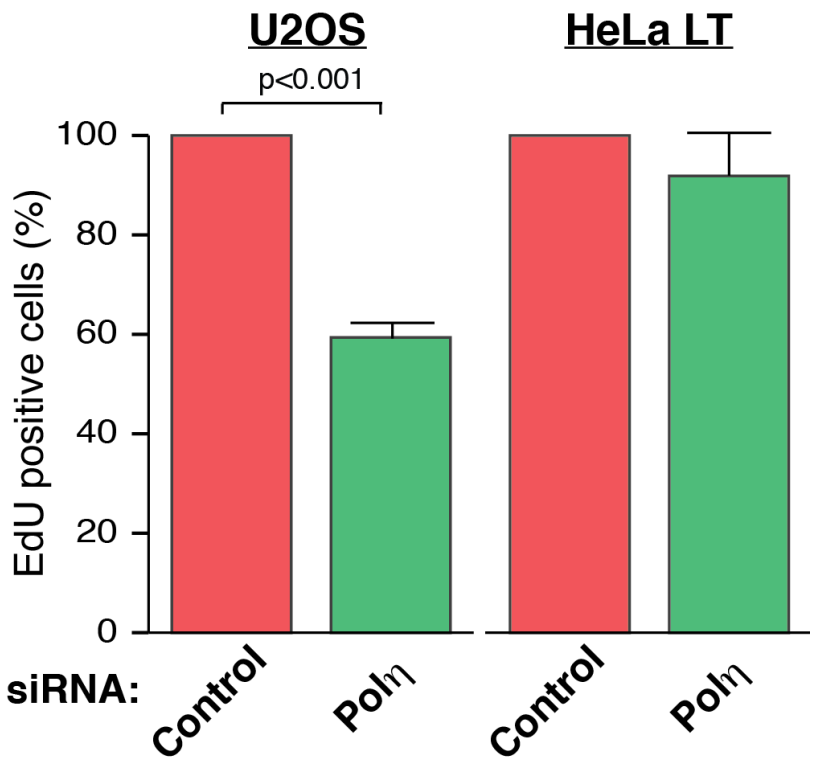


Polη initiates DNA synthesis from D-loops

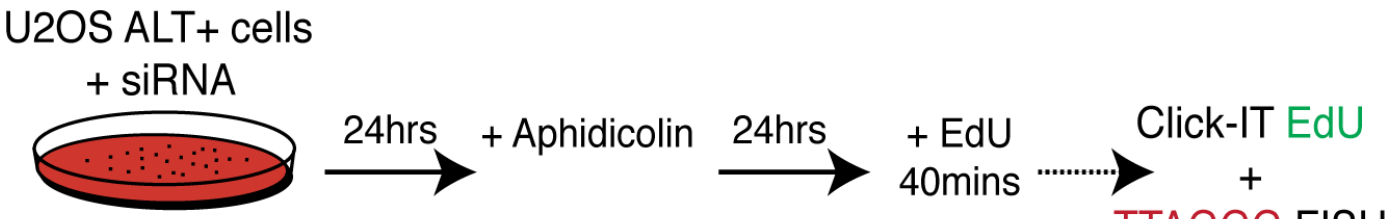


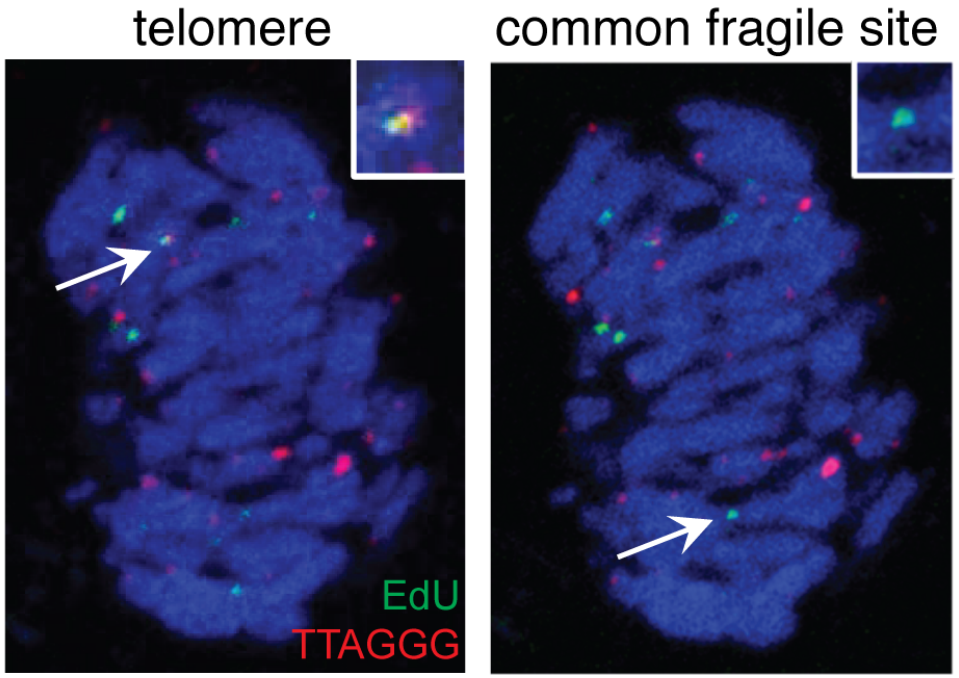


Delayed DNA synthesis after Polη kd

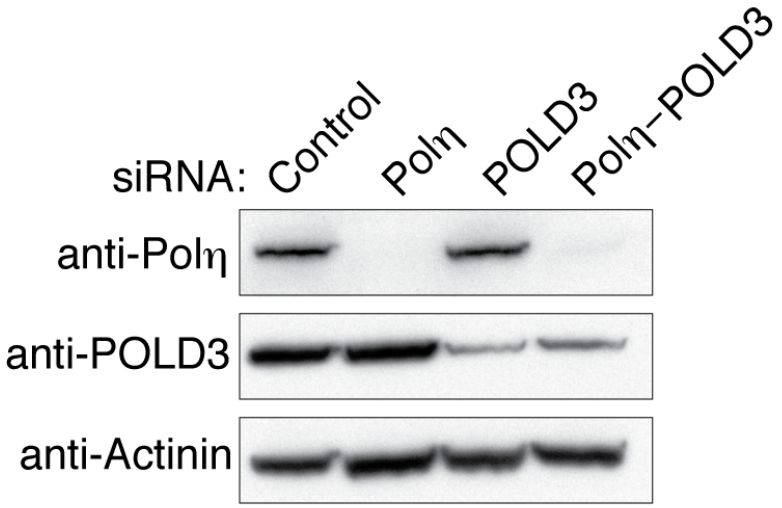


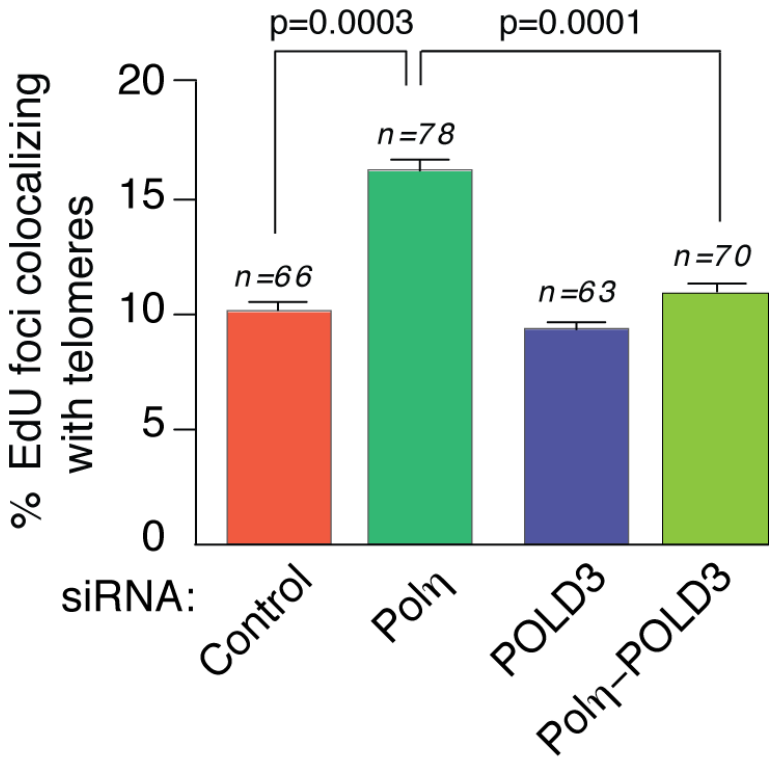
Measuring Late DNA synthesis





