



Transcription stress-associated telomere dysfunction & cellular senescence



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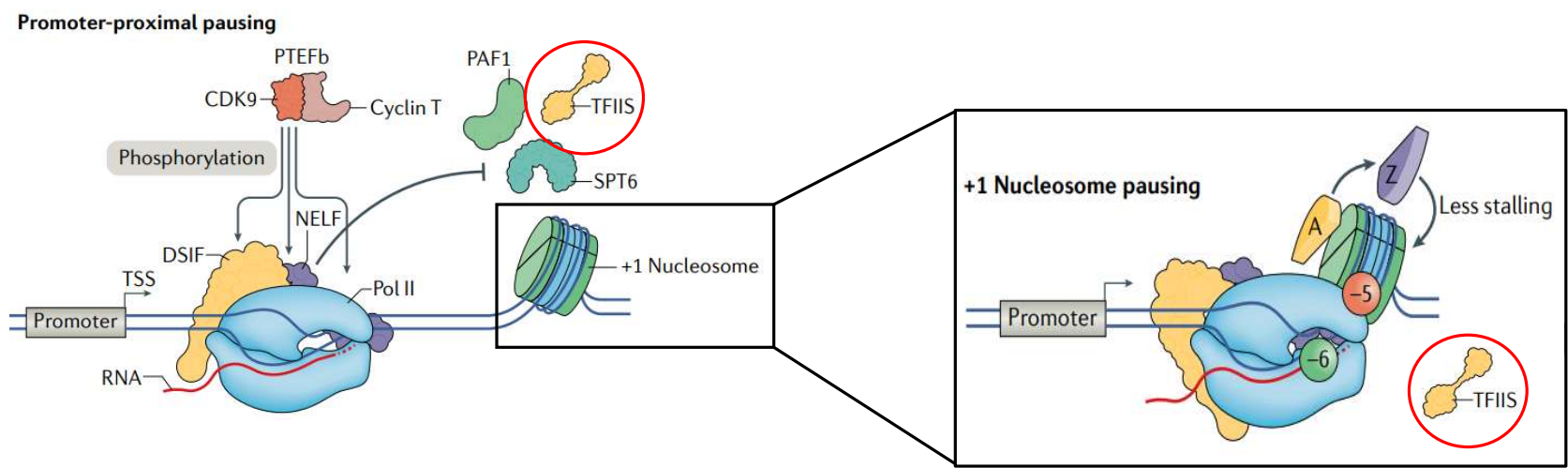
October 21, 2024



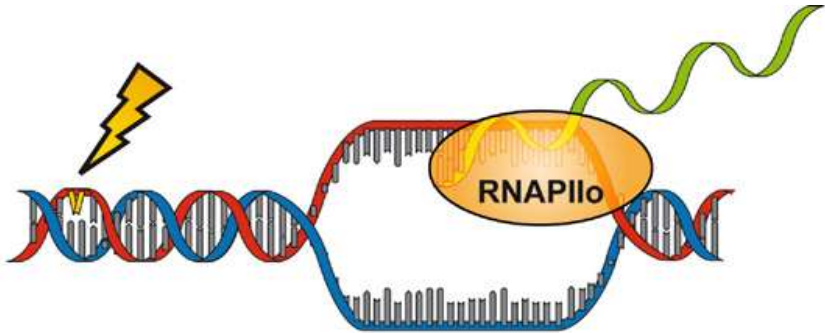
Intrinsic barriers to transcript elongation

RNA PolII along genes:

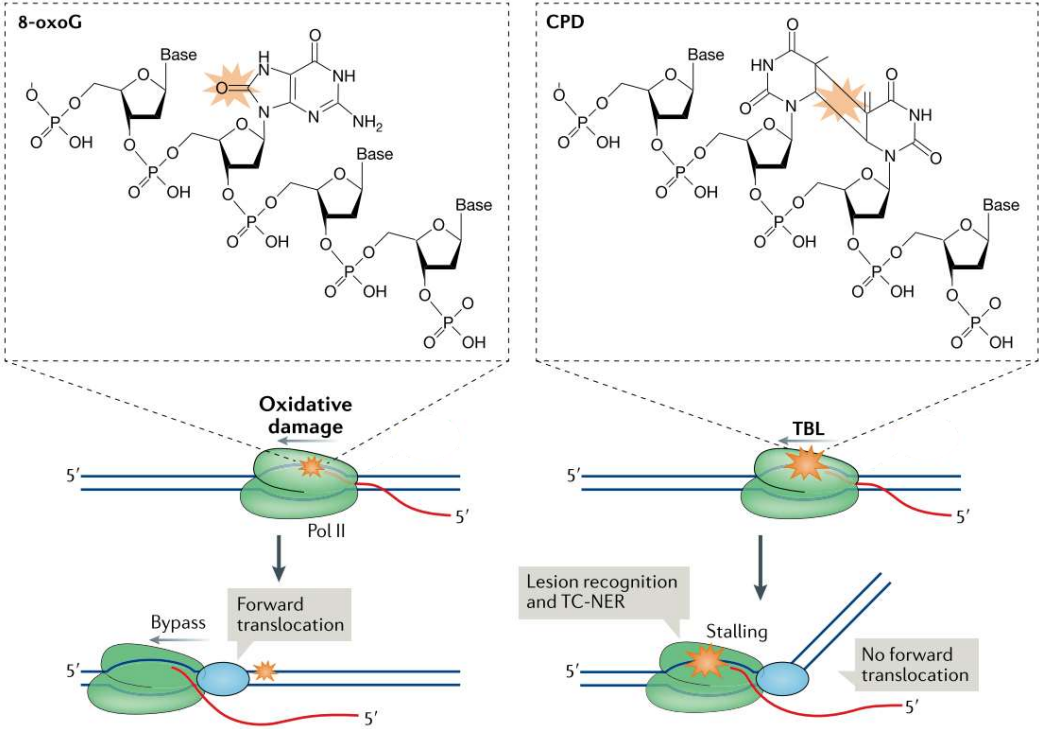
- 1. Downstream of the TSS (Promoter-proximal pausing)
 - 2. Poly(A) sites (Transcription termination)
 - 3. Physical obstacles (Nucleosomes, Specific sequences)
- Gene regulation & RNA processing



Transcription & Transcription-blocking lesions (TBLs)



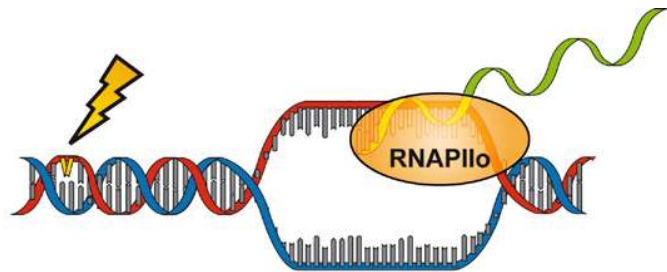
Type of lesion	Transcription-blocking lesion	Causes
Structural lesions	6-4PP and CPD photoproducts	Ultraviolet light
	Abasic sites	Depurination, BER intermediate
DNA crosslinks	Platinum–DNA crosslinks	Chemotherapy
	Psoralen–DNA crosslinks	Phototherapy
Bulky adducts	BPhDE adducts	Pollutant BPhDE
	BPDE adducts	Smoke from organic matter
	AAF adducts	Carcinogen AAF
	4NQO–DNA adducts	4NQO
	DNA–protein crosslinks	Aldehydes, topoisomerase inhibitors
Oxidative lesions	Cyclopurines	Oxidative stress
Toxins	Illudin S adducts	Mushroom toxin
	Aristolactam–DNA adducts	Herbal toxin aristolochic acid



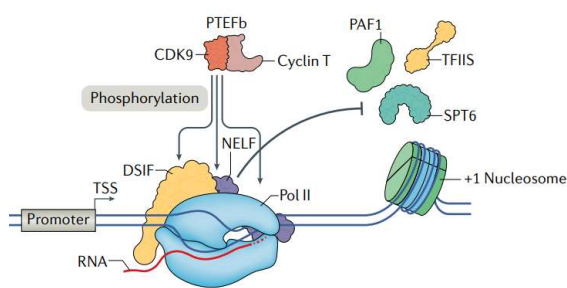
Transcription stress

“Transcription stress”

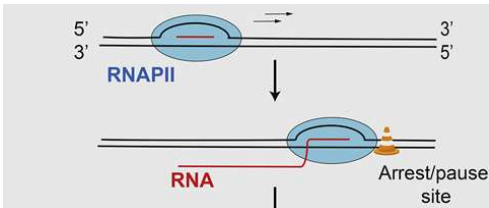
Impact of DNA damage on transcription accuracy and efficiency



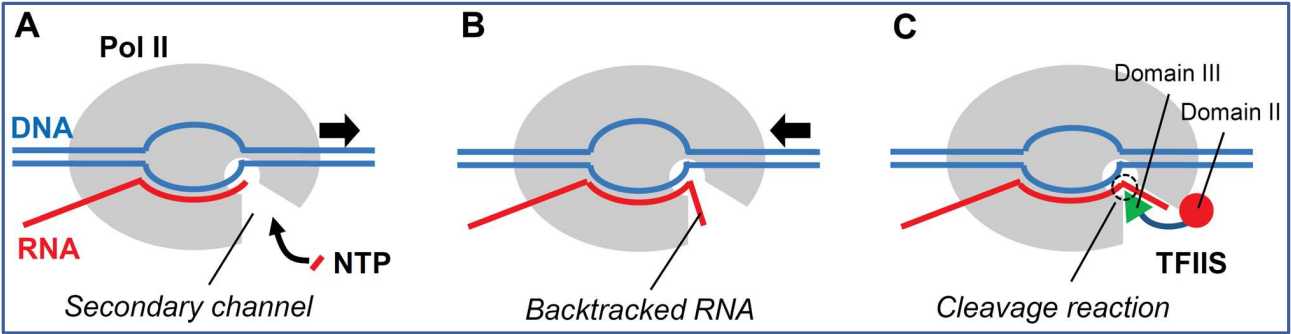
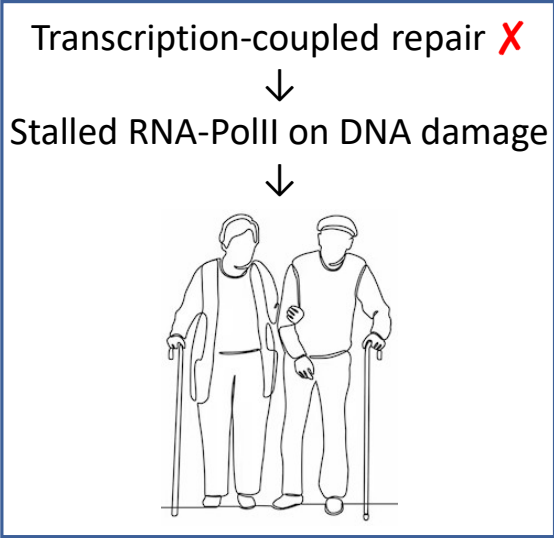
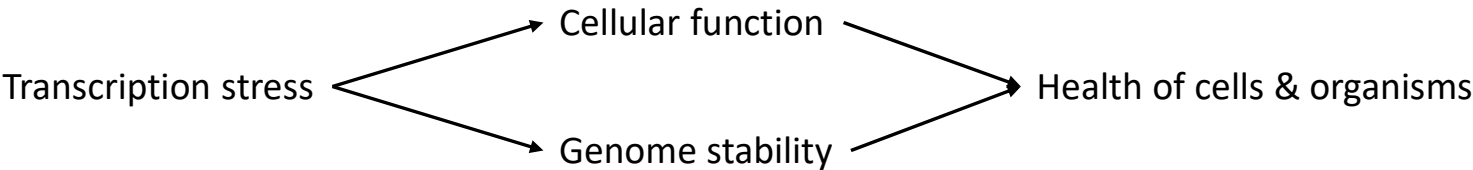
Genome-wide transcription regulation



Transcription stalling & transcription arrest



Transcription stress



🎯 How RNA-PolII stalling can trigger physiological outcomes that threaten cell viability?

