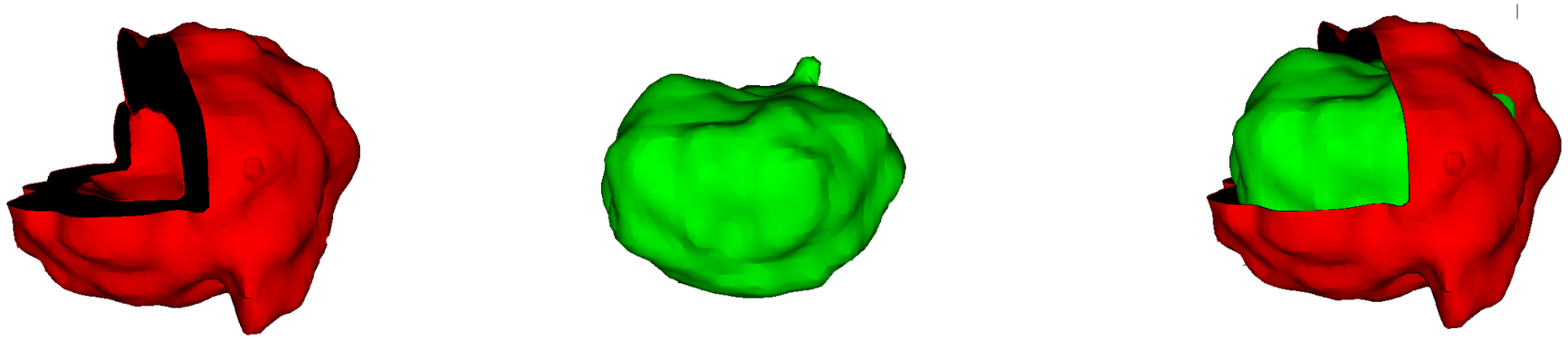
Targeting the alternative lengthening of telomeres (ALT) pathway in cancer cells

Karsten Rippe

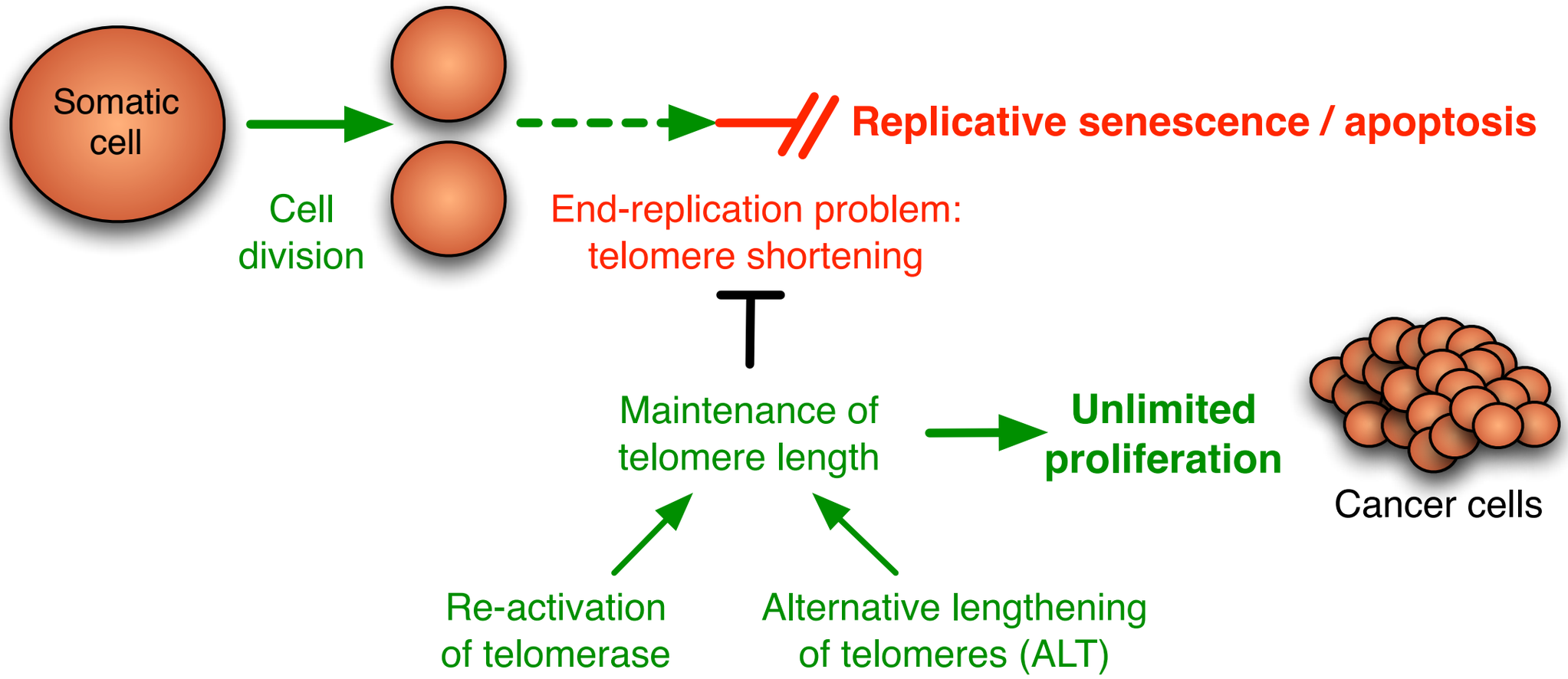


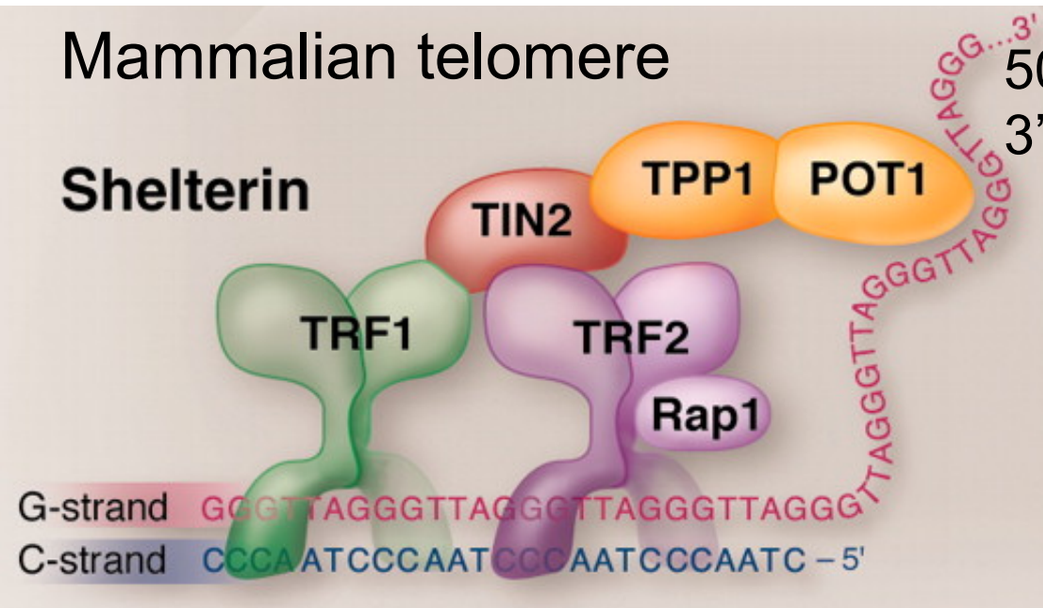
Research Group *Genome Organization & Function*
Deutsches Krebsforschungszentrum
& BioQuant, Heidelberg