POLD3 dependent mitotic DNA synthesis





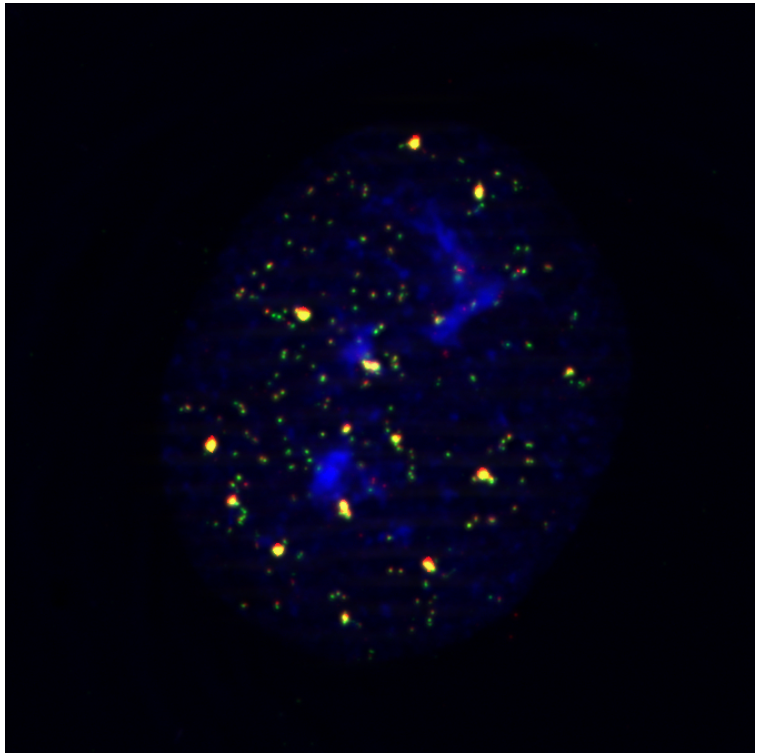
Effects of Polη loss for ALT activity

Markers of ALT activity

- ALT associated PML Bodies (APBs)
- Extra-Chromosomal Telomeric DNA
- Telomere-Sister Chromatid Exchanges

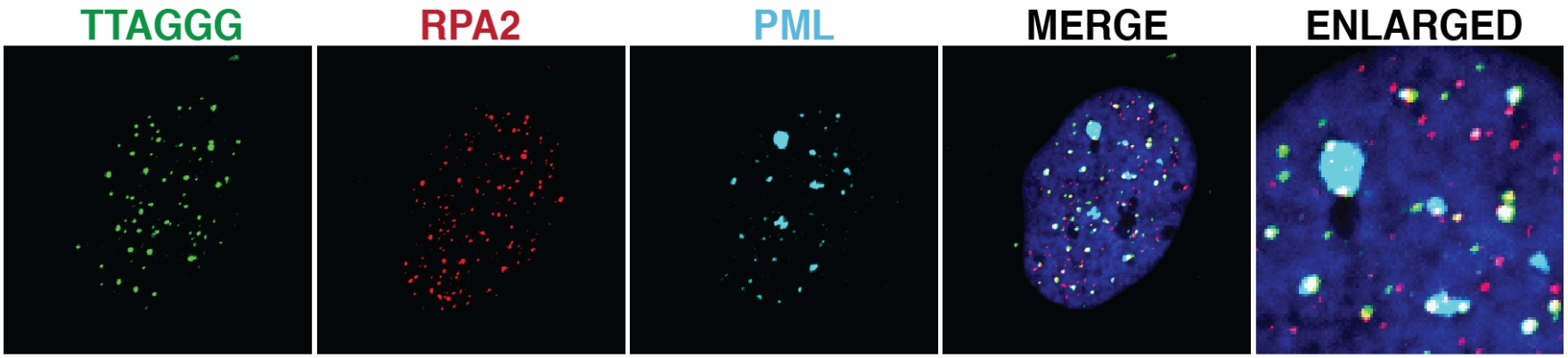
APBs: ALT associated PML bodies