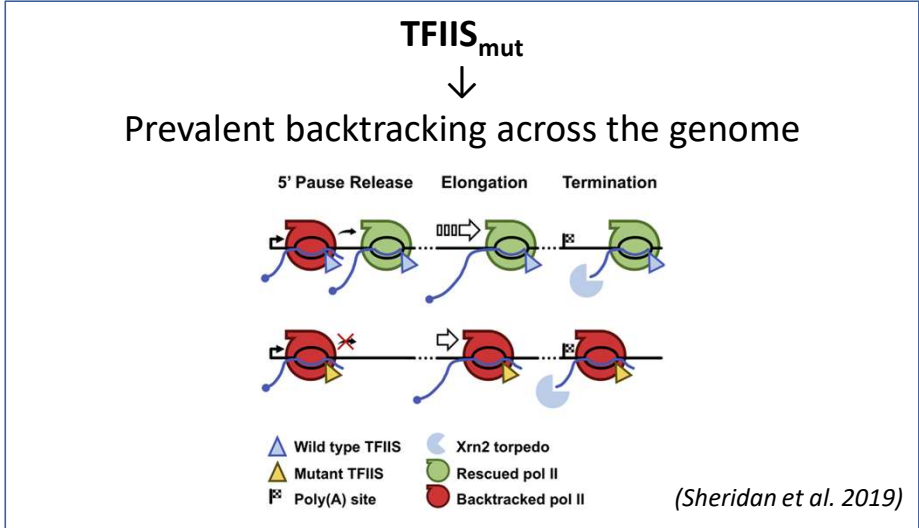
Backtracking and the role of Transcription elongation factor S-II (TFIIS)

Transcription elongation factor S-II → The first Pol II-associated transcription factor to be purified (Sekimizu et al. 1976)

In vitro Transcription Assays + TFIIS → Elevated levels of RNA synthesis —————→ The first known Pol II elongation factor (Rappaport et al. 1987, Reinberg & Roeder 1987)

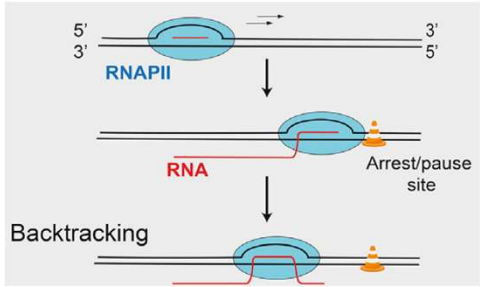
TFIIS exerts its effect following elongation arrest → Strongly stimulating RNA cleavage activity of Pol II (Reines et al. 1992, Izban et al. 1992)

Role of TFIIS in rescuing arrested and backtracked elongation complexes (Cheung & Cramer 2011, Lisica et al. 2016)



TFIIS_{mut}:

- Global transcript elongation defects
- Increased R-loop formation
- Genome instability, including the formation of DNA DSBs



(Zatreanu et al. 2019)

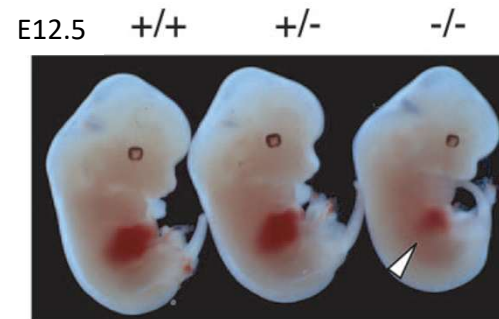
- RNA polymerase II bypass of oxidative DNA damage is regulated by transcription elongation factors (Charlet-Berguerand et al. 2006)
- RNA polymerase II bypasses 8-oxoguanine in the presence of transcription elongation factor TFIIS (Kuraoka et al. 2007)

TCEA1^{fl/fl} mouse

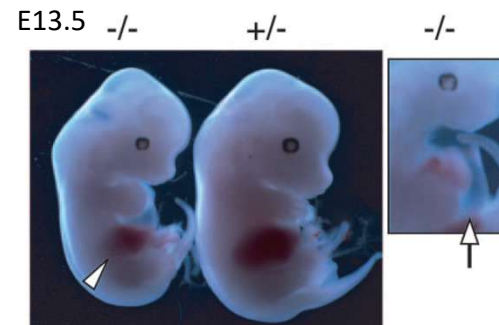


Intrinsic transcription stress

- **TFIIS** null mutants are embryonic lethal in mice

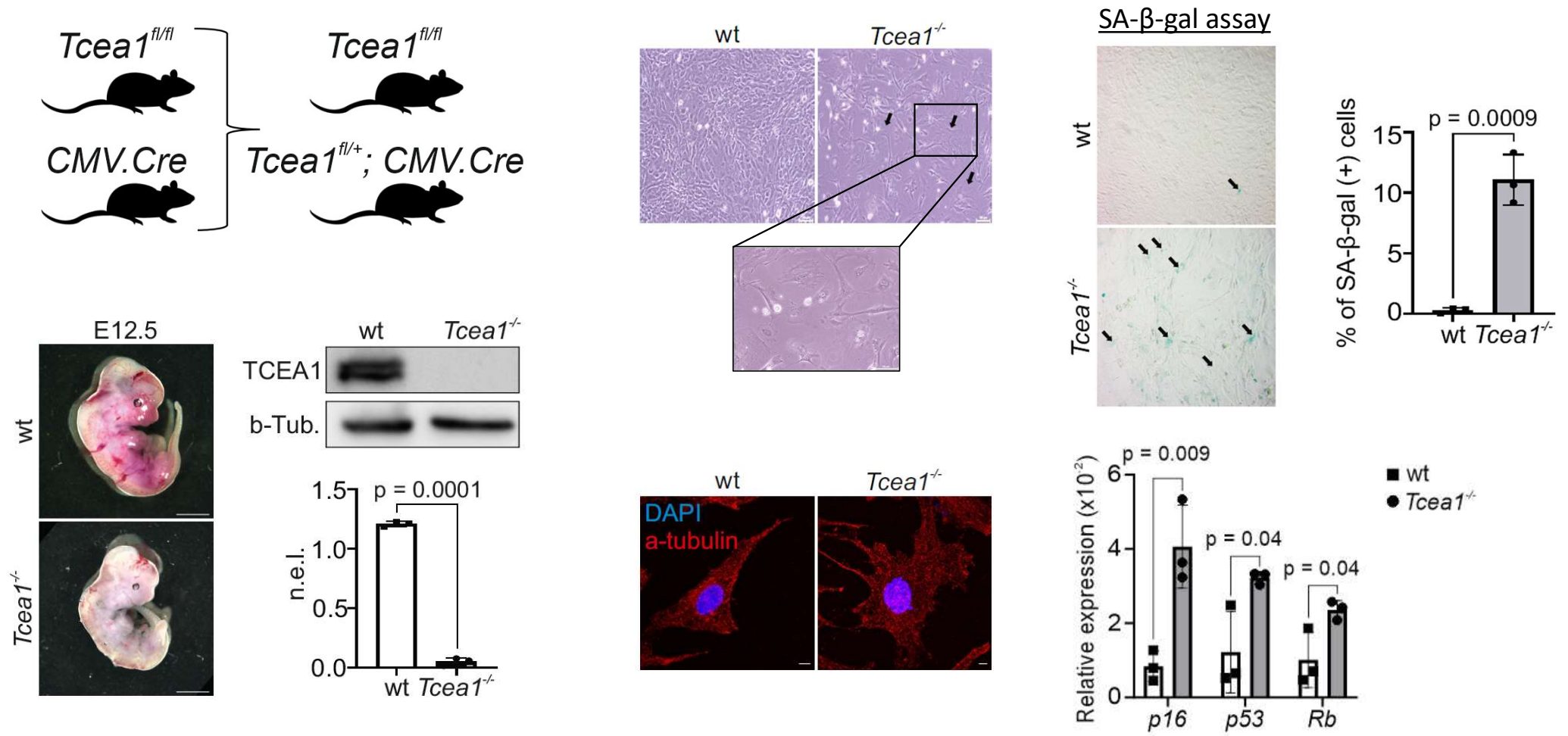


- Fetal liver hypoplasia
- Tail curling



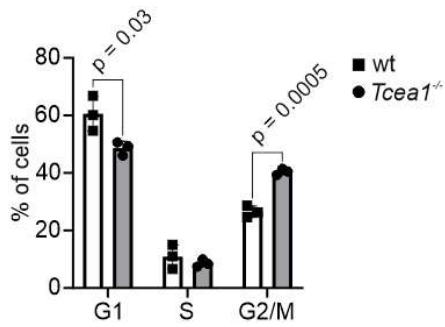
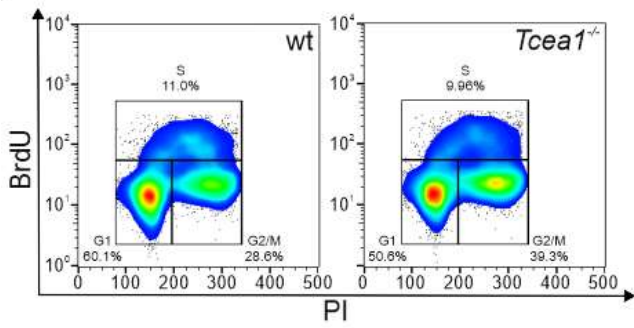
- Smaller
- Pericardial edema

Loss of TFIIS in Mouse Embryonic Fibroblasts (MEFs) results in early cellular senescence

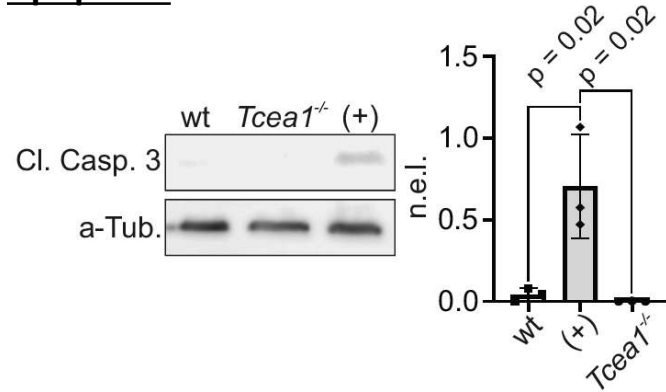


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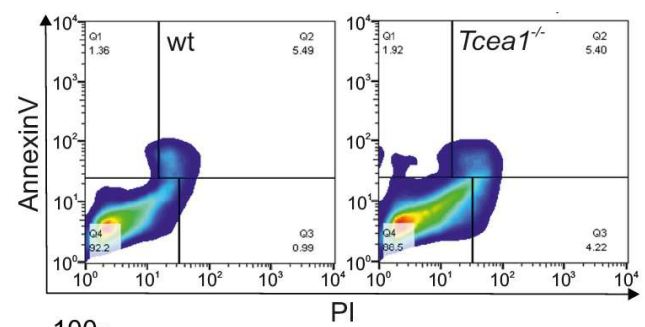
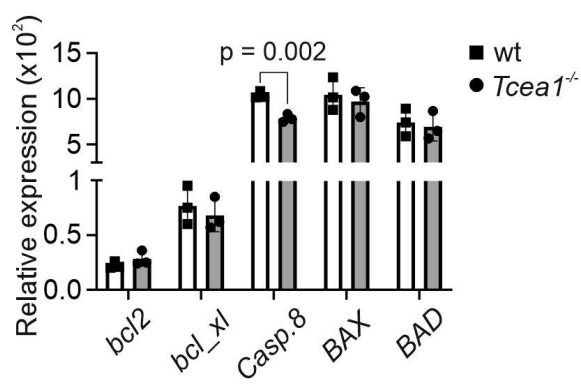
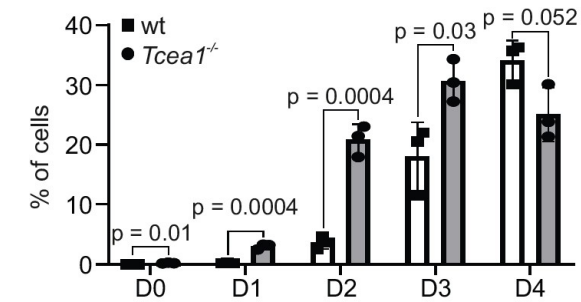
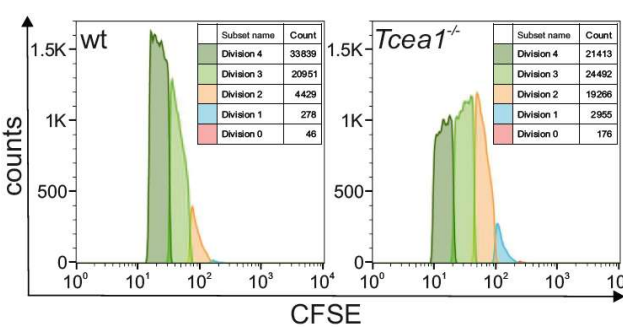
Cell cycle assay