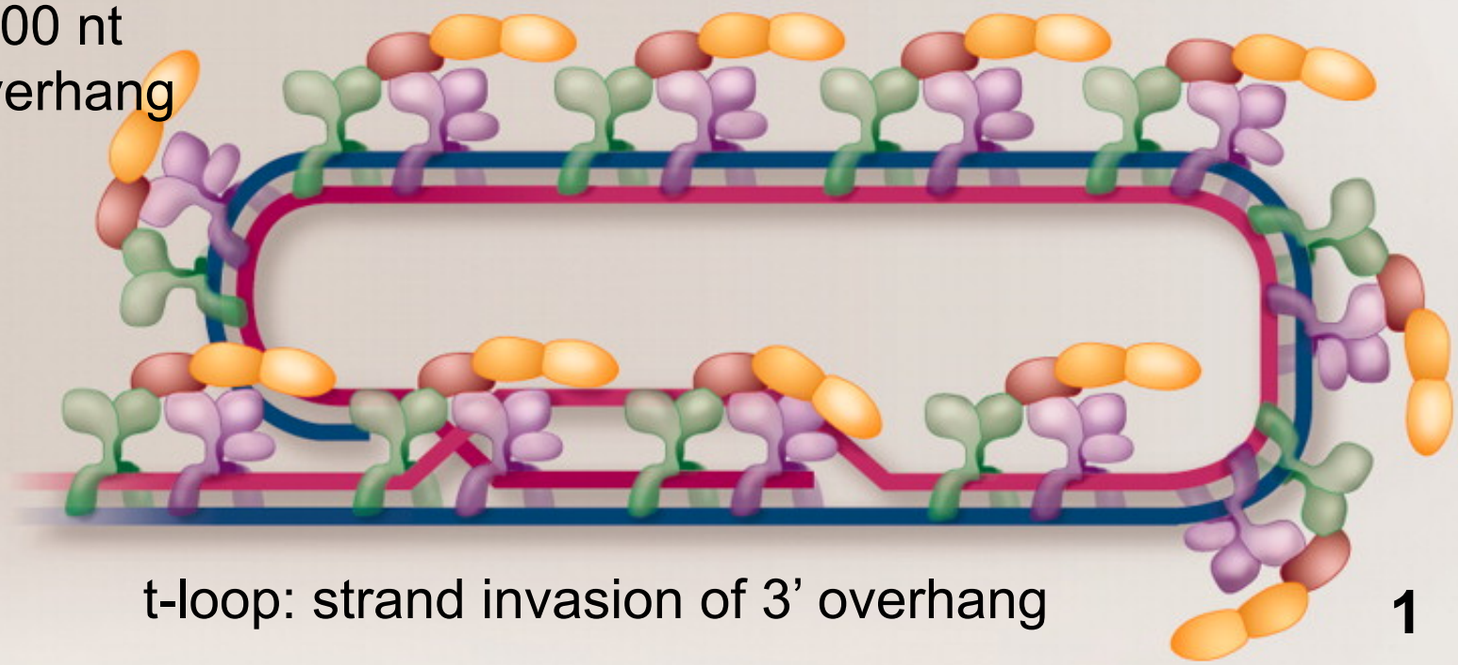
dkfz.

BioQuant
MODEL base of LIFE

Maintaining telomeres is crucial for unlimited proliferation





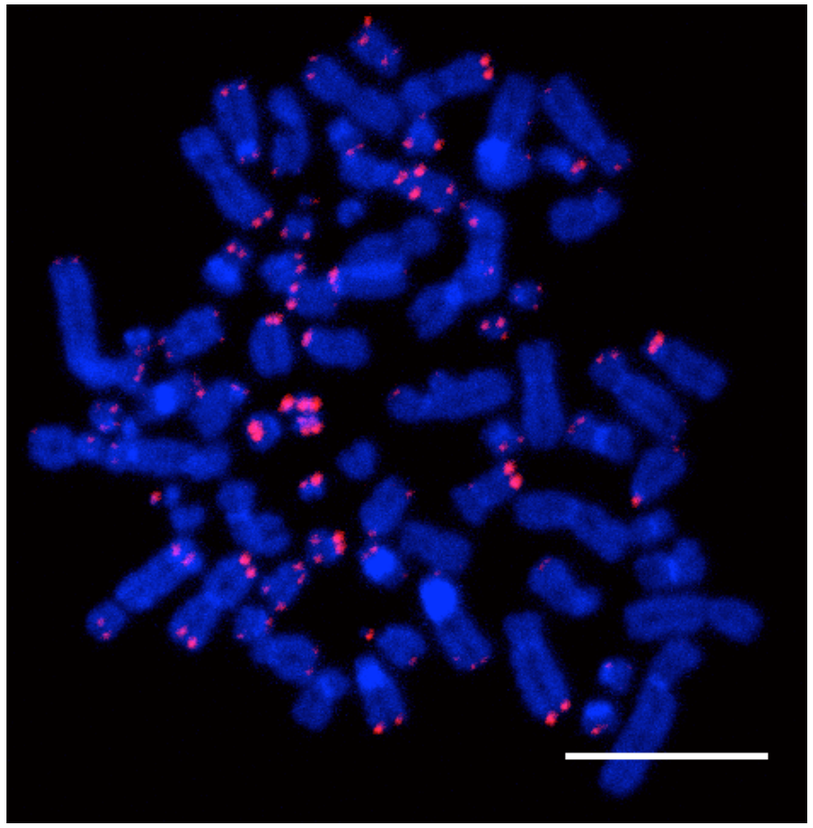


Alternative lengthening of telomeres (ALT)

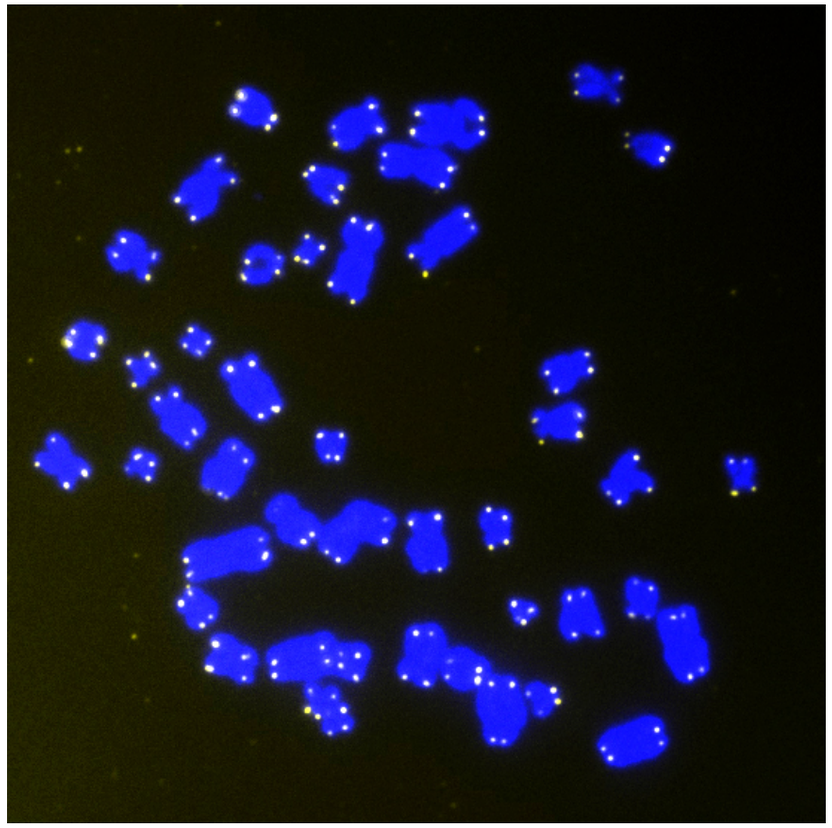
Maintaining telomere length without telomerase in ~30% of sarcomas and ~10% of carcinomas

- DNA repair/recombination based mechanism
- heterogenous telomere repeat length
- **A**LT-associated **P**ML **B**odies (APBs)
  = complexes of PML bodies at telomeres

Metaphase FISH of telomere repeats

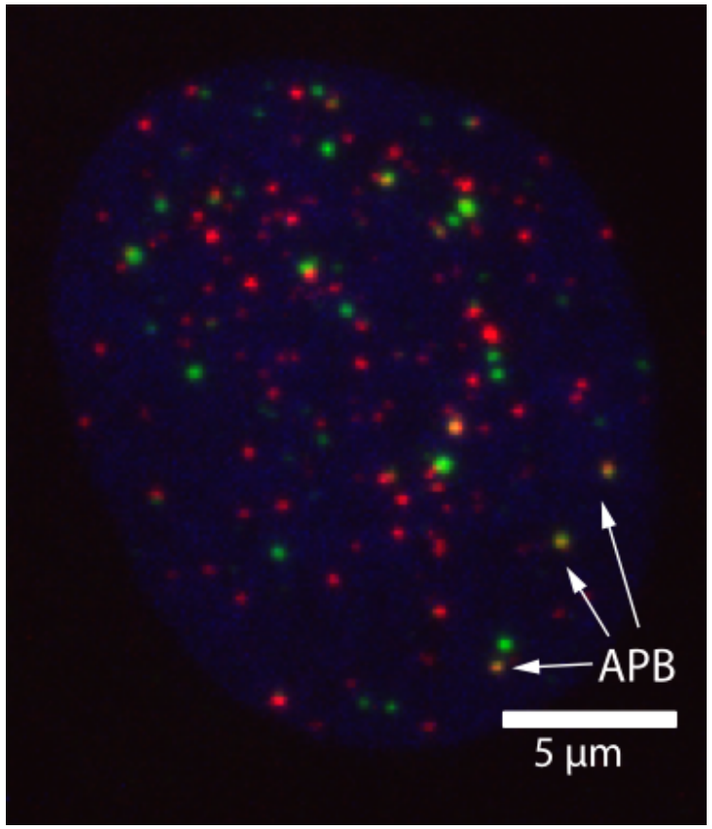


U2OS cells, ALT(+)