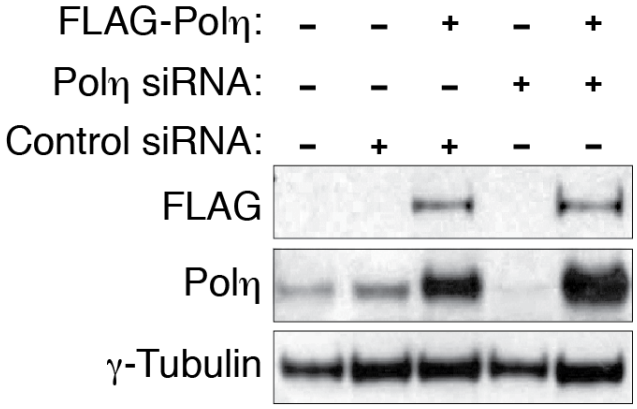
Yeager et al., Cancer Research (1999)

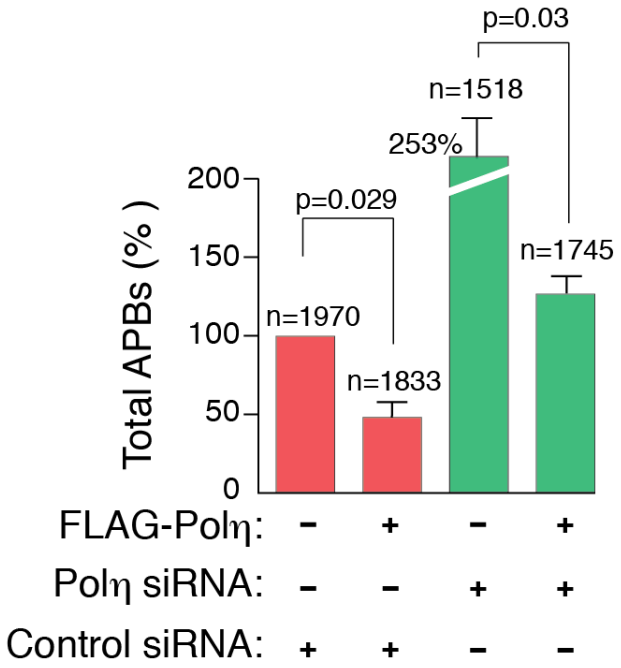


- Present in 5-10% of cells
- Function is unknown
- Co-localization of PML, Telomeric DNA, telomeric and DNA repair proteins
  (TRF1, TRF2, RAD51, RPA, BLM, BRCA1)
- Recombination Factories?
- Biomarker of ALT activity