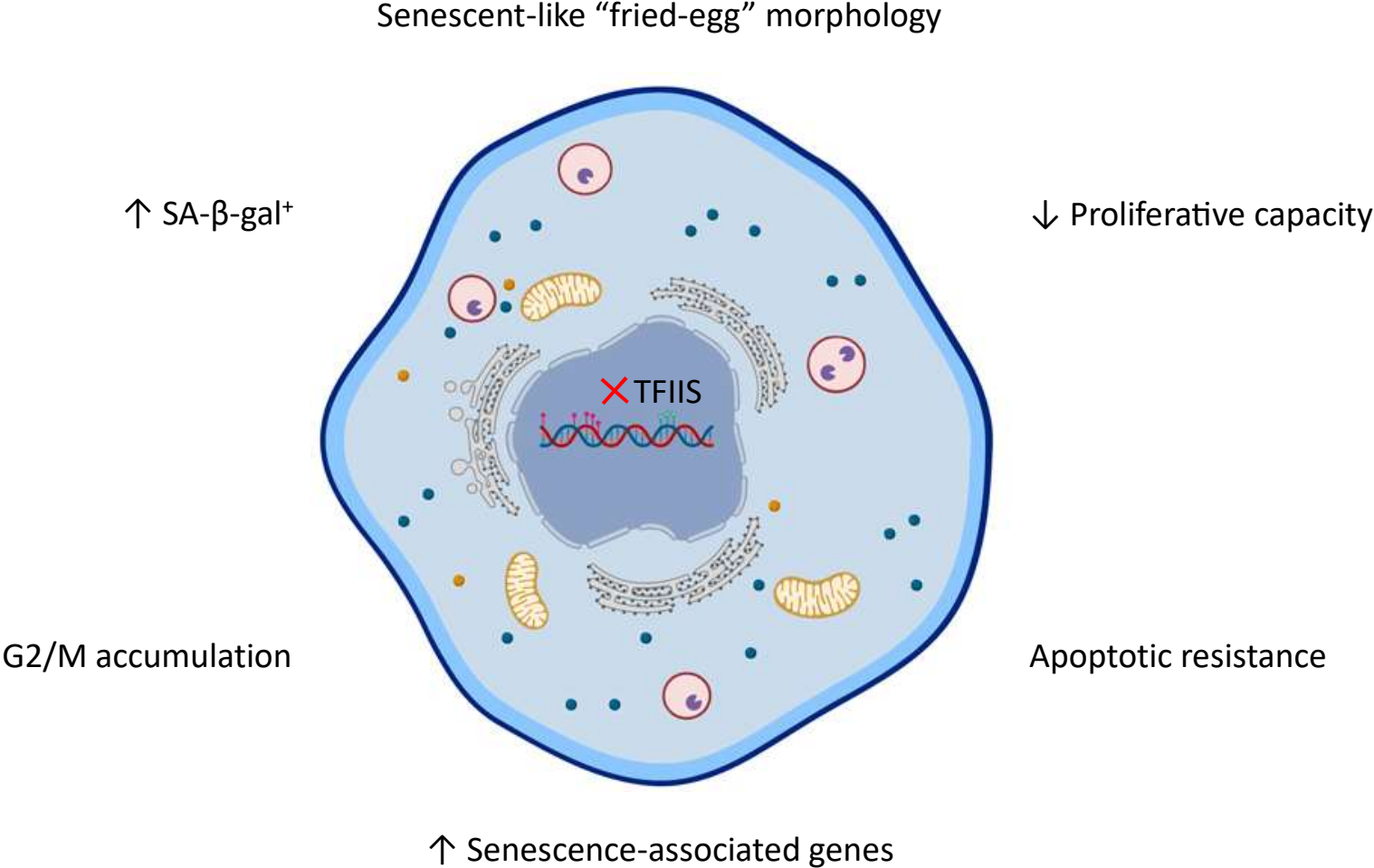
Apoptosis



CFSE proliferation assay

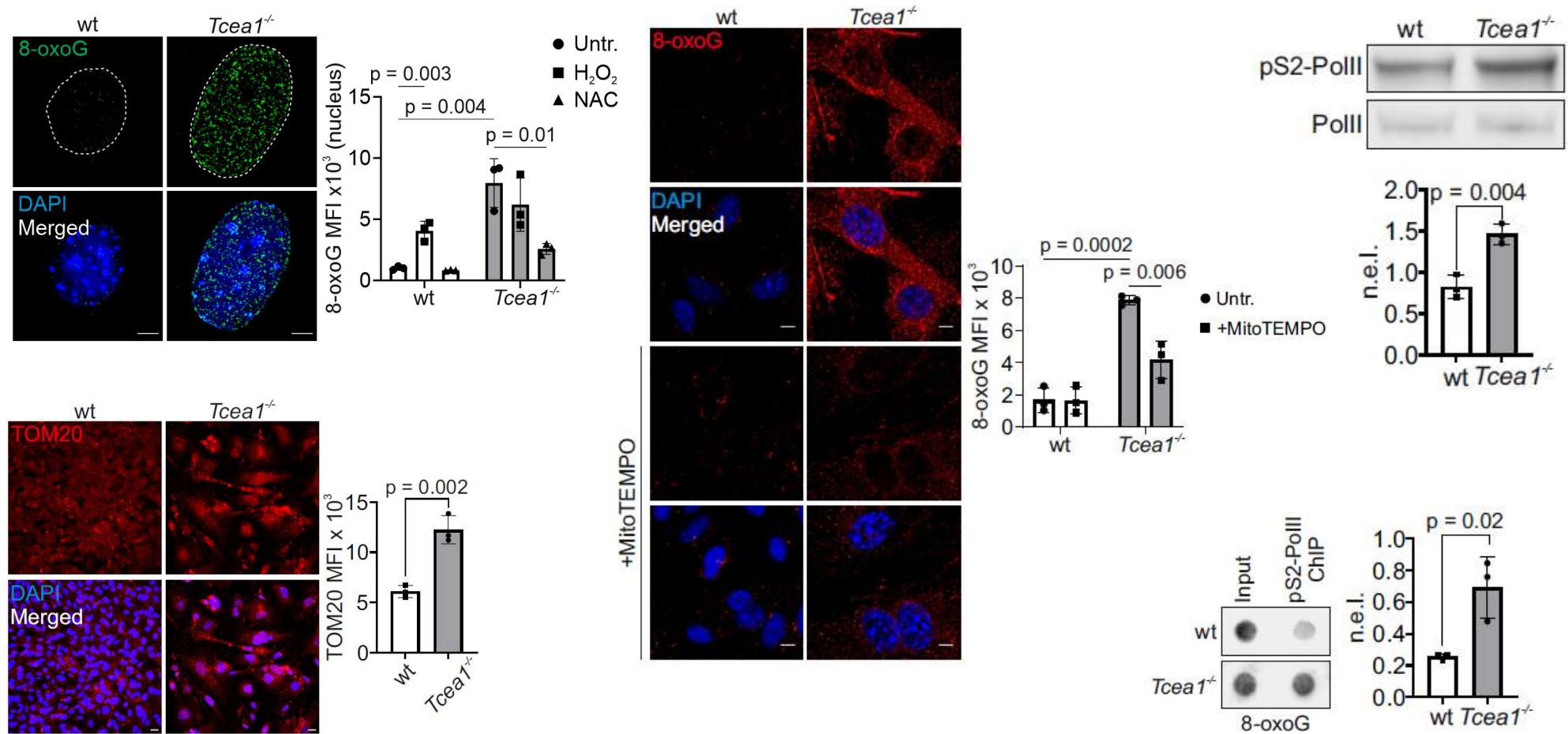


Loss of TFIIS in Mouse Embryonic Fibroblasts (MEFs) results in early cellular senescence

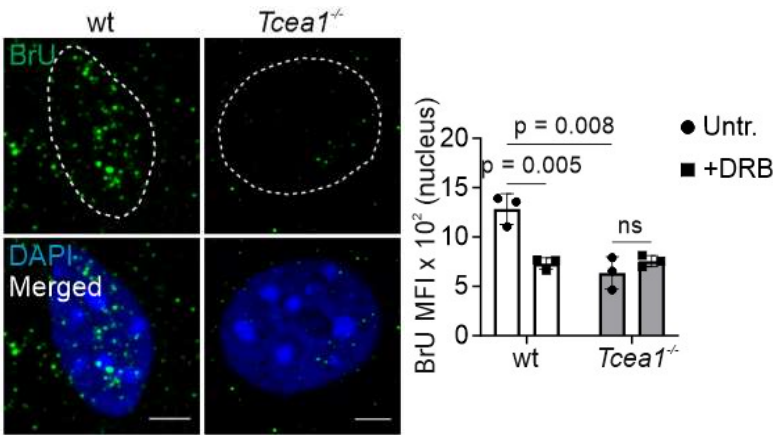
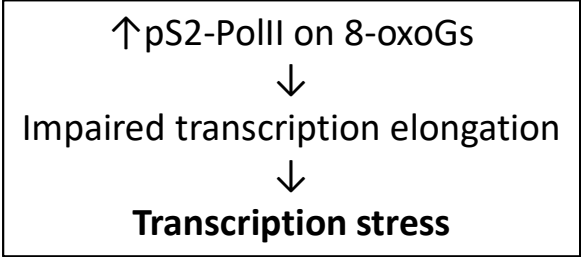
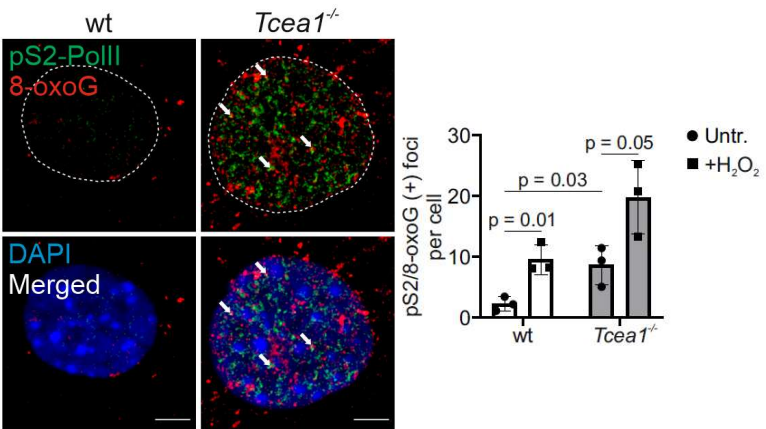


pS2-RNAPII accumulation on 8-oxoG lesions in *Tcea1*^{-/-} MEFs

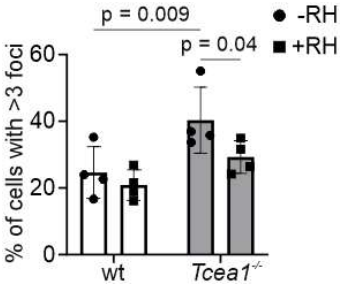
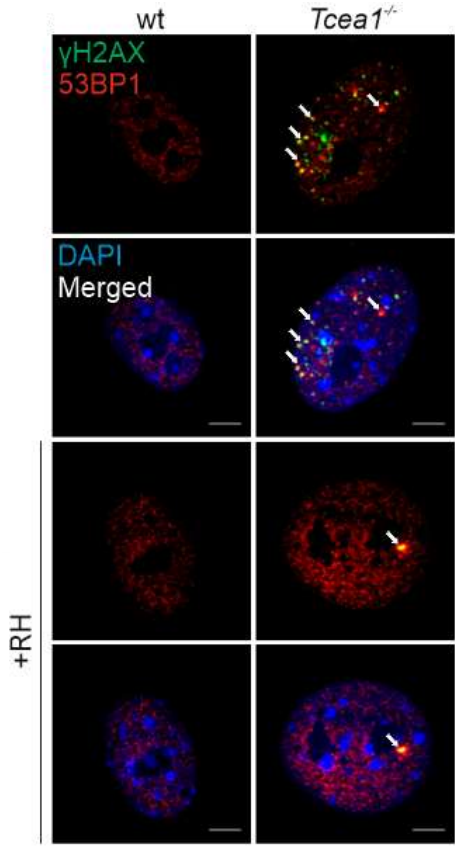
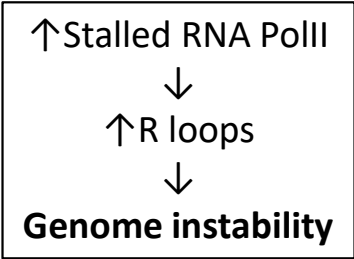
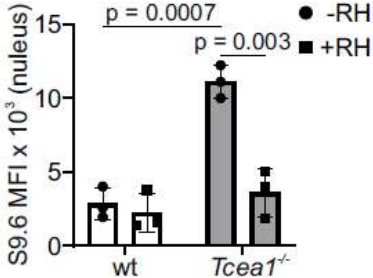
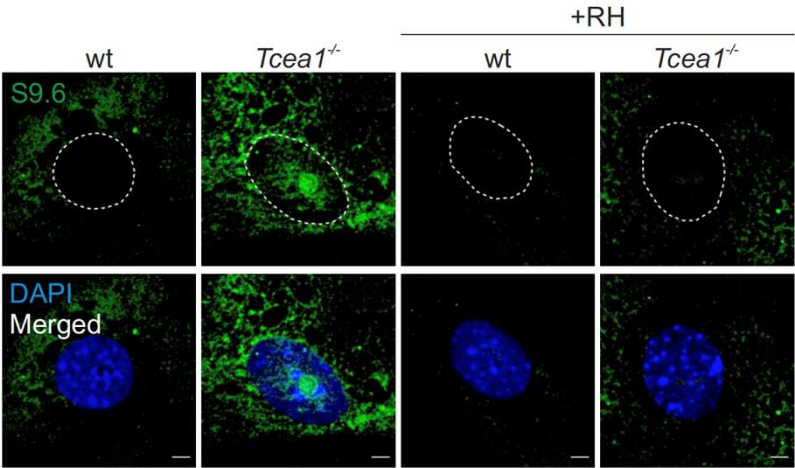
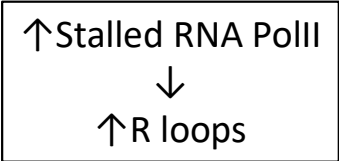
Senescent cells → ↑ROS → 8-oxoG