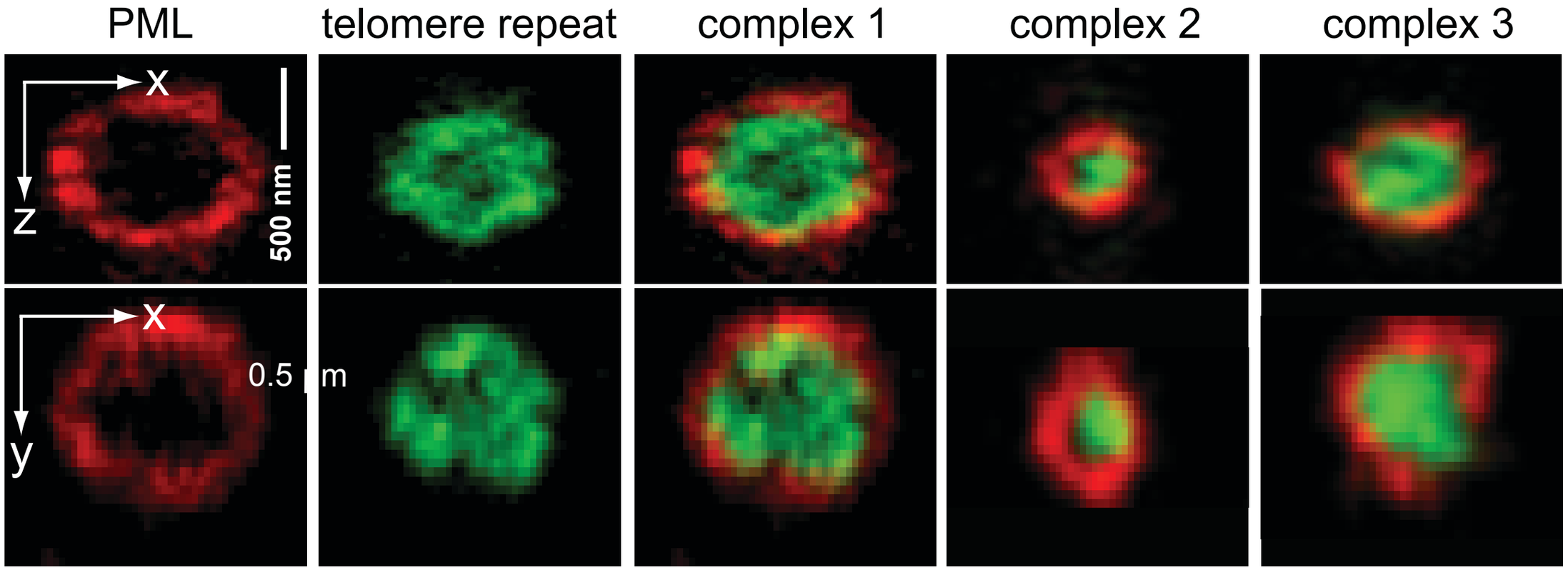
human lymphocytes, ALT(-)

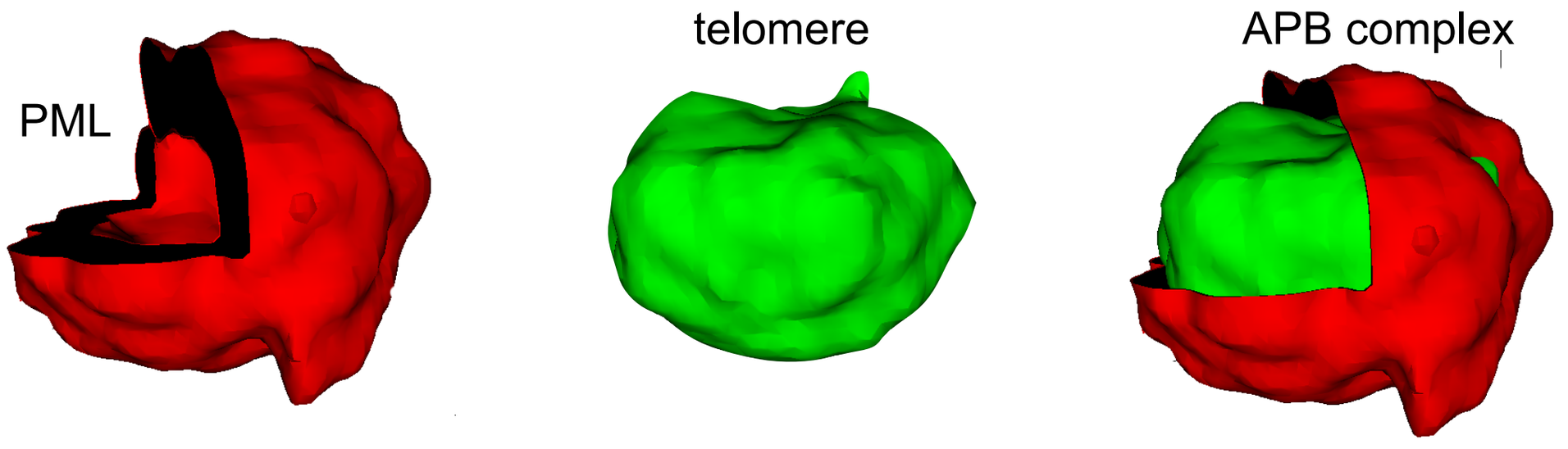
Immunostaining of telomeres and PML



APBs in U2OS cells

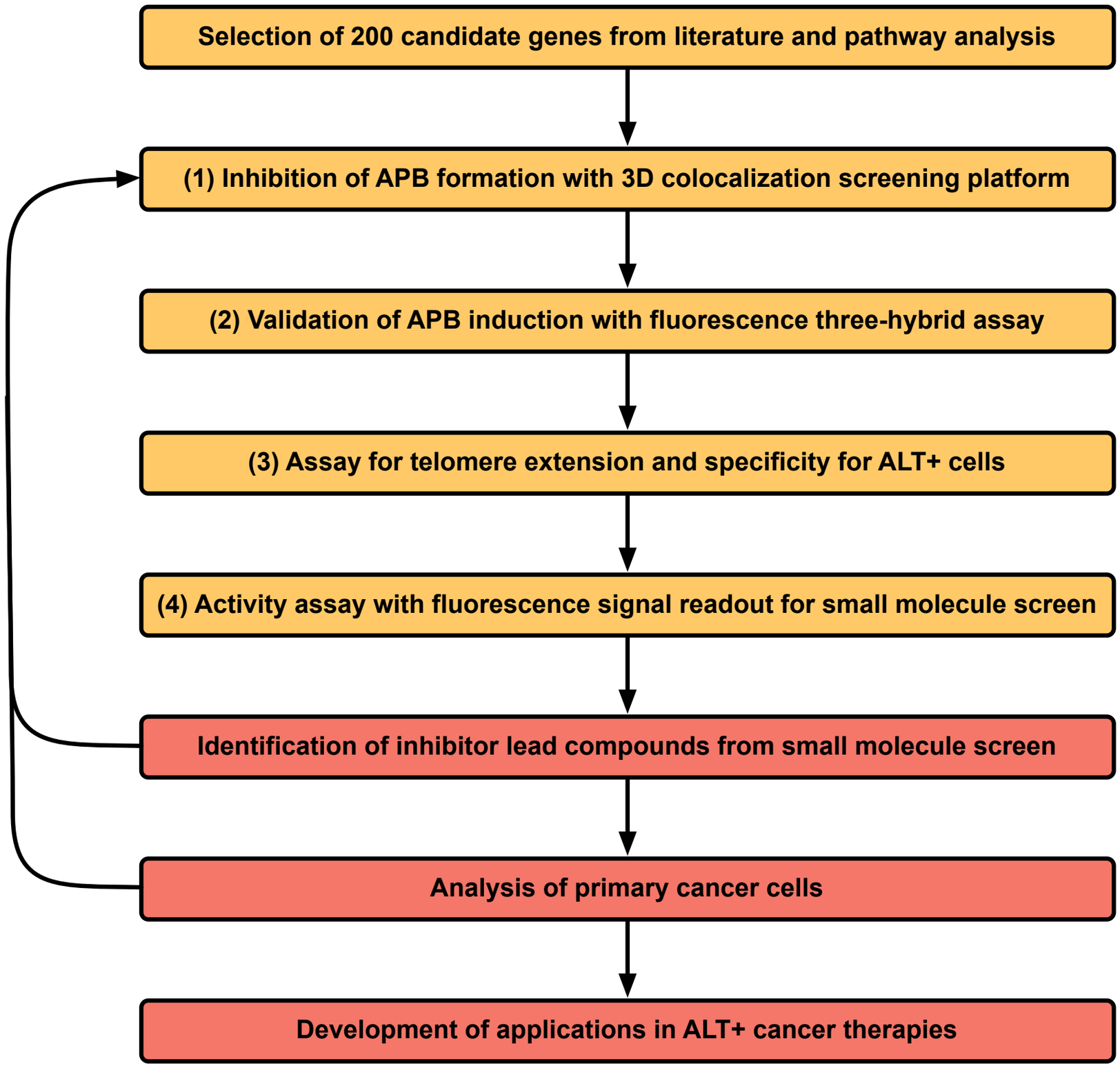
High resolution imaging of APBs in U2OS cells





Lang, Jegou, Chung, Richter, Udvarhelyi, Münch, Cremer, Hemmerich, Engelhardt, Hell, & Rippe (2010). *J. Cell Science* **123**, 392-400.