Elevated APBs upon Polη depletion



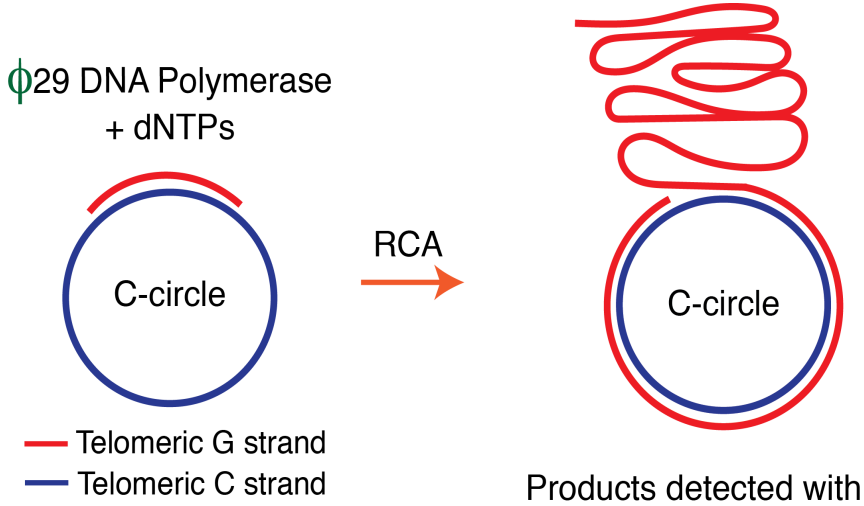




Elevated ECTR upon Polη depletion

C-circles

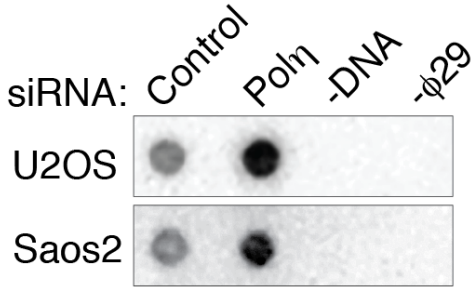
Henson et al., Nature Biotech (2009)

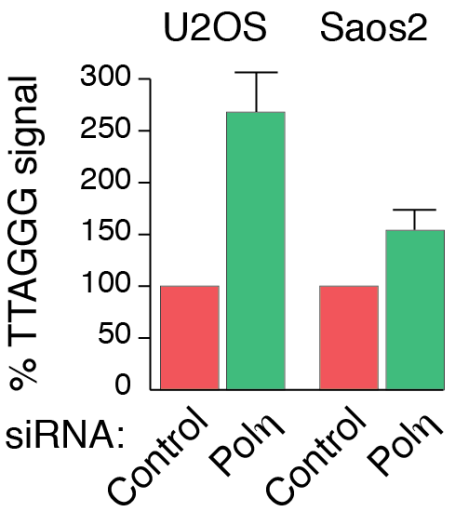


Products detected with
P^{32}-(CCCTAA)

native blot:
single stranded C-circles

denatured blot:
double stranded T-Circles





Alternative Lengthening of Telomeres (ALT)