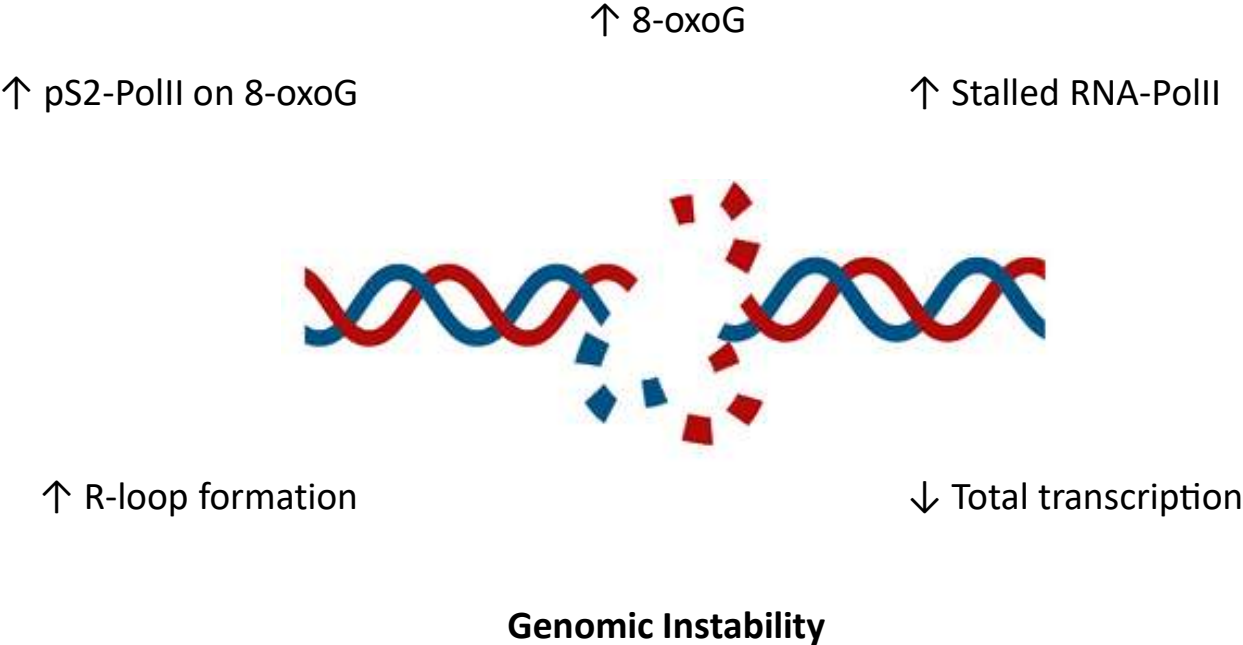
Tcea1 deletion triggers the formation of R loops and leads to genomic instability in MEFs



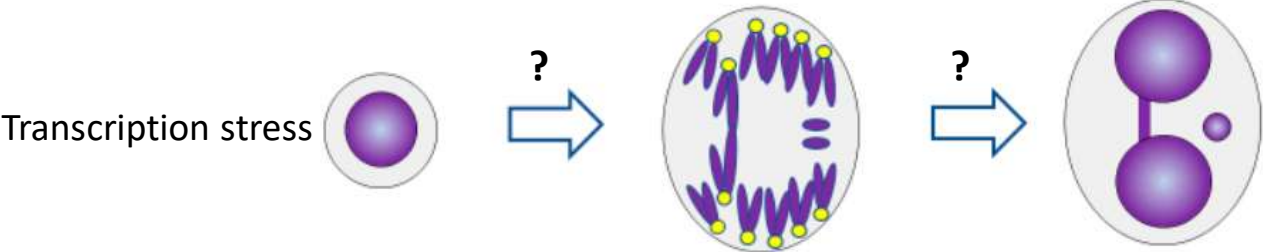
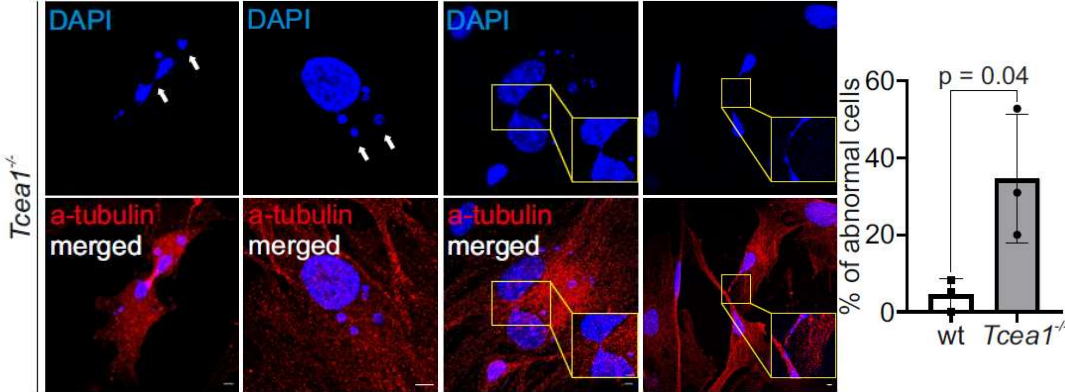
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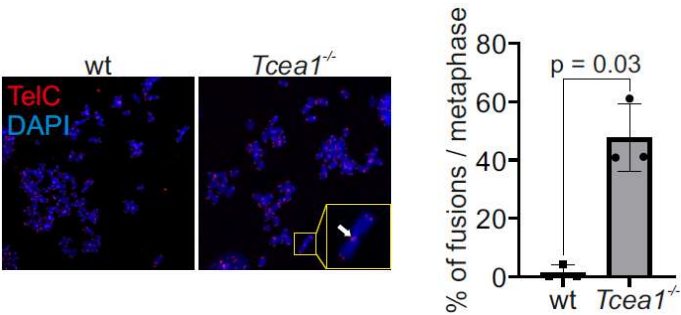
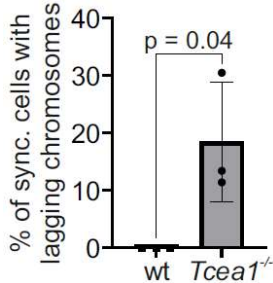
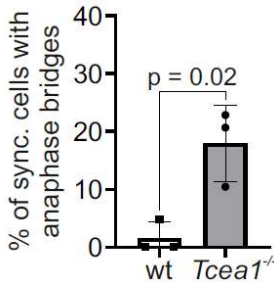
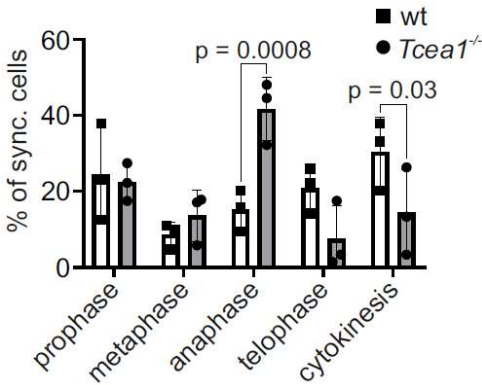
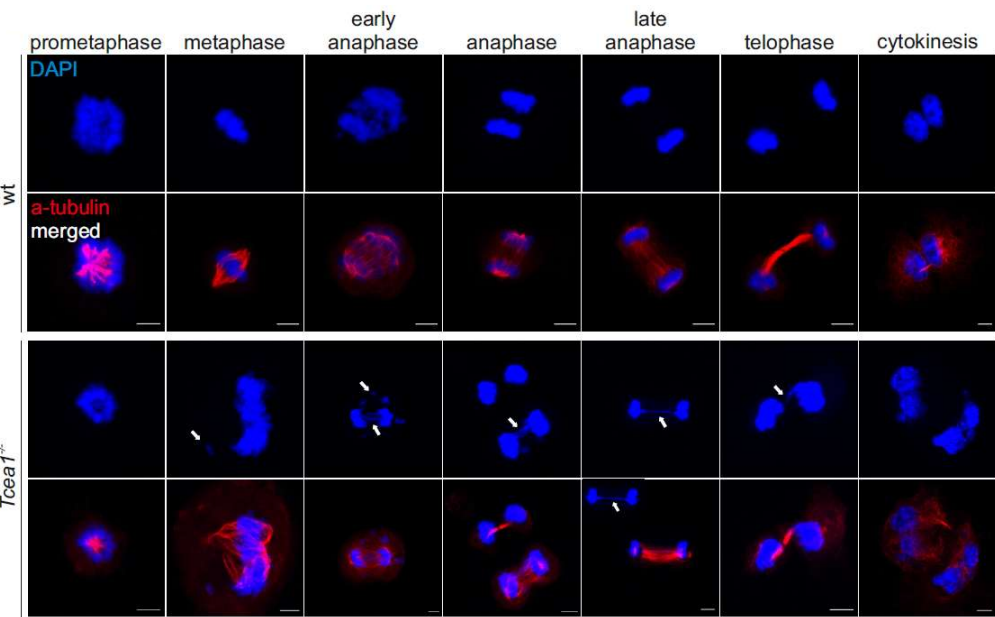
***Tcea1* deletion triggers the formation of R loops and leads to genomic instability in MEFs**



Tcea1^{-/-} MEFs form chromatin bridges

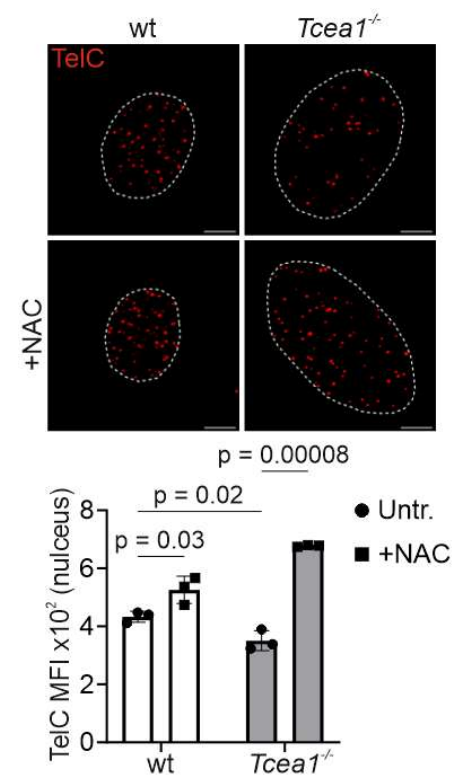
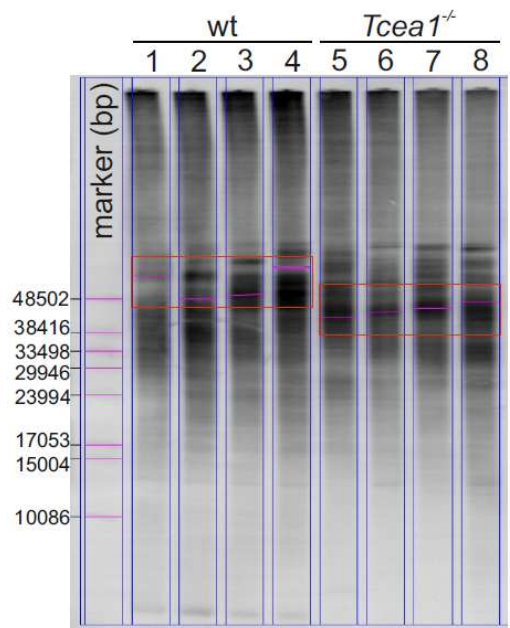
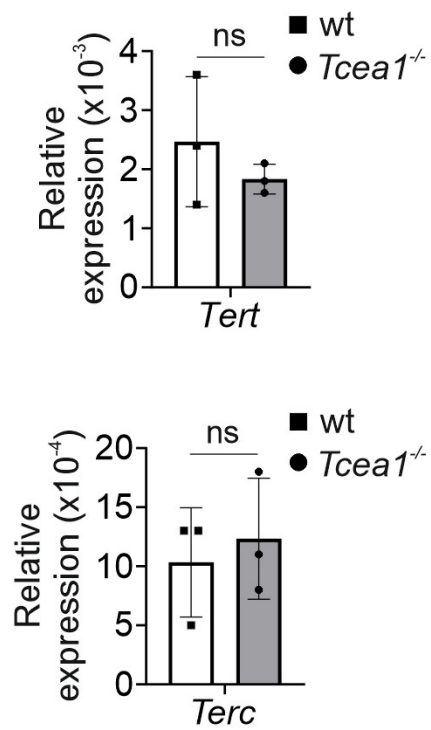