Project workflow



current status

done

15 candidates from siRNA knock-down of 100 genes

10 proteins validated that can initiate APB formation

confirmed for MMS21

in progress for MMS21

planned

planned

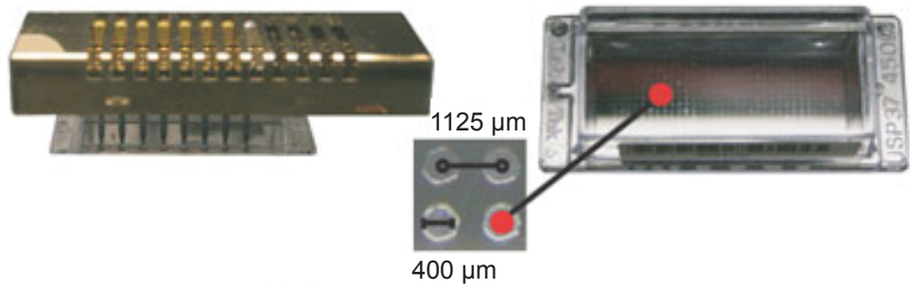
planned (long term)

(1) The 3D co-localization screening platform

Preparation of siRNA transfection mix

1 h

Spotting of siRNA microarray



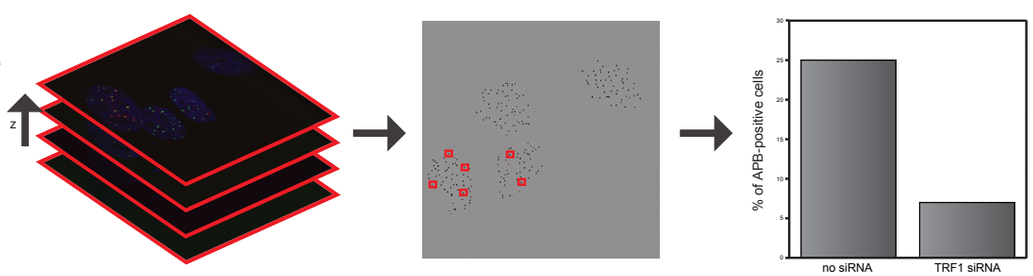
72 h

cell seeding

Automated confocal image acquisition

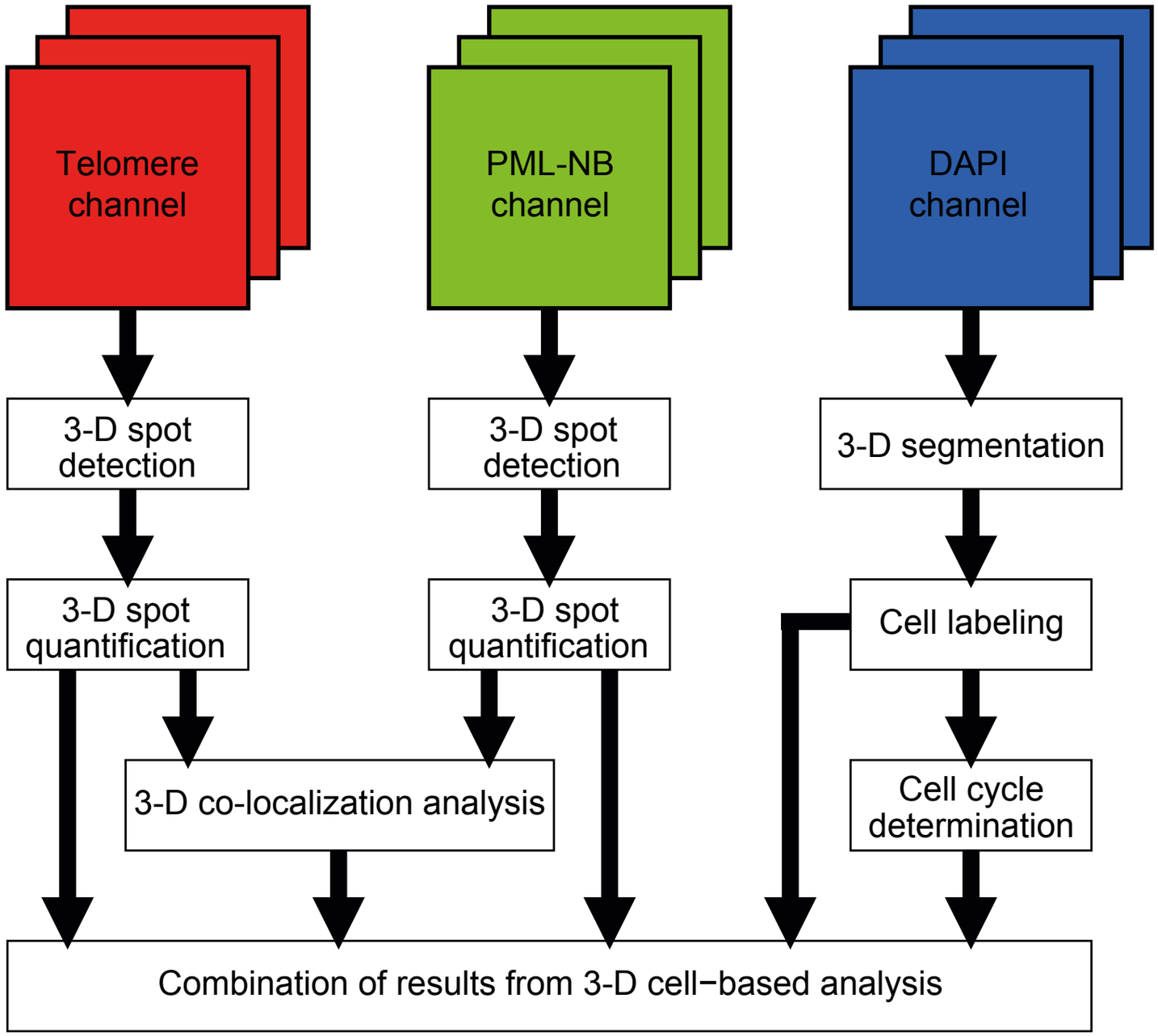
~ 2000 confocal stacks/45 Gb per slide
~ 35 000 images/gene