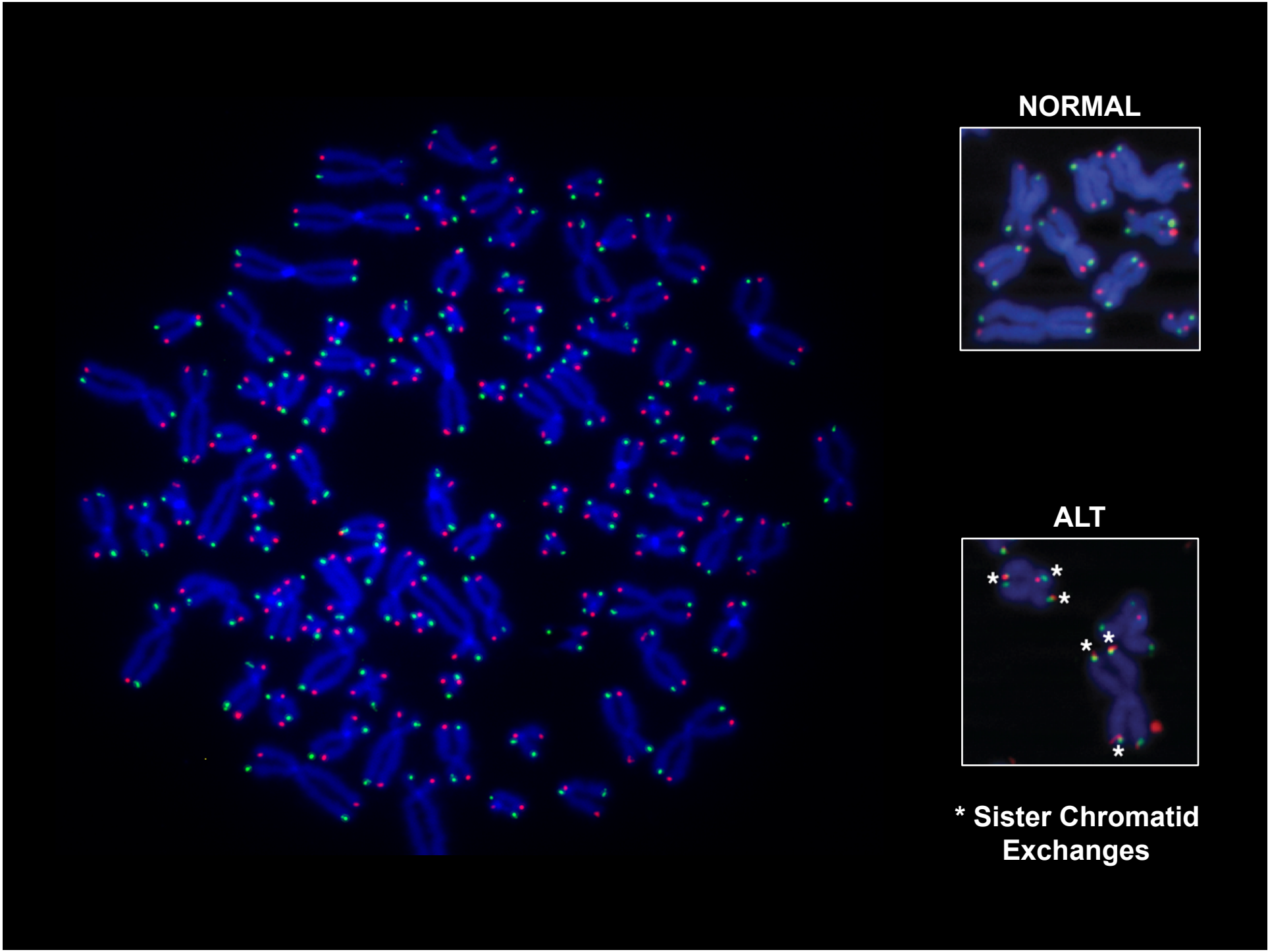
Molecular Hallmarks of ALT

Telomeric Recombination

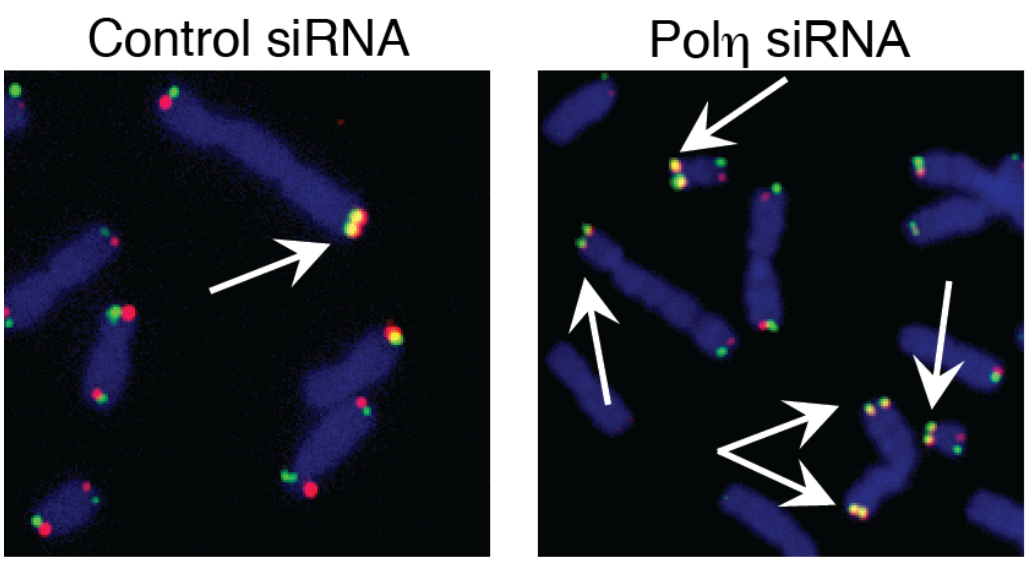
Chromosome Orientation FISH

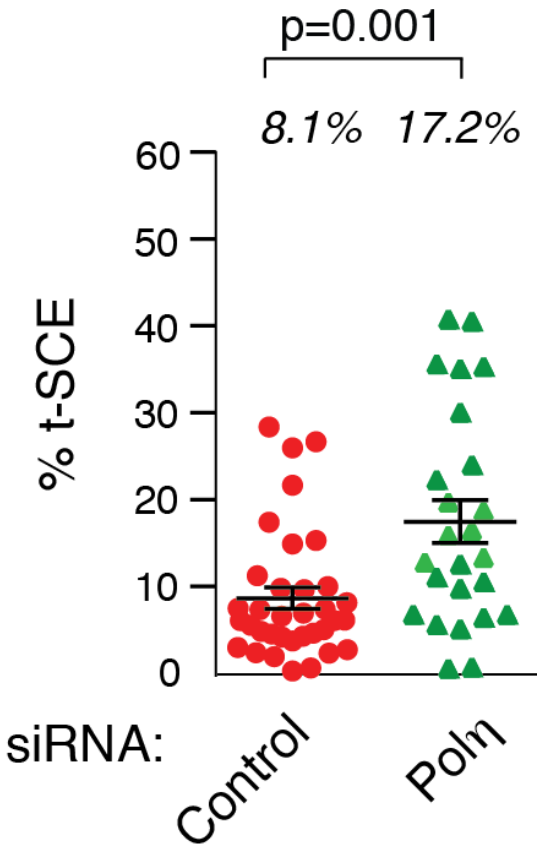
- **BrdU and BrdC added to cell culture medium for ONE cycle**
- **Incorporated into newly replicated DNA strands**
- **Degraded by UV exposure and exonuclease treatment**