A defect in TFIIS triggers aberrant mitosis & telomere fusions

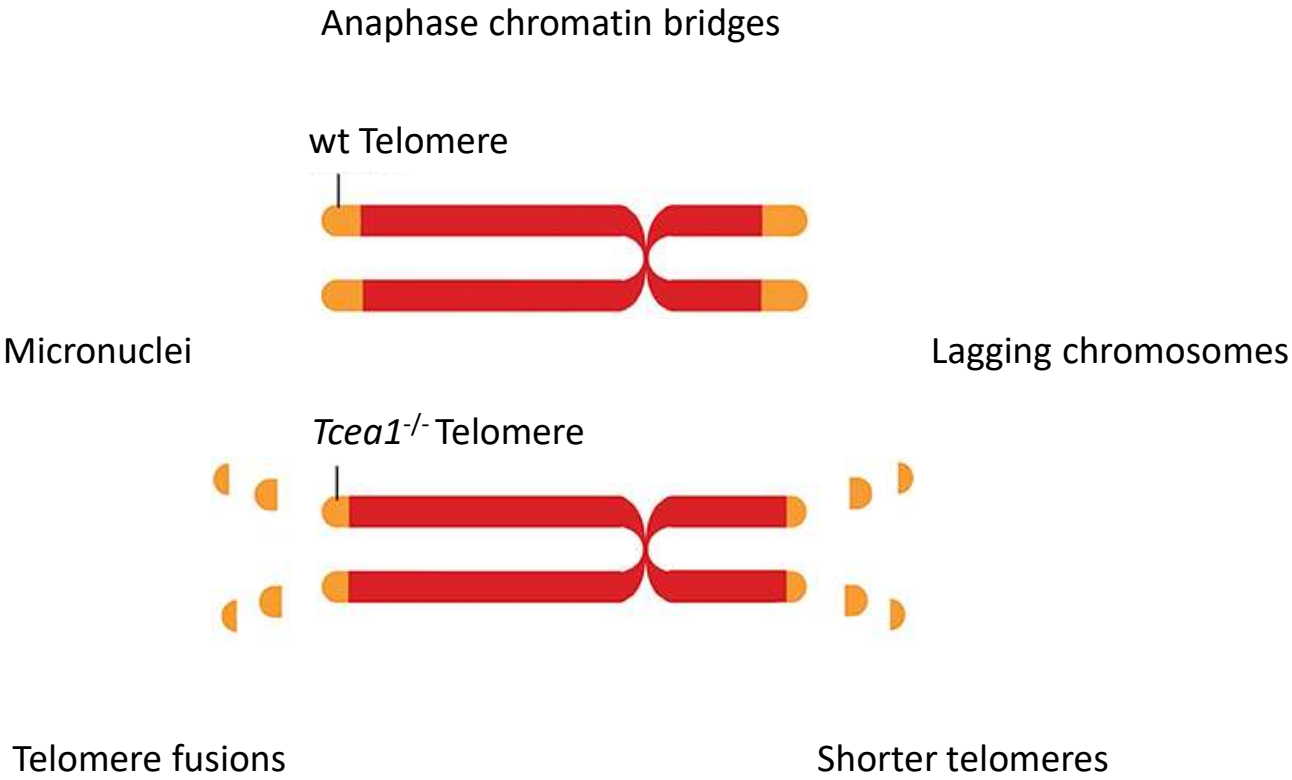


- Double-strand breaks (DSBs)
- Errors during DNA replication
- Aging-related Telomere erosion

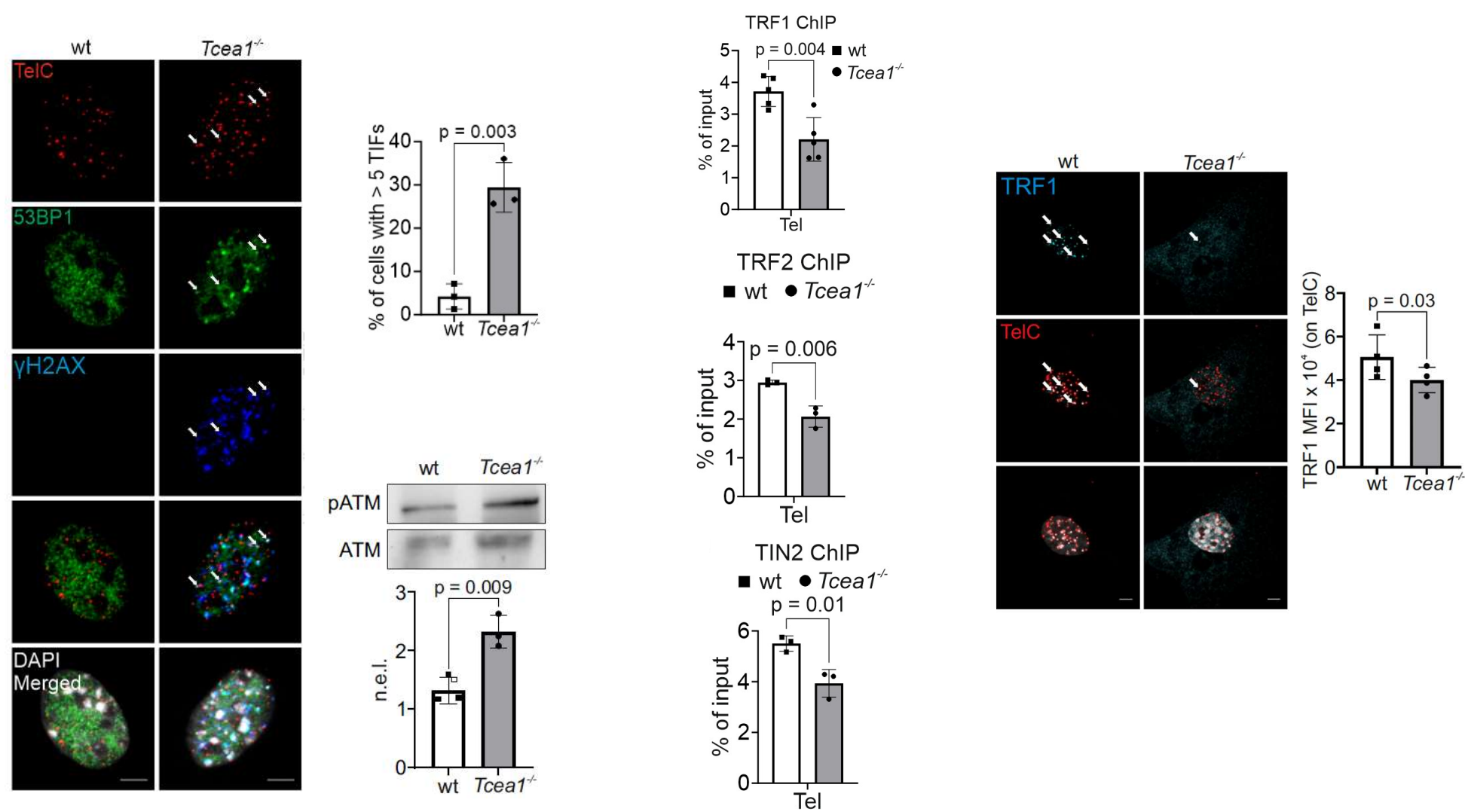
A defect in TFIIS triggers telomere attrition



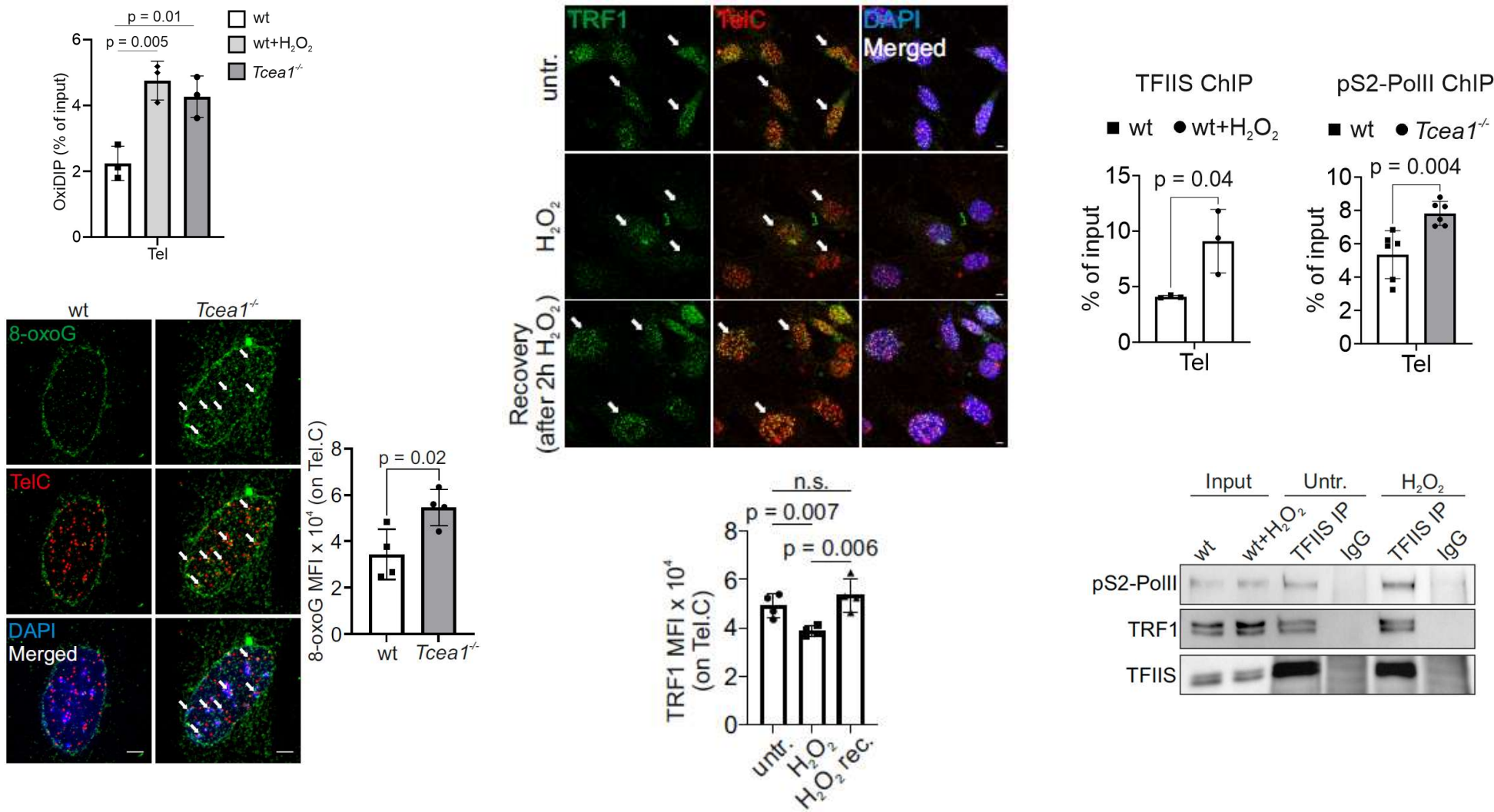
A defect in TFIIS triggers telomere attrition, telomere fusions & aberrant mitosis