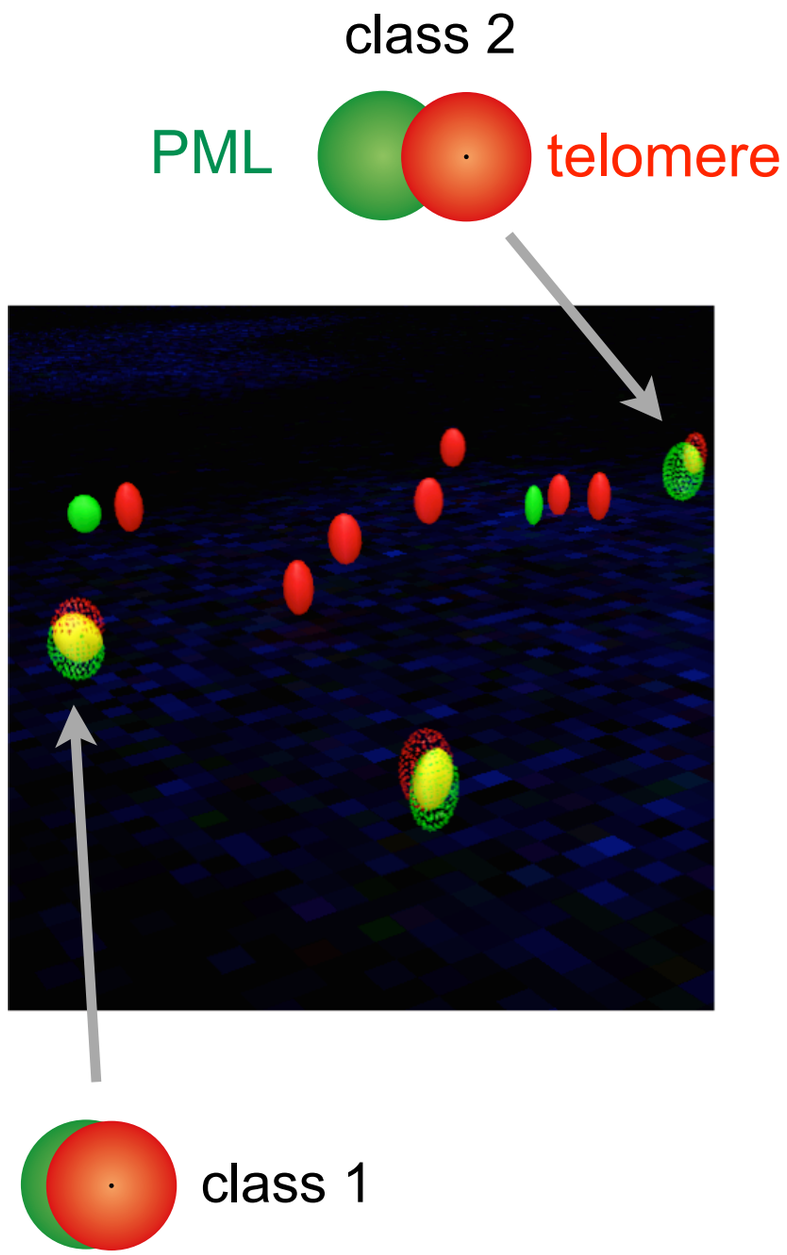
Automated image analysis



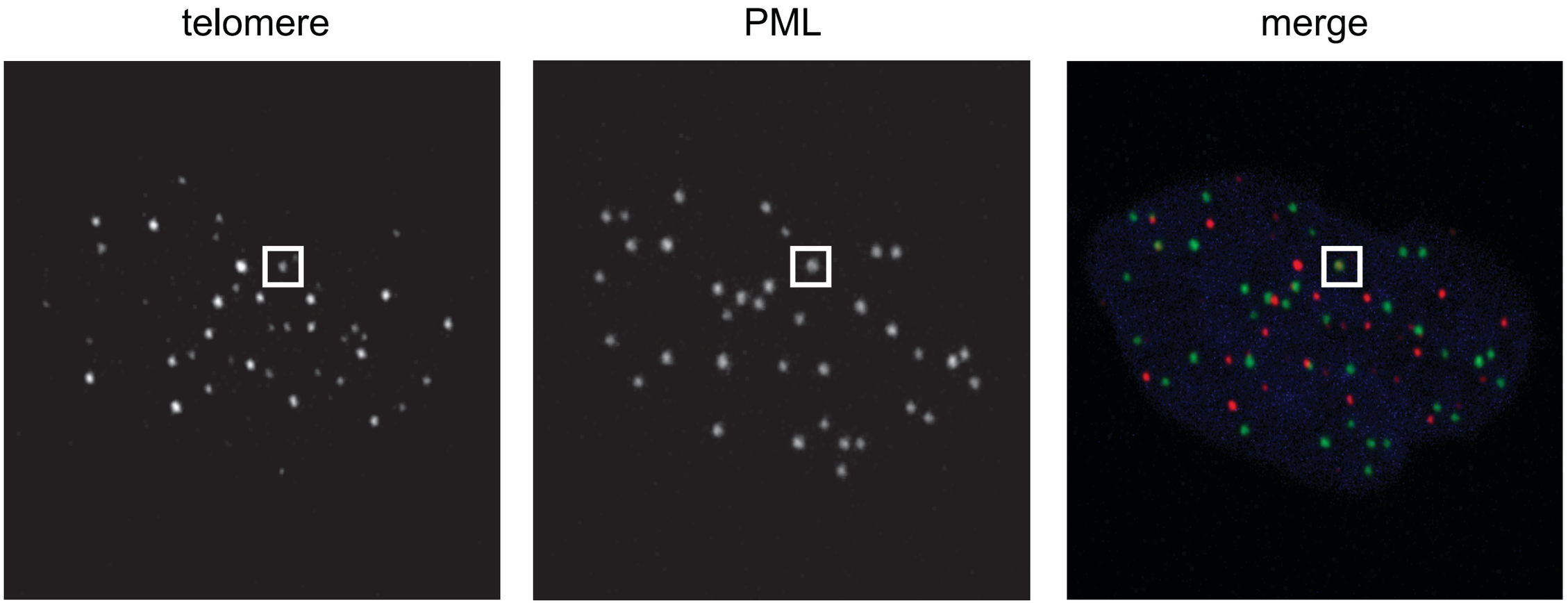
Osterwald, Wörz, Reymann, Sieckmann, Rohr, Erfle & Rippe (2011). *Biotechnol. J.*, under revision

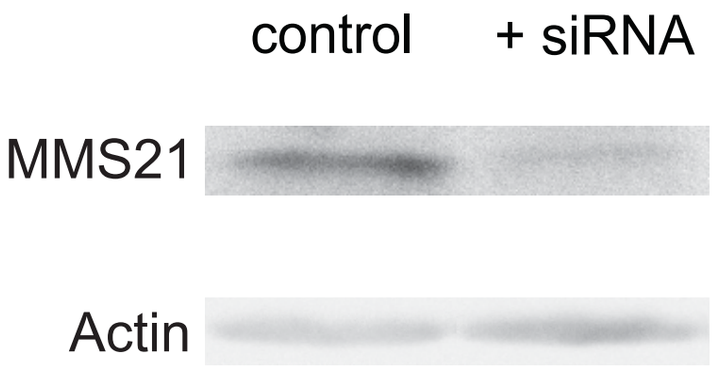
Automated high-content image analysis and APB quantification