TAGGTAGTAG TAGGTAGTAG TAGGTAGTAG TAGGTAGTAG

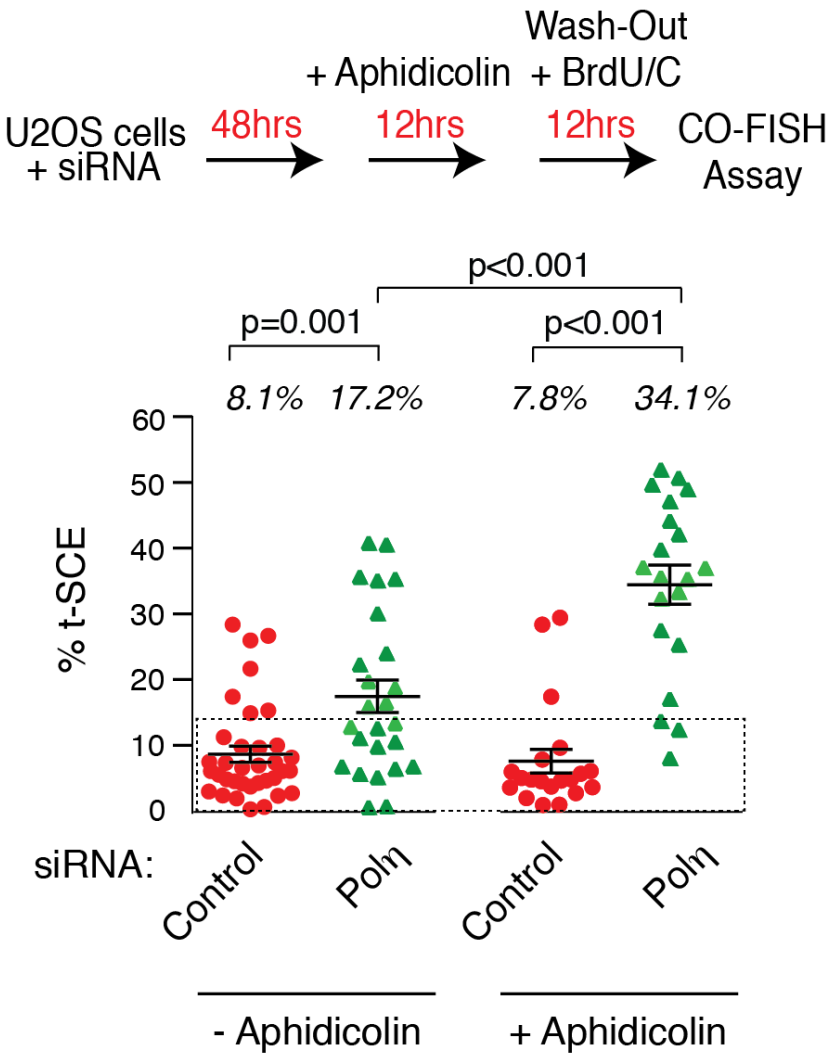

NORMAL
ALT
* Sister Chromatid Exchanges

Elevated t-SCEs upon Polη depletion

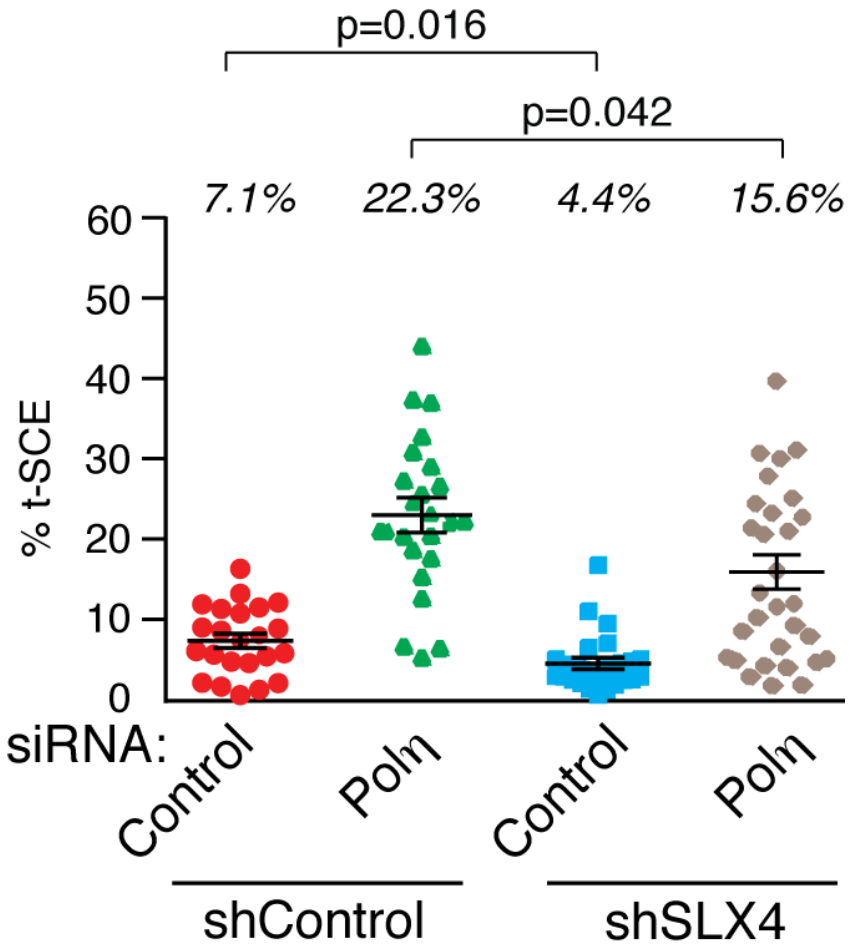




Elevated t-SCEs upon Polη depletion



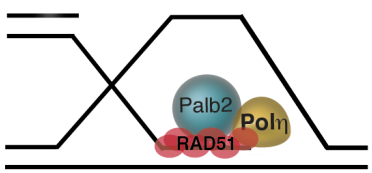
Partial suppression of t-SCEs by SLX4 kd



Proposed Model for Polη function

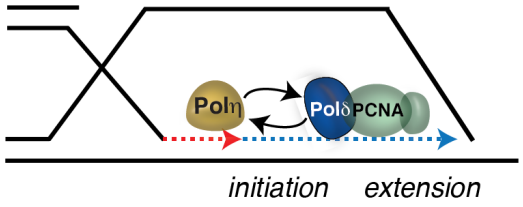
Polη Recruitment
by Rad18-^{Ub}-PCNA

Stabilization
by HR factors
(and TRF1?)