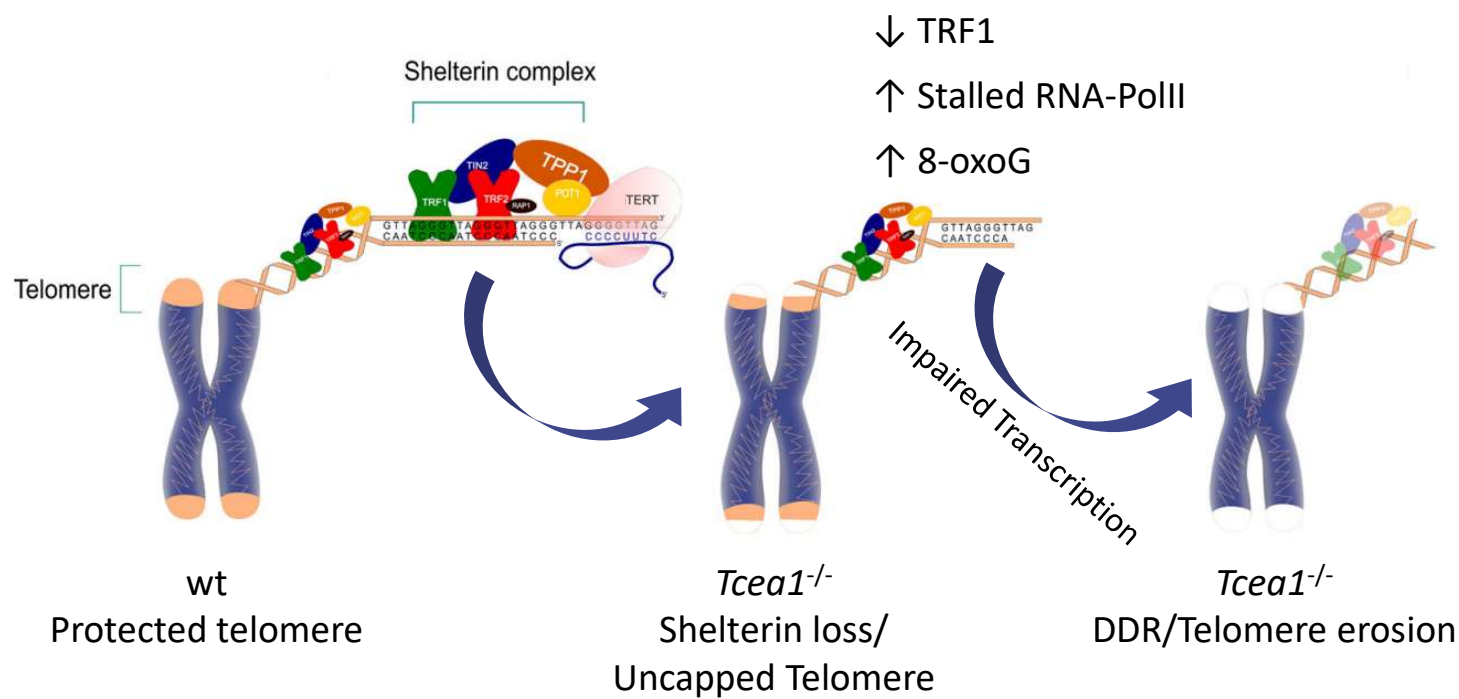
A defect in TFIIS associates with telomere uncapping and DDR activation



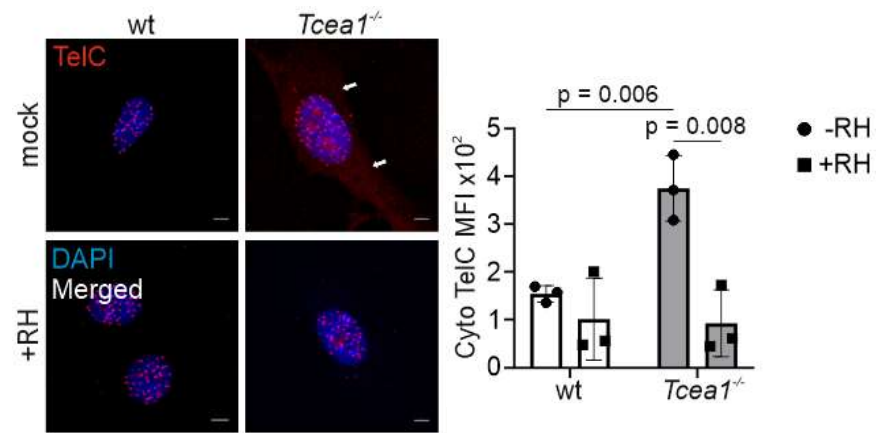
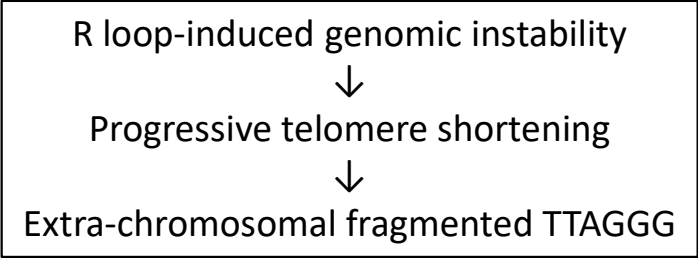
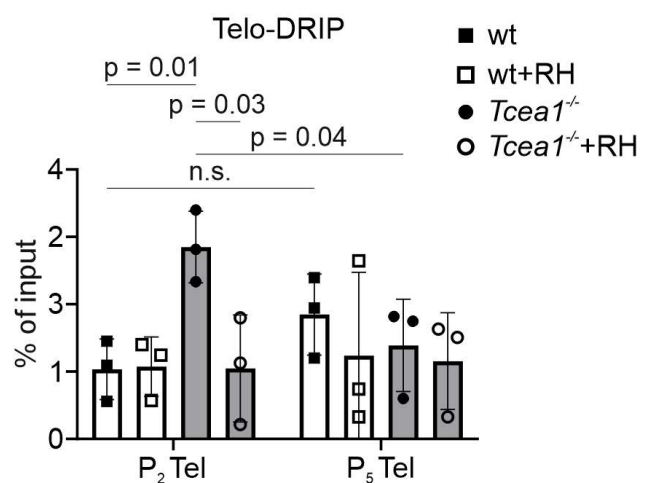
TRF1 interacts with pS2-RNAPII and is released from telomeres upon oxidative stress



A defect in TFIIS associates with telomere uncapping and DDR activation



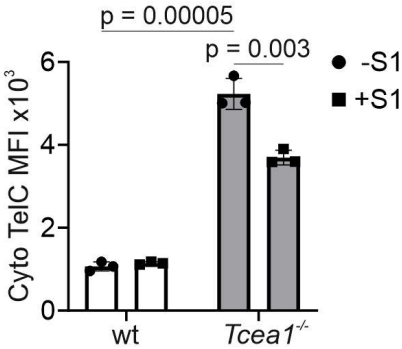
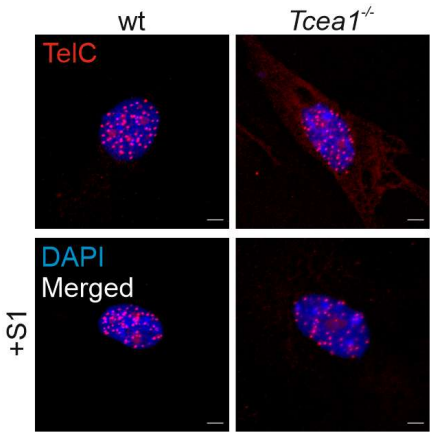
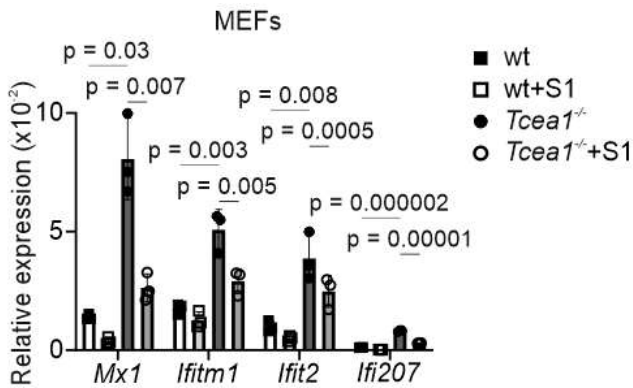
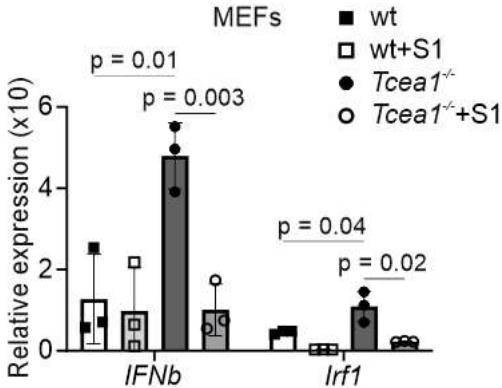
R loop-derived cytosolic telomeric fragments in *Tcea1*^{-/-} MEFs



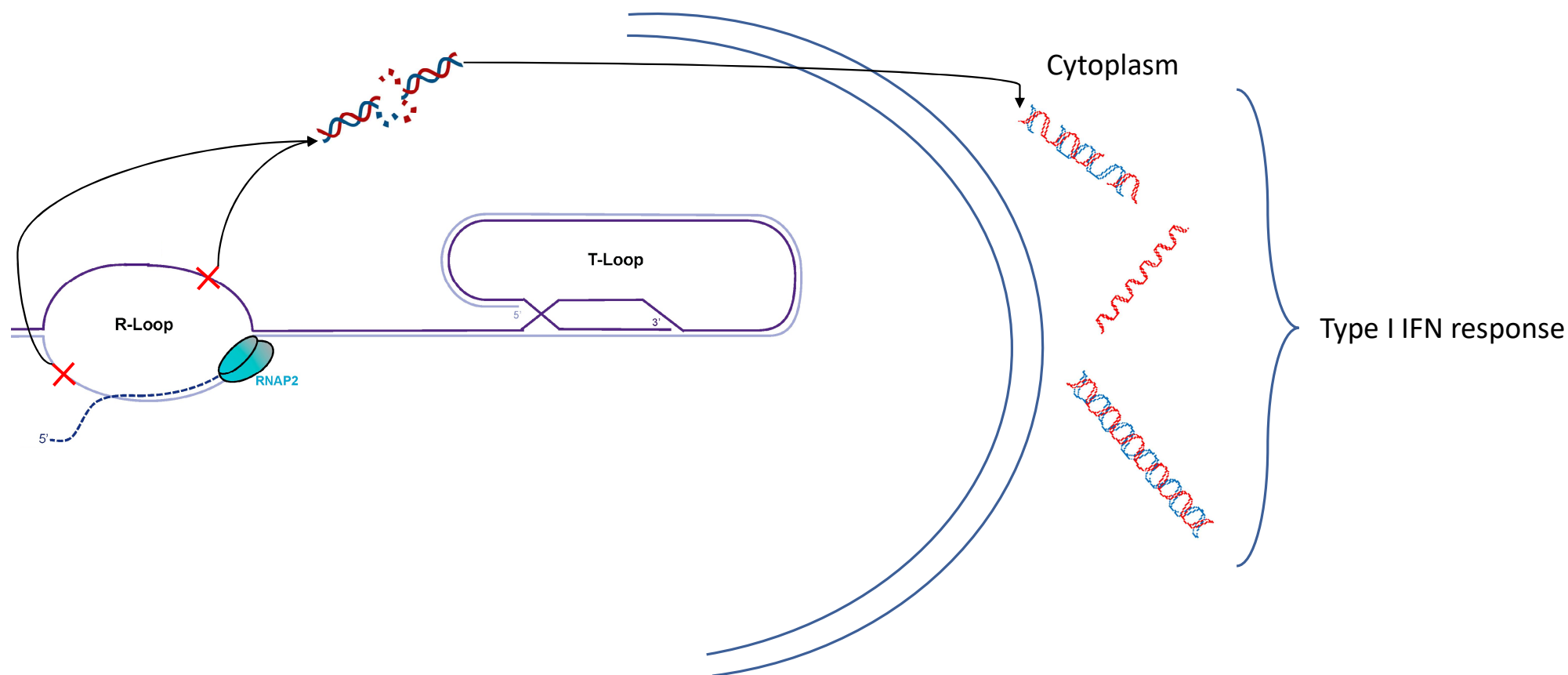
R loop-derived cytosolic telomeric fragments in *Tcea1*^{-/-} MEFs



