

# A tale of two genomes:

*DNA repair in the mitochondria and the nucleus*

Skirball Institute of  
Biomolecular Medicine

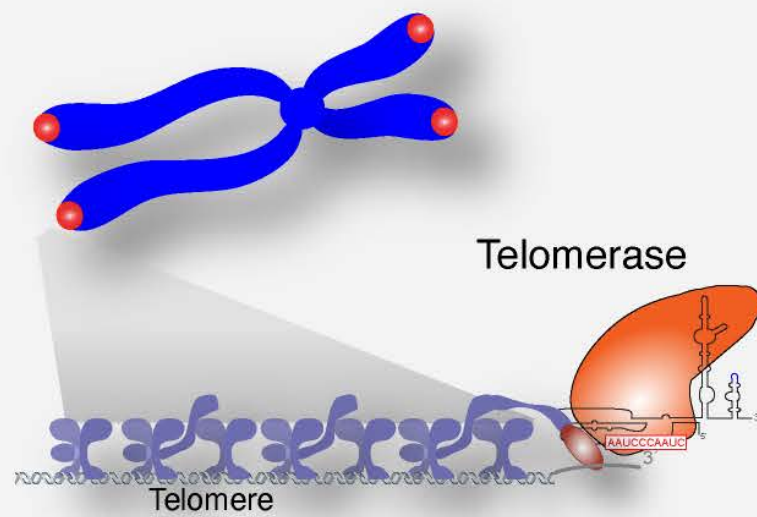


Memorial Sloan Kettering  
Cancer Center

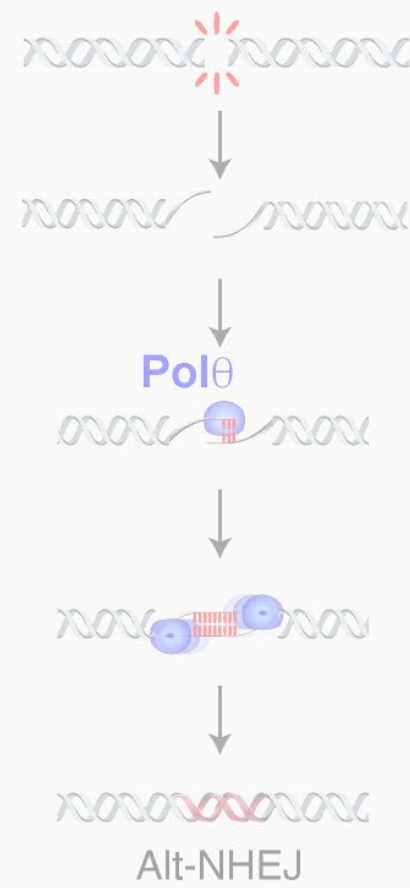
Agnel Sfeir  