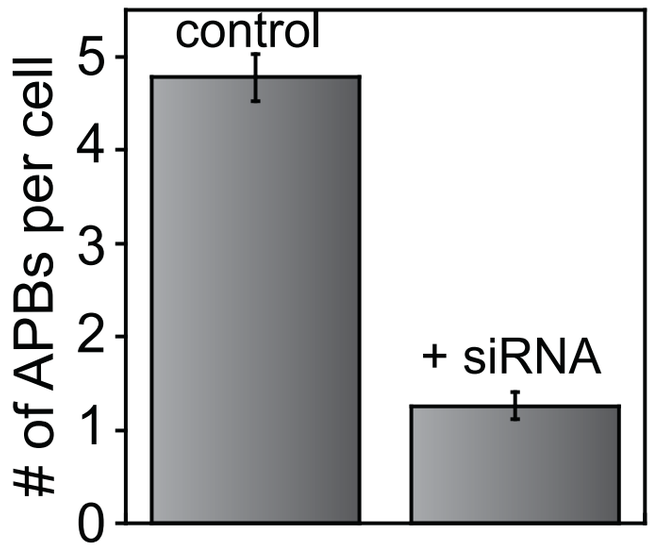


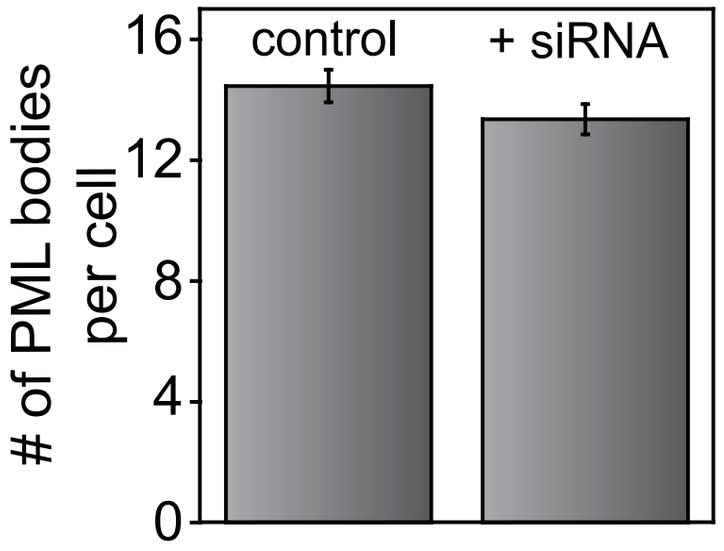


SUMO ligase MMS21 knock-down inhibits APB formation

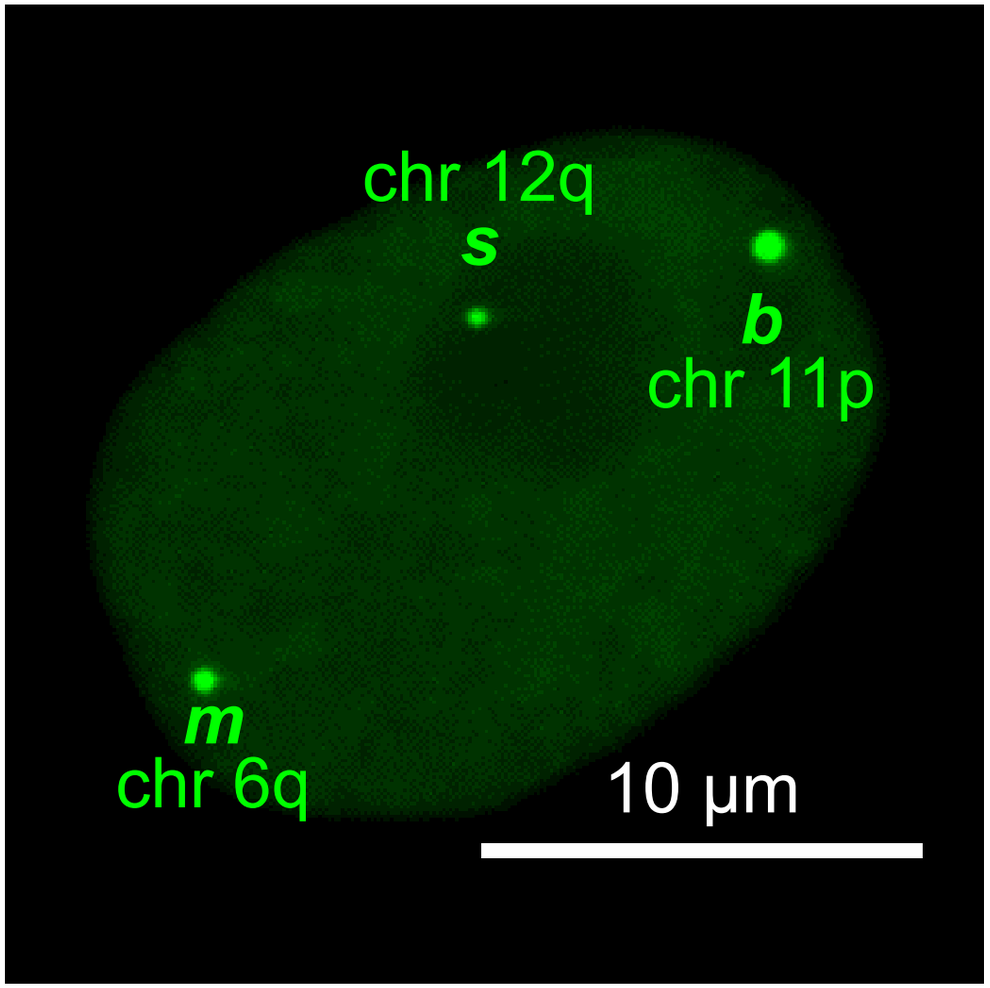




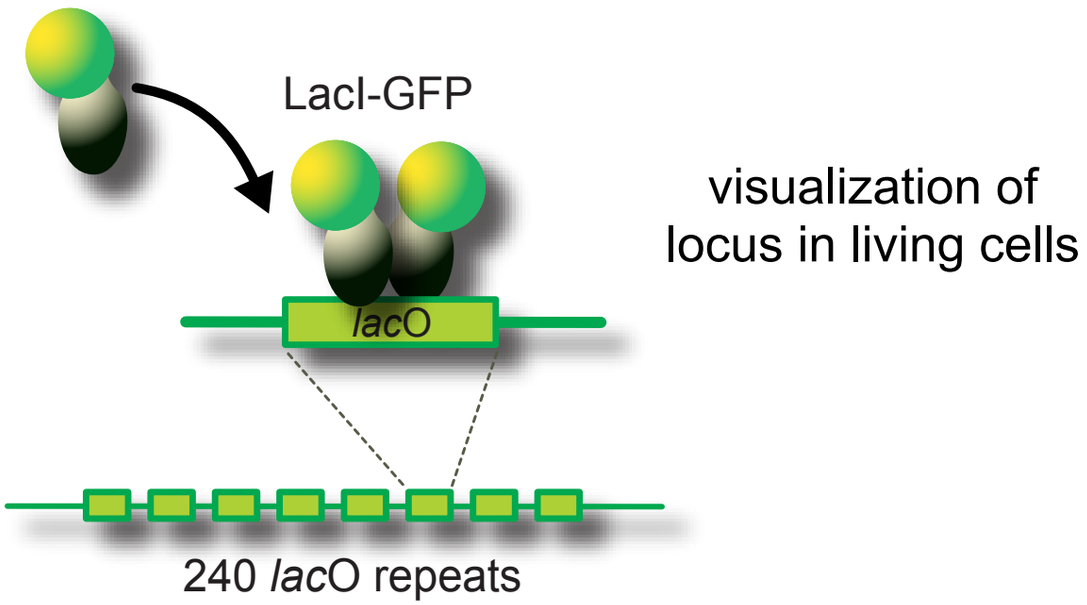




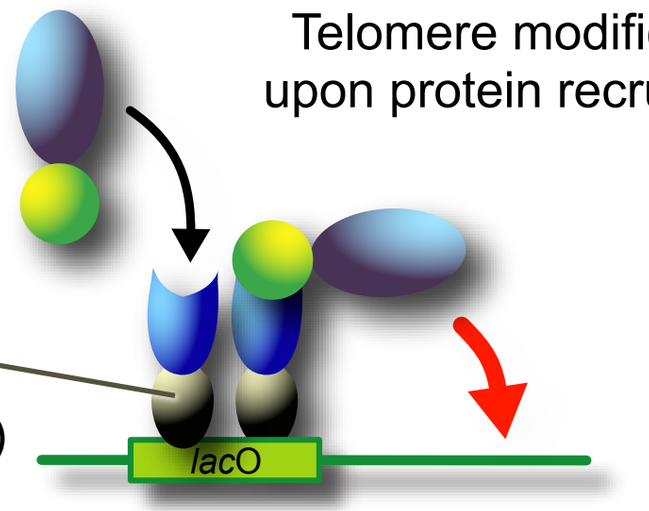
(2) Validating APB formation and function in a U2OS cell line with *lac*O repeat integration at three telomeres



integrated *lac*O repeats at telomere 12q, 11p and 6q



visualization of locus in living cells

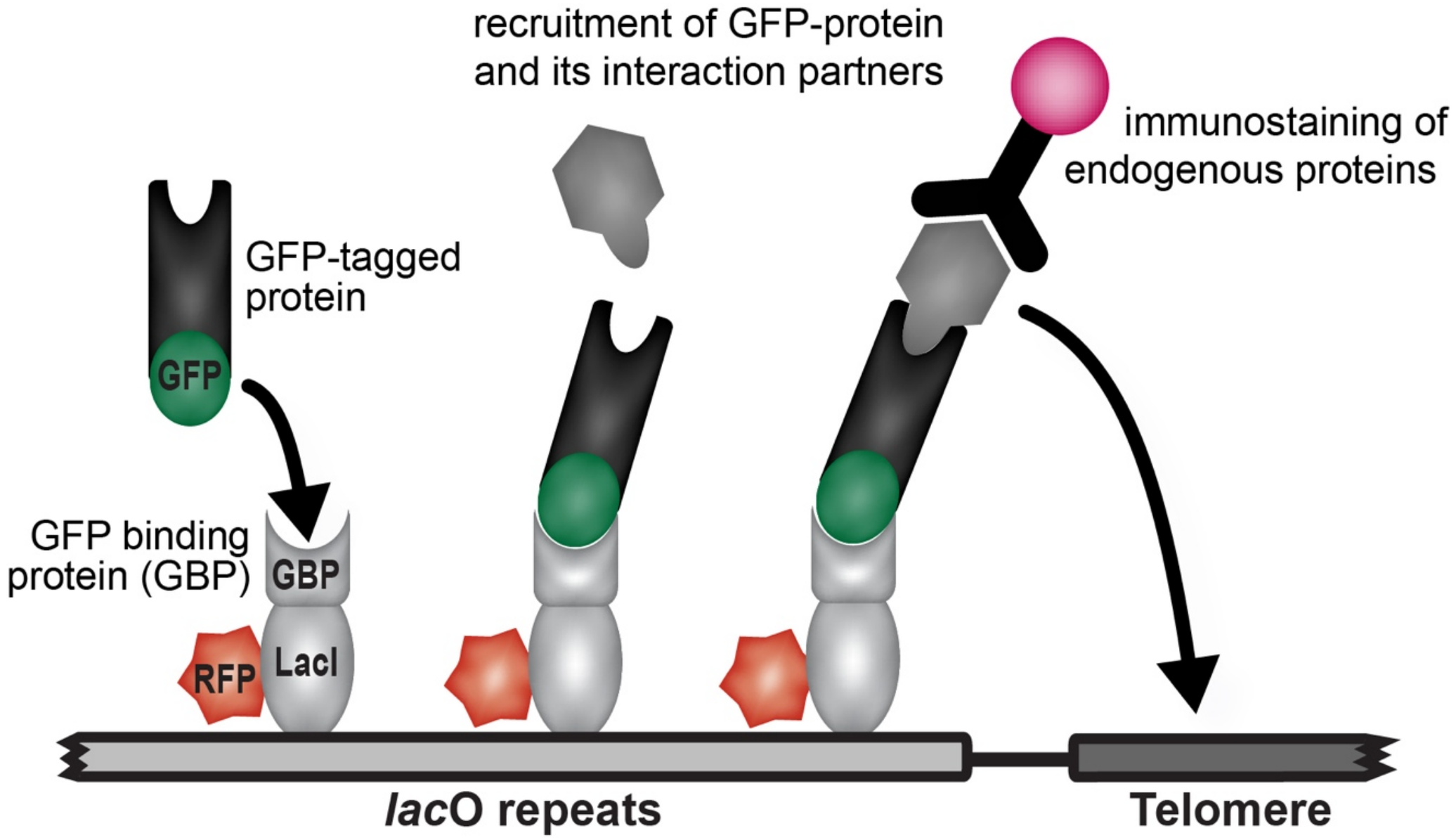


Telomere modification upon protein recruitment

antibody against GFP fused to LacI (Heinrich Leonhardt)