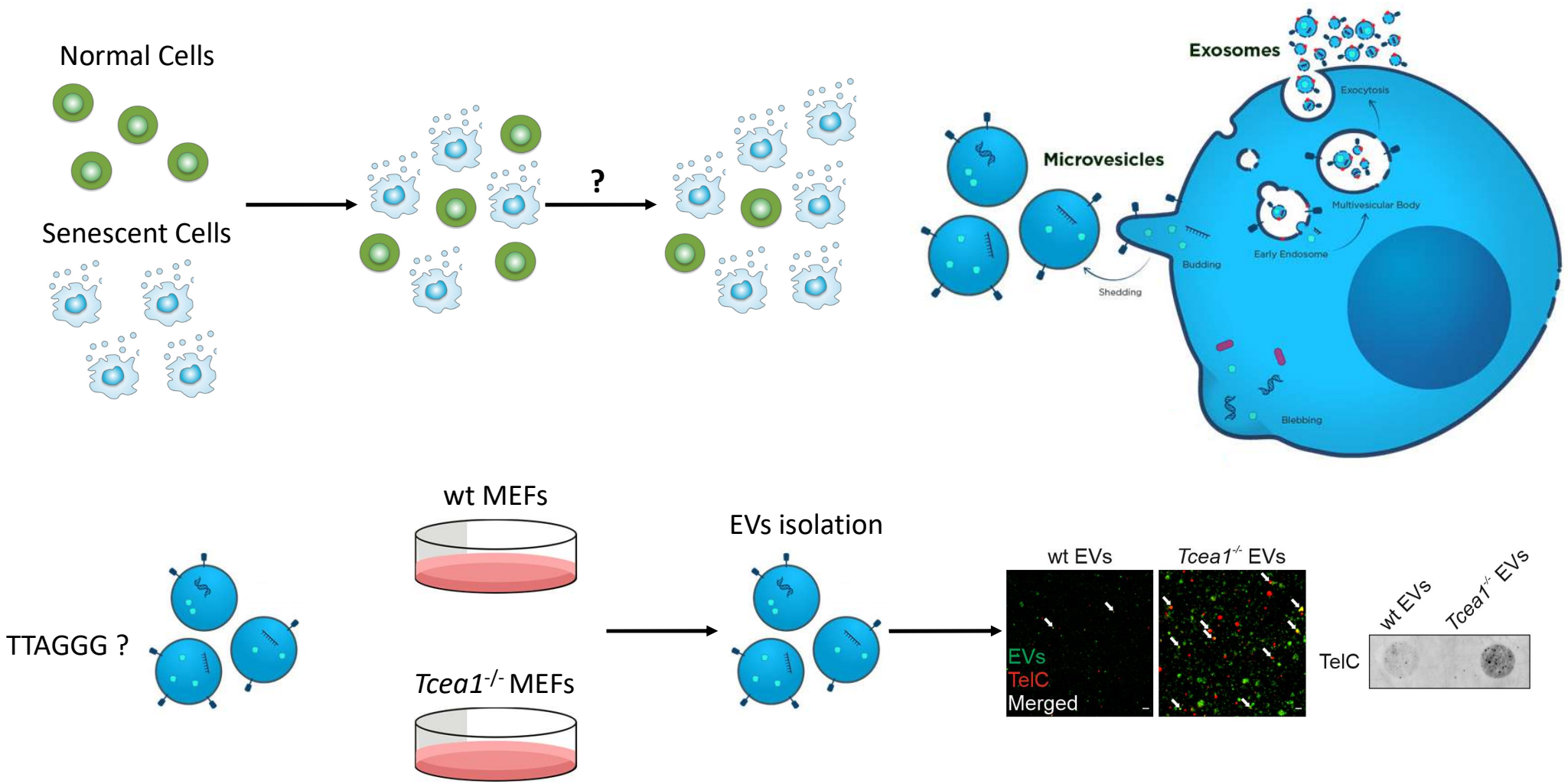
Viral-like response → Type I interferon response



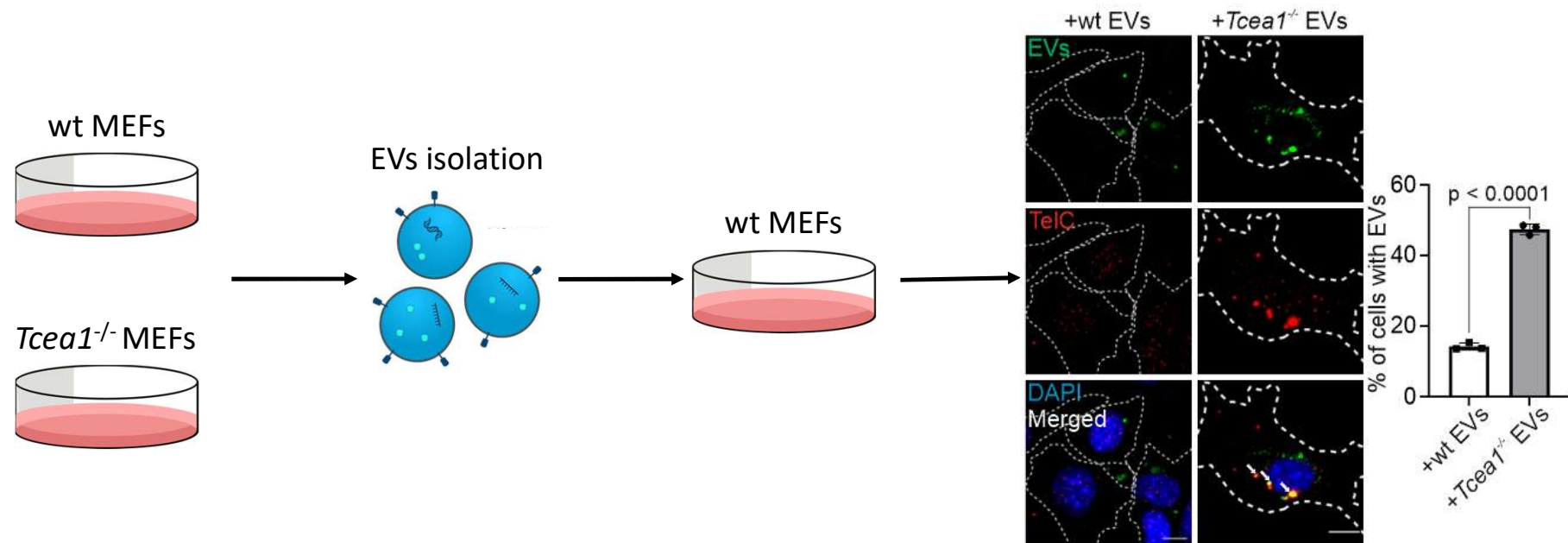
R loop-derived cytosolic telomeric fragments mount an inflammatory response



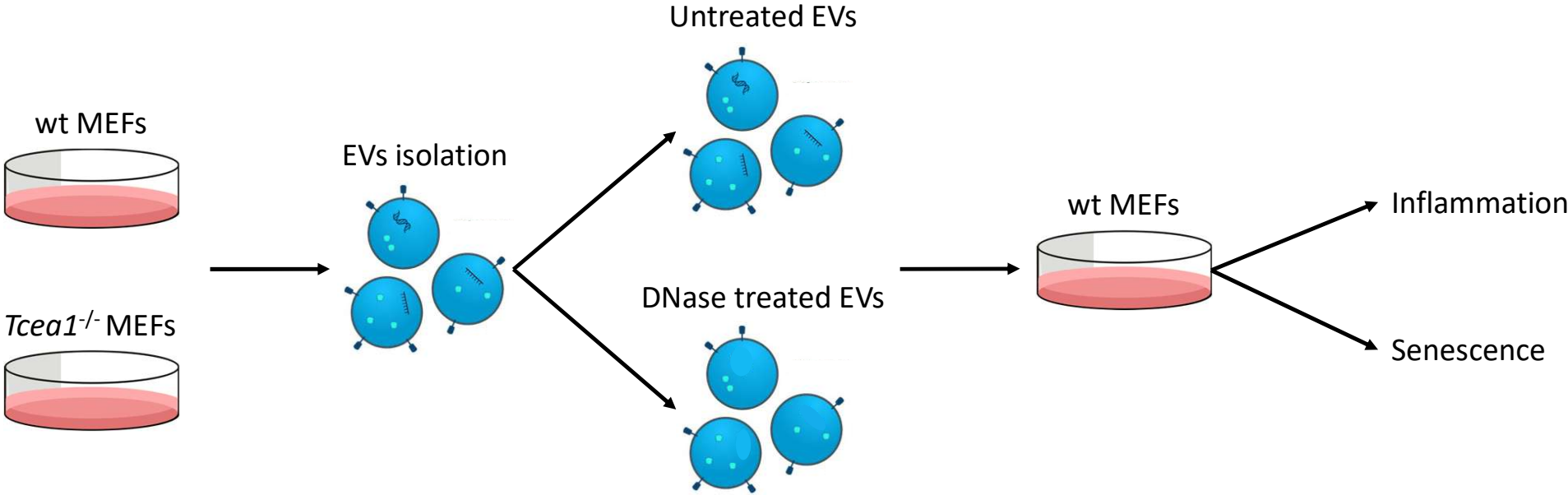
Tcea1^{-/-} MEFs secrete EVs loaded with telomeric DNAs