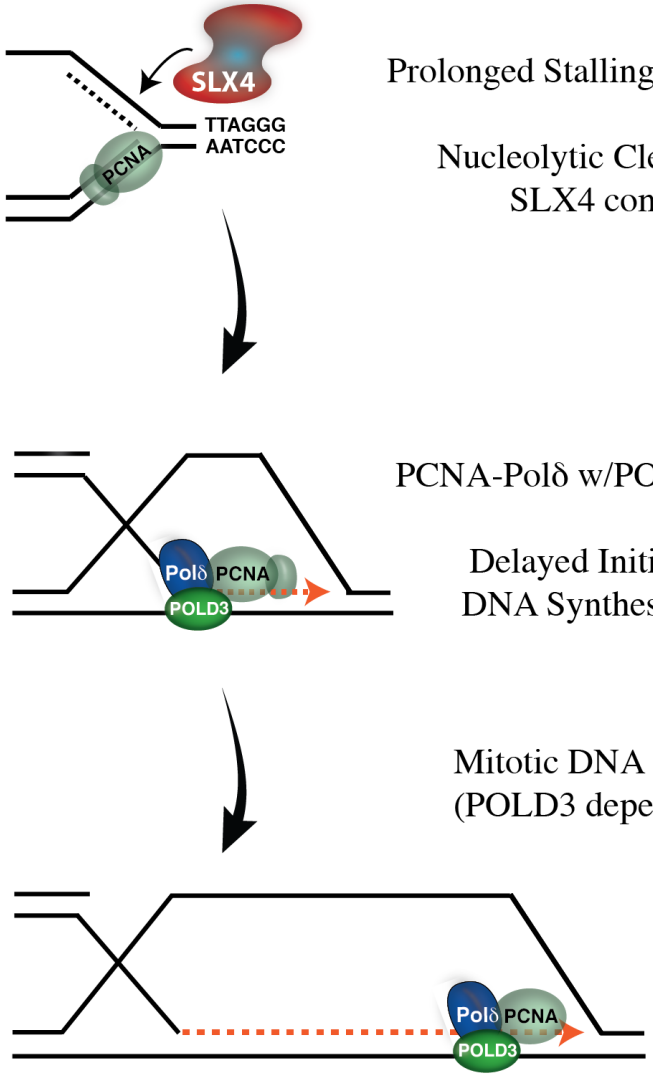
Late S-phase

Polη initiates DNA
synthesis, Switch to Polδ



S-G2 phase

Proposed Model for Polη function



Prolonged Stalling and Collapse

Nucleolytic Cleavage by SLX4 complex

PCNA-Polδ w/POLD3 loading

Delayed Initiation of DNA Synthesis (BIR)

Mitotic DNA synthesis (POLD3 dependent)

Late S-phase

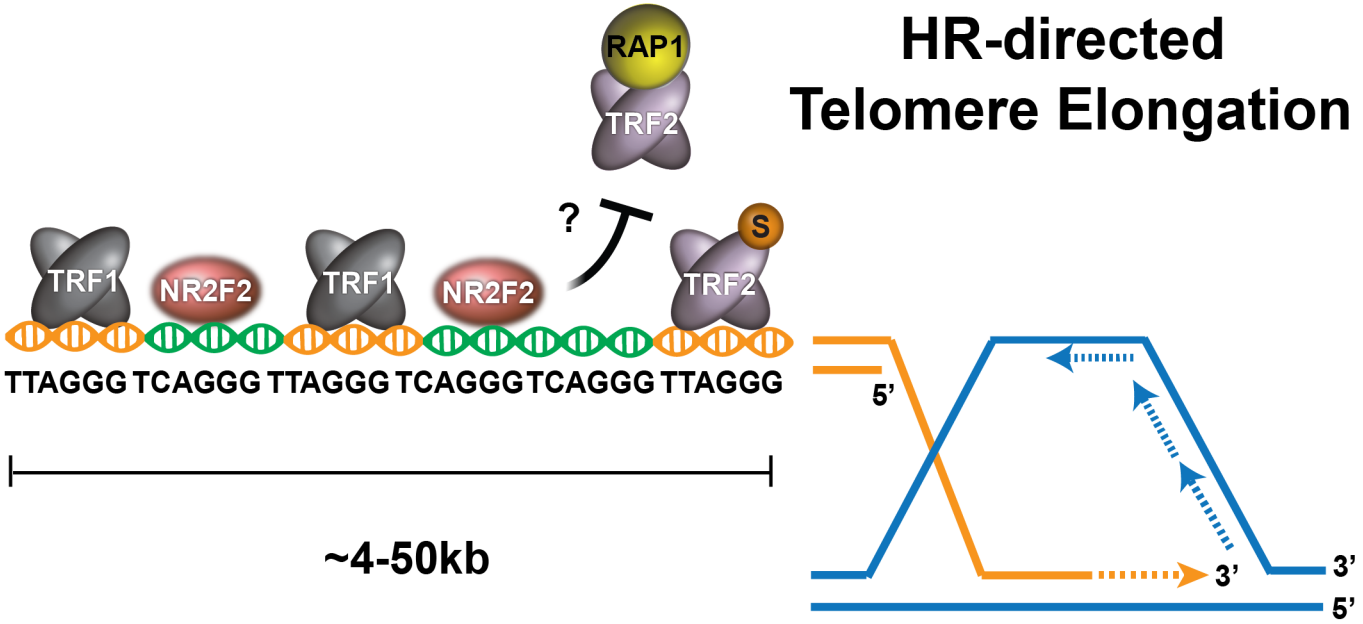
G2 phase

Pro-Metaphase

Pros and cons of Polη use in ALT

- ✓ **PCNA independent initiation of DNA synthesis**
- ✓ **Polη suggested to act in SDSA pathway; no crossovers**
- ✓ **Polη preferable to DSBR pathway; crossovers**
- ✓ **Prevents excessive BIR; crossovers and mutagenesis**
- ***X*** **Polη is error prone on undamaged DNA**
  - *Base substitution rate of 1 per 18-360 nucleotides*
  - *Preference for T-C transitions (Matsuda et al., Nature 2000)*
    (Johnson et al. JBC, 2000)

What is the origin of variant repeats at ALT telomeres



Hilda Pickett, NSMB (2014)
Jerome Dejardin, Cell (2015)

ACKNOWLEDGEMENTS

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Laura Garcia
Jonathan Barosso
Justin Roncaioli
SongMy Hoang

Collaborators

Patricia Opresko
Arindam Bose
Simon Watkins
Jean-Seb Hoffmann
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Elodie Bournique

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St. Baldrick's FOUNDATION
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