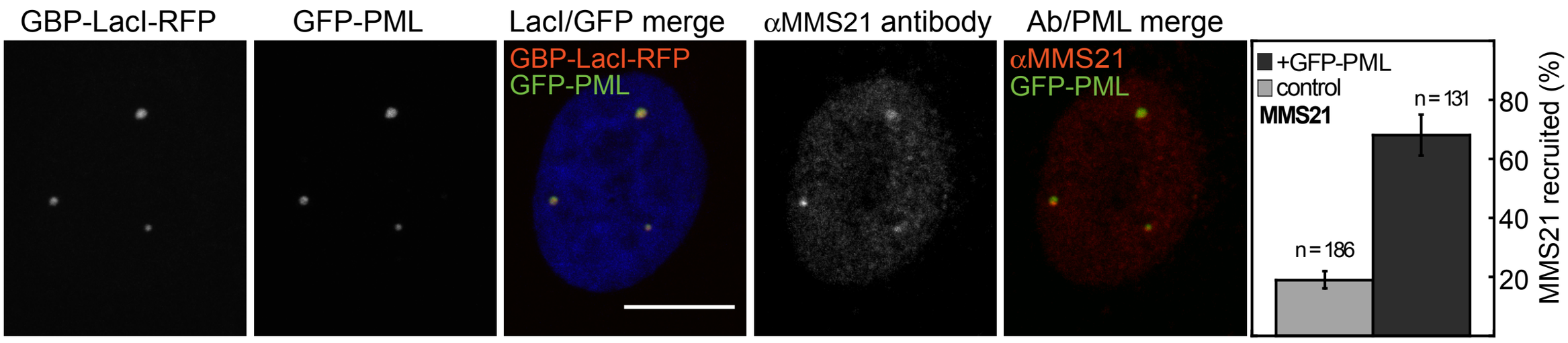
Jegou, Chung, Heuvelmann, Wachsmuth, Görisch, Greulich-Bode, Boukamp, Lichter & Rippe (2009). Mol. Biol. Cell 20, 2070-2082.

Protein interaction analysis in U2OS cells with fluorescence three-hybrid assay

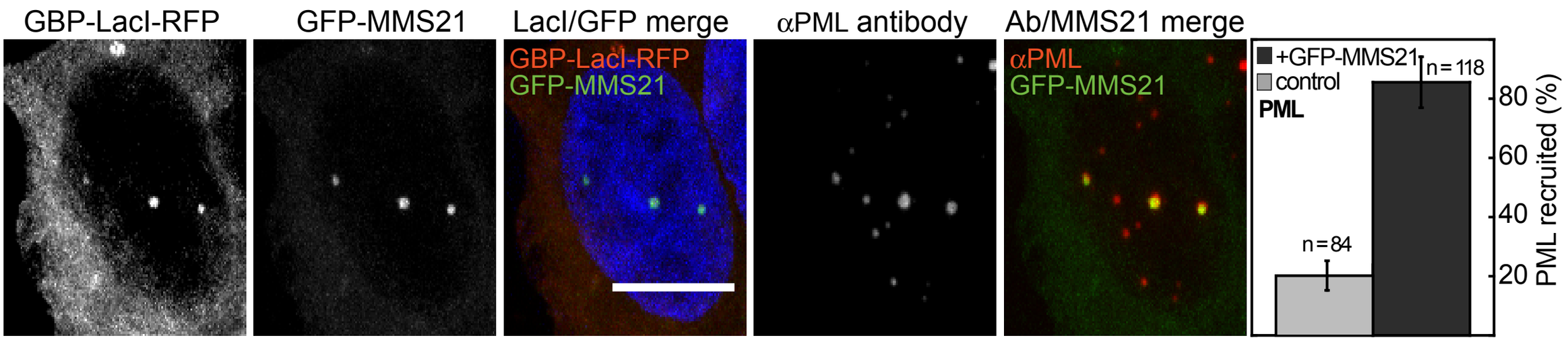


Chung, I., Leonhardt, H. & Rippe, K. (2011). *J. Cell Sci.*, under revision

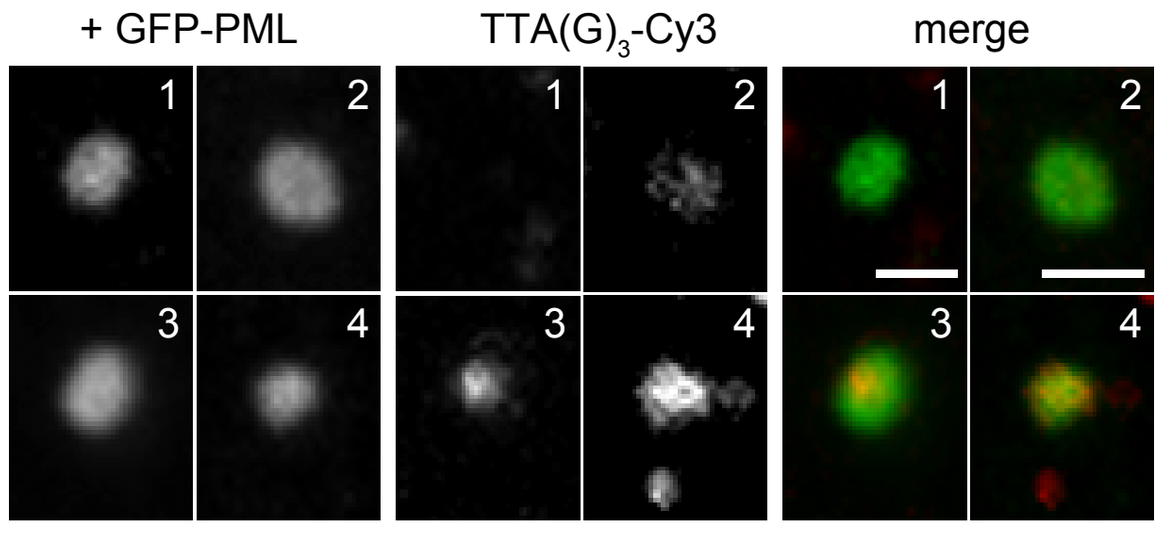
Endogenous MMS21 is a component of de novo formed APBs



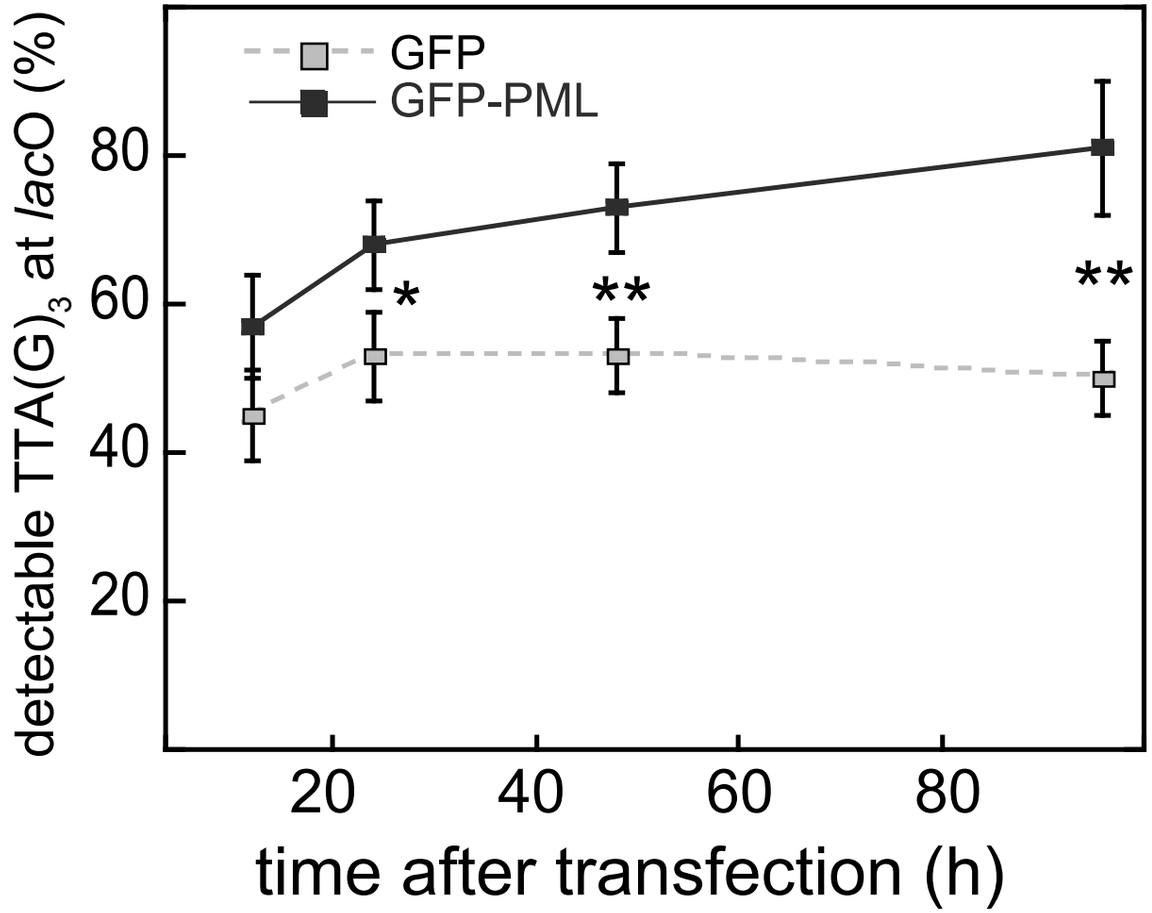
MMS21 is highly efficient in inducing APB formation