Tcea1^{-/-} MEFs secrete EVs loaded with telomeric DNAs

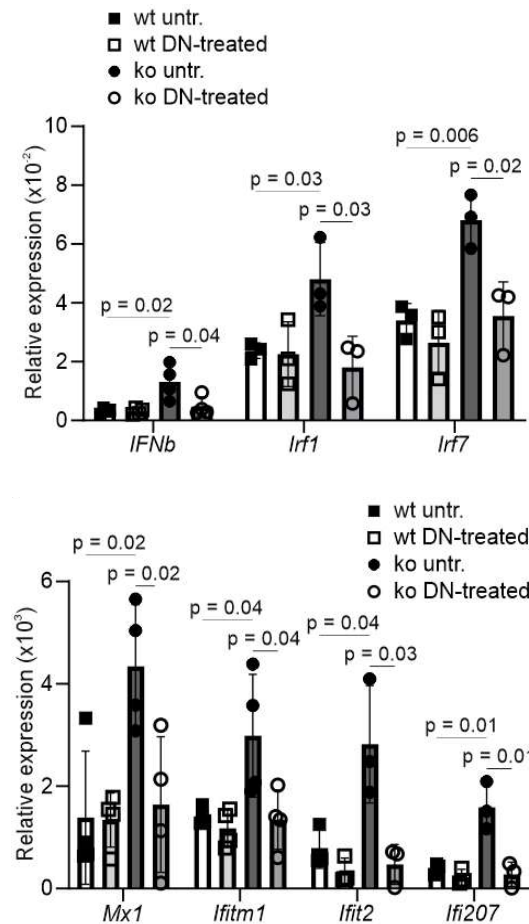


***Tcea1*^{-/-} MEFs secrete Tel-loaded EVs that trigger inflammation & “bystander” senescence**

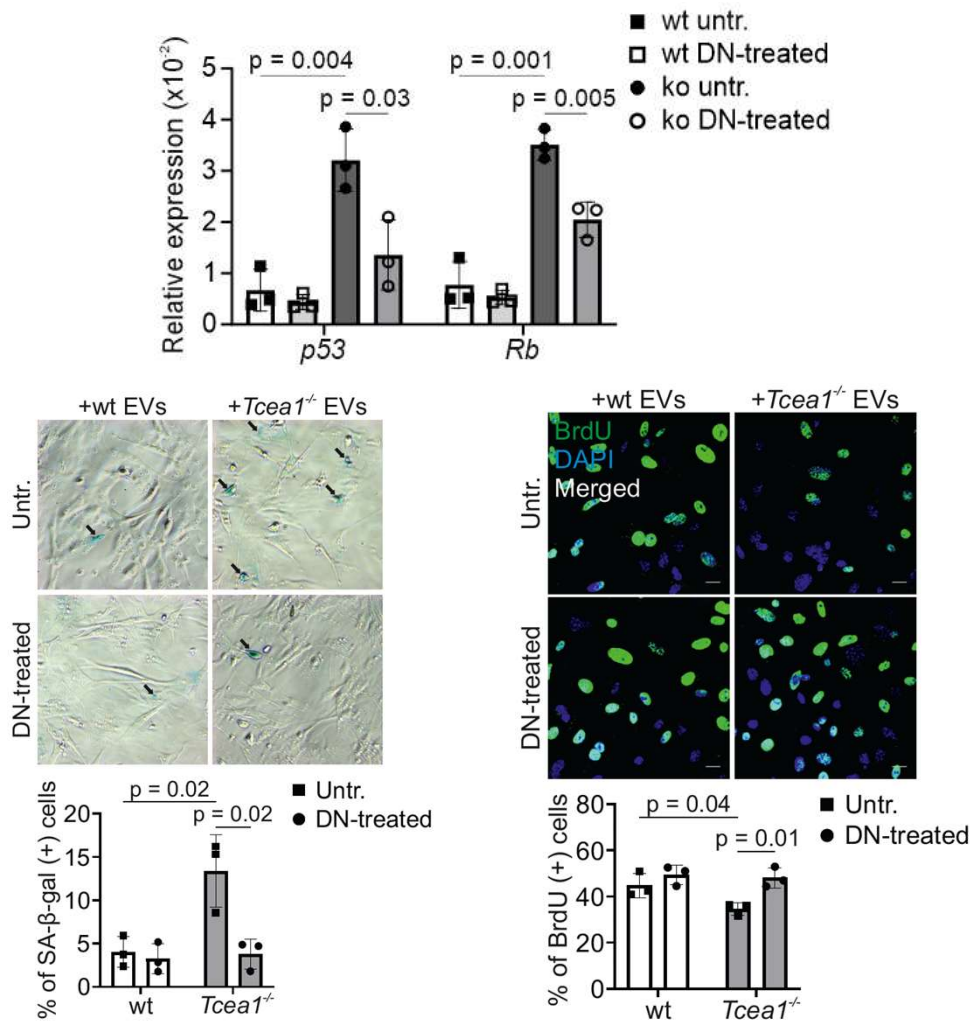


Tcea1^{-/-} MEFs secrete Tel-loaded EVs that trigger inflammation & “bystander” senescence

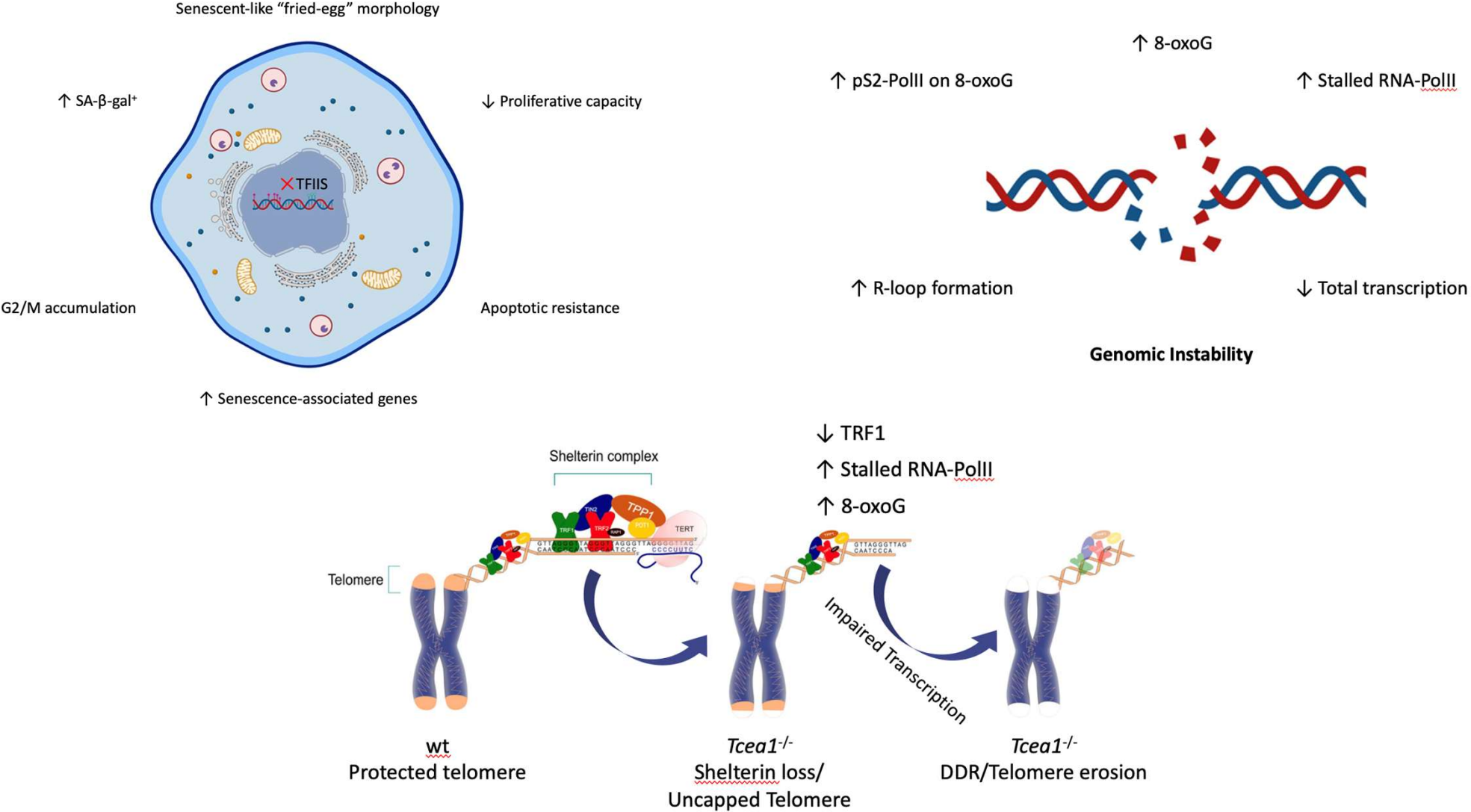
Inflammation



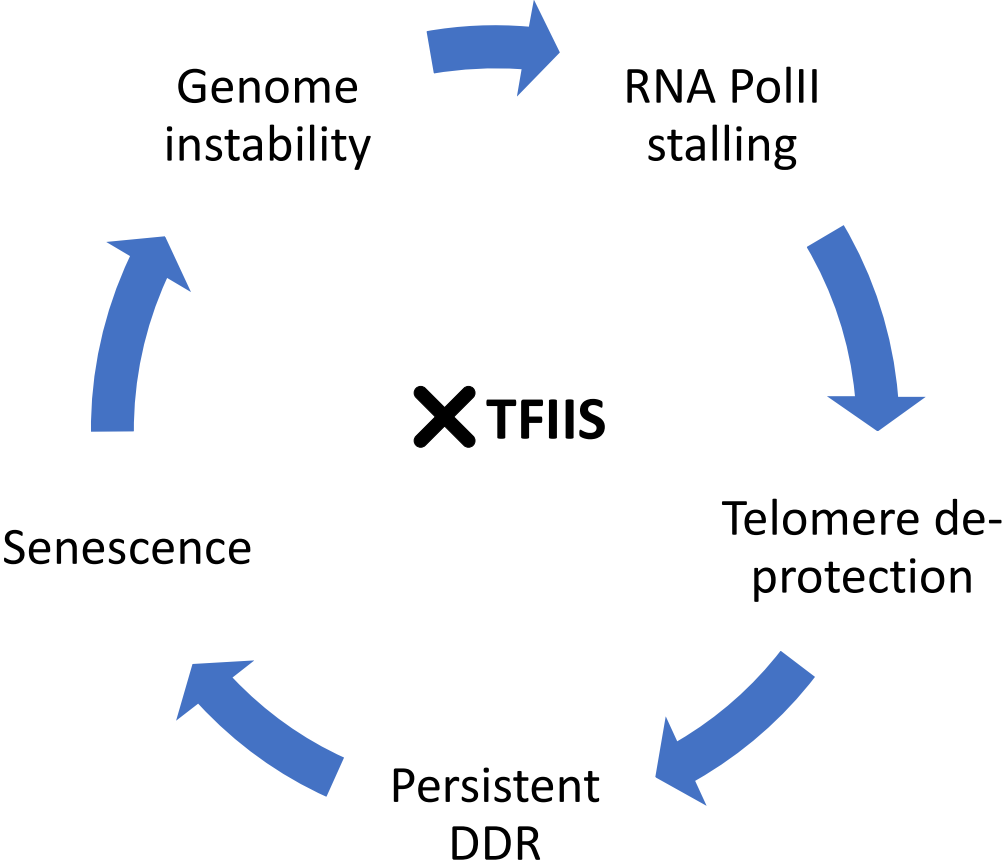
Senescence



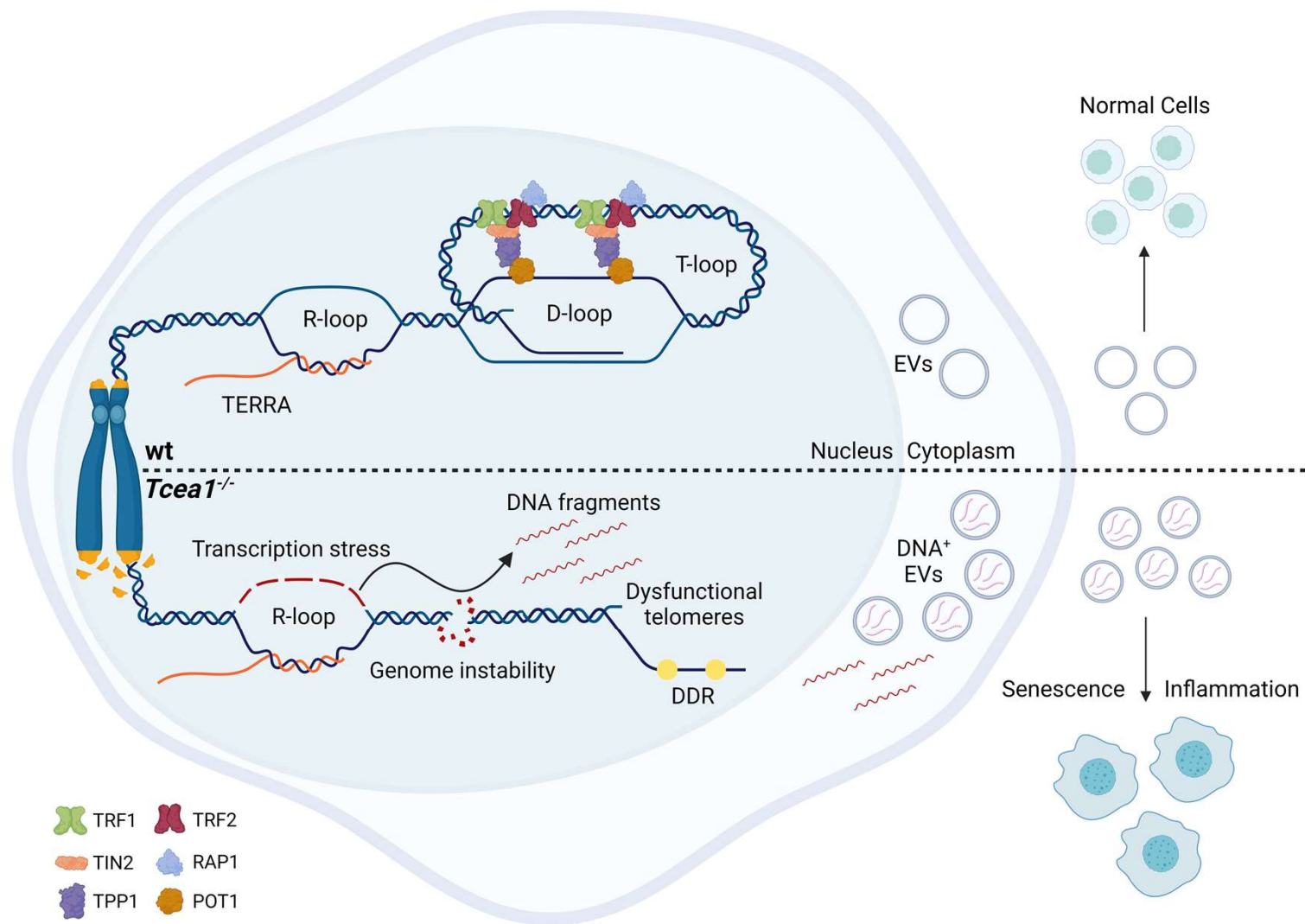
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Transcription stress associated DNA damage induces telomere dysfunction & cellular senescence



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- **Despoina Giamaki**

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- **Björn Schumacher**



- **Brian Luke**



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