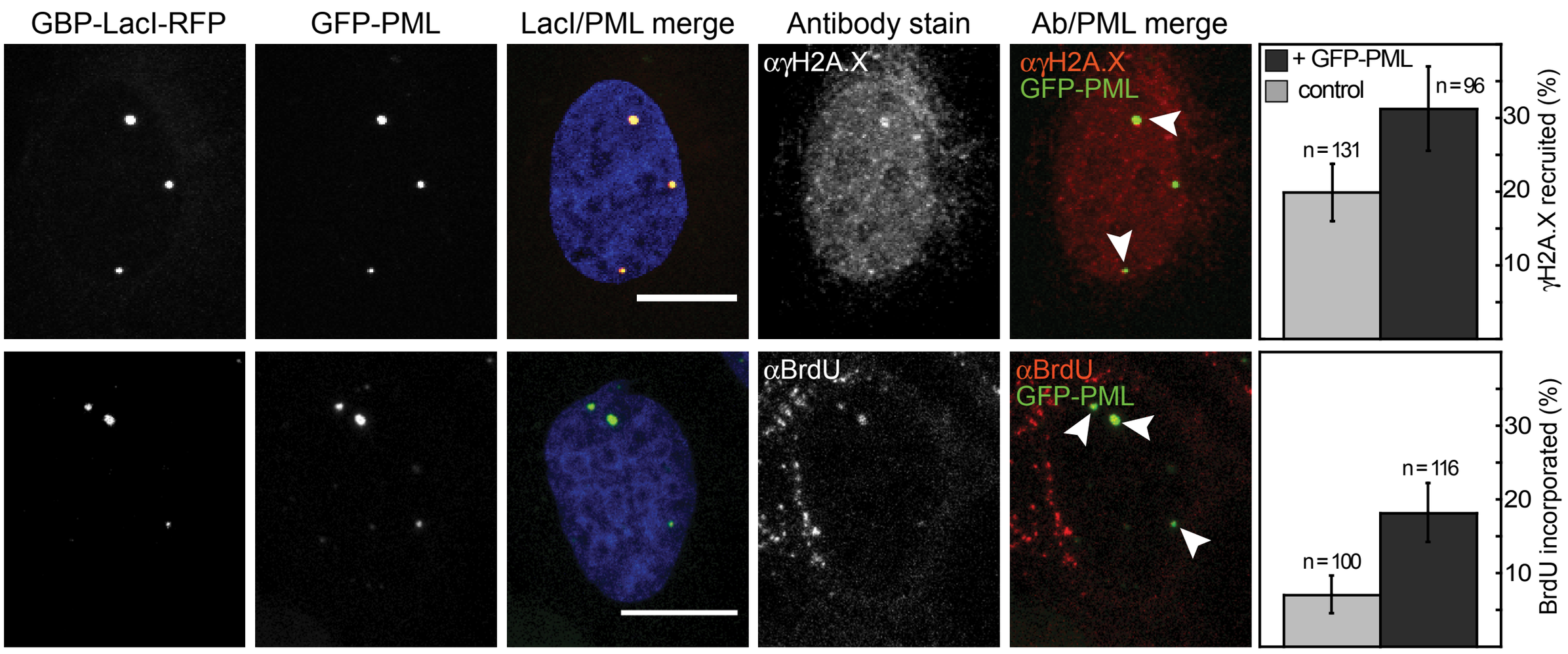
(3) De novo formation of APBs induces telomere elongation



telomeric *lac*O



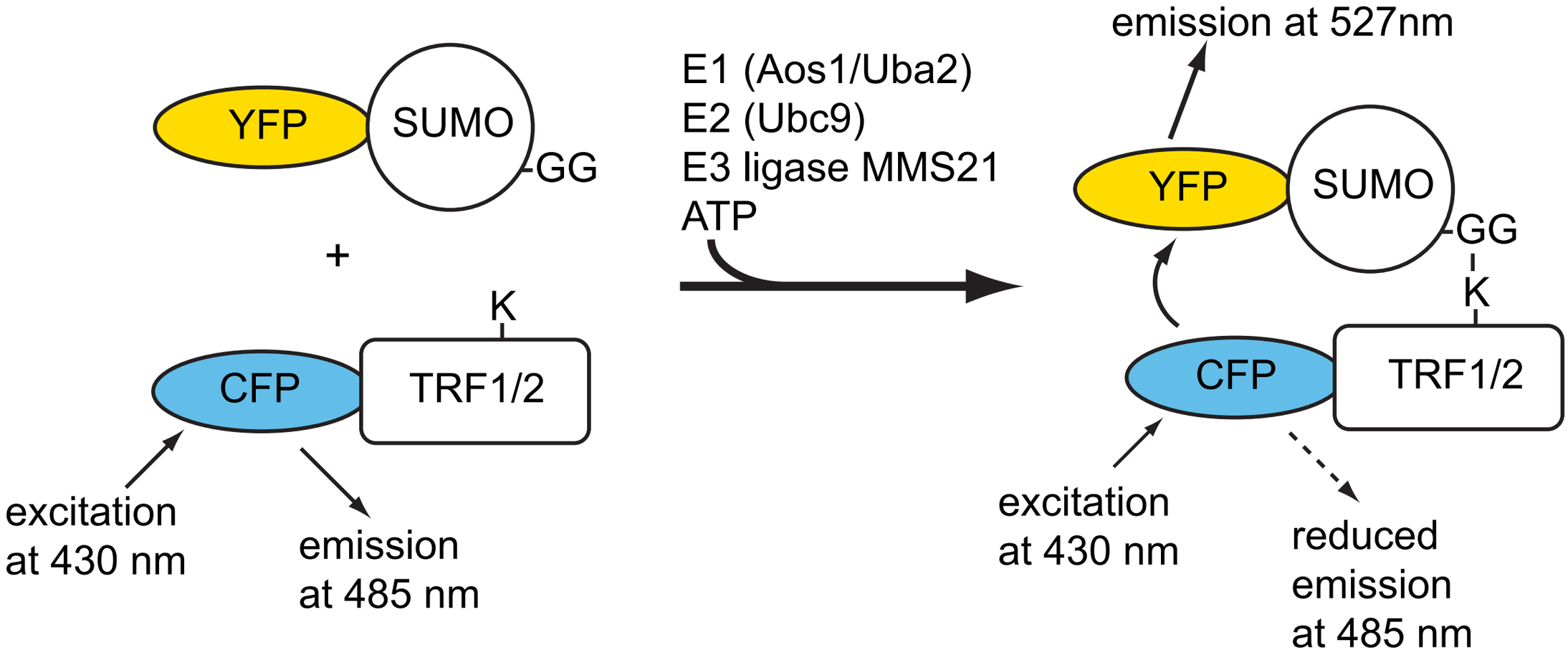
De novo formed APBs are sites of DNA repair synthesis



Other functional assays after candidate protein knock-down/inhibition:

- telomere shortening
- induction of senescence/apoptosis in ALT(+) but not ALT(-) cells
- reduced telomere recombination (sister chromatid exchange)

(4) MMS21 activity assay with fluorescence readout for high-throughput screening of small molecule inhibitors



FRET sumoylation assay by Stankovic-Valentin et al. (2009). *Methods Mol. Biol.* **497**, 241-251

APBs and the ALT pathway an excellent target for novel therapeutic approaches against cancer

- ALT operates in 30% of sarcomas and 10% of carcinomas
- ALT can emerge during inhibition of telomerase in cancer cancer
- APBs are a unique target since they form **only** in cancer cells
- ALT(+) can be reliably diagnosed via APB quantification with our 3D platform
- APBs are functional intermediates of telomere elongation in ALT(+) cells
- MMS21 is a novel and highly promising drug target for inhibiting ALT
- 10 more protein component of functional APBs under investigation
- set of unique tools for validation of APB formation and ALT function available
- specificity: knock-down of APB protein MMS21 induces senescence and apopotosis in ALT(+) cells after ~20 divisions but has no effect in ALT(-) cells

Inn Chung
who makes
APBs...

in collaboration with
Jürgen Reymann
Holger Erfle
Stefan Wörz
Karl Rohr

Current ALT pathway work in the Rippe lab is conducted by

...and
Sarah Osterwald
who breaks them!

Stefan Hell
Hans Engelhardt
Peter Lichter

at DKFZ & BioQuant