June 8, 2021



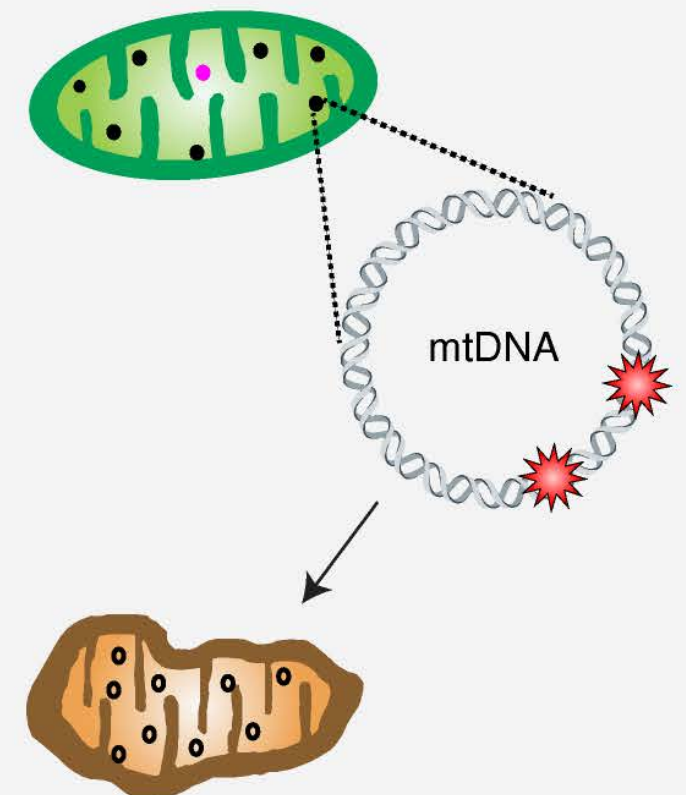
## Telomere maintenance



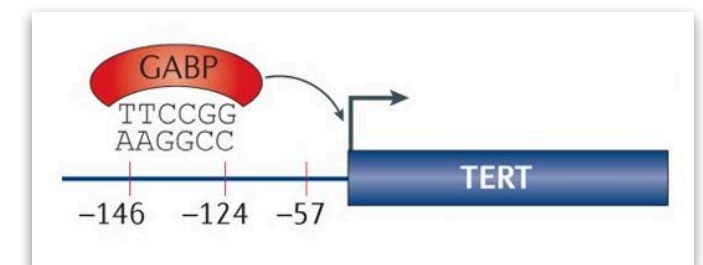
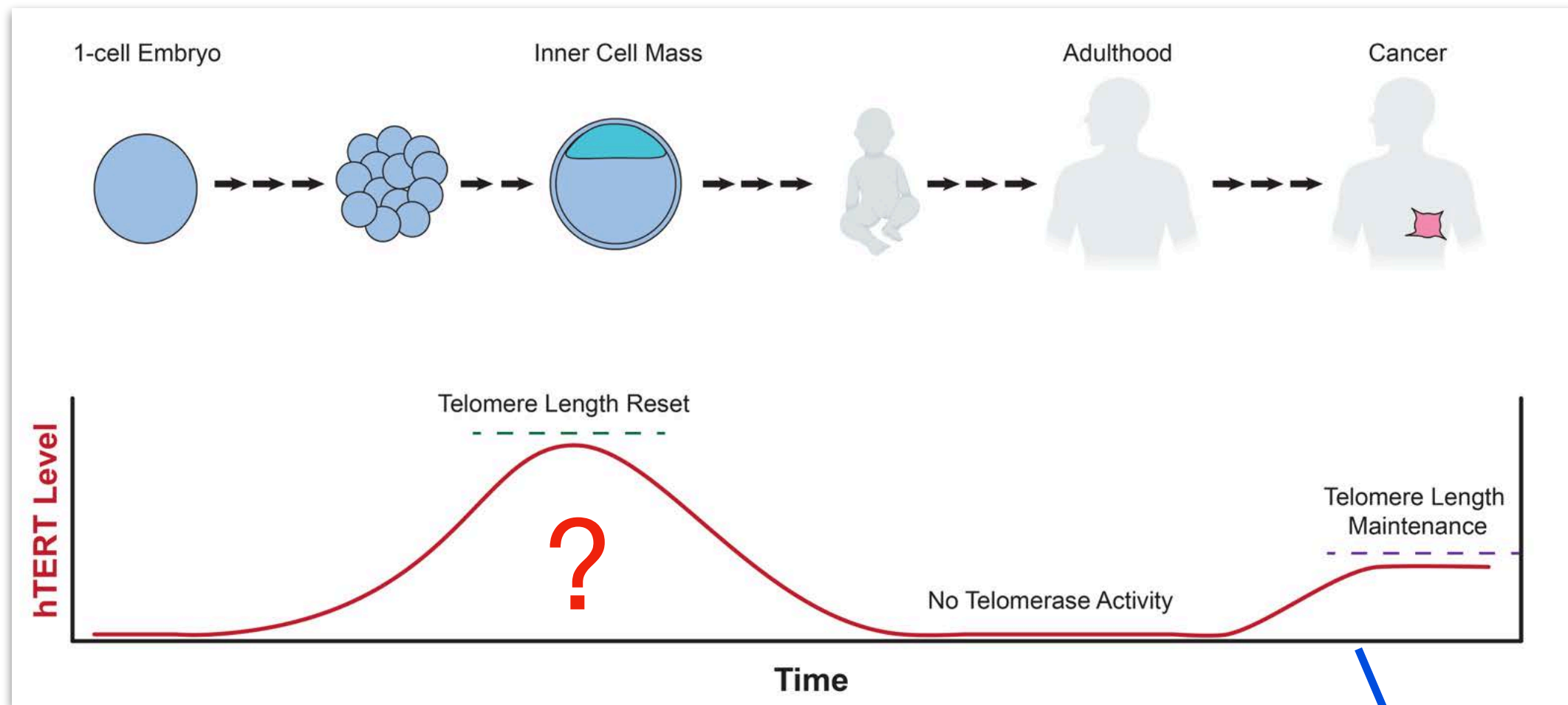
## DNA repair



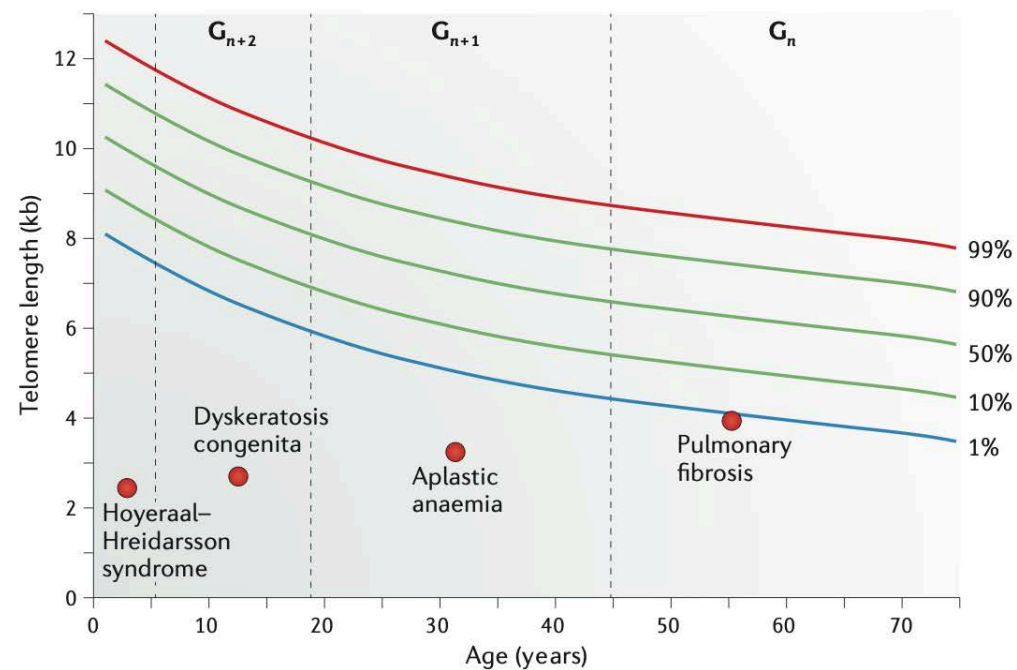
## Mitochondrial genome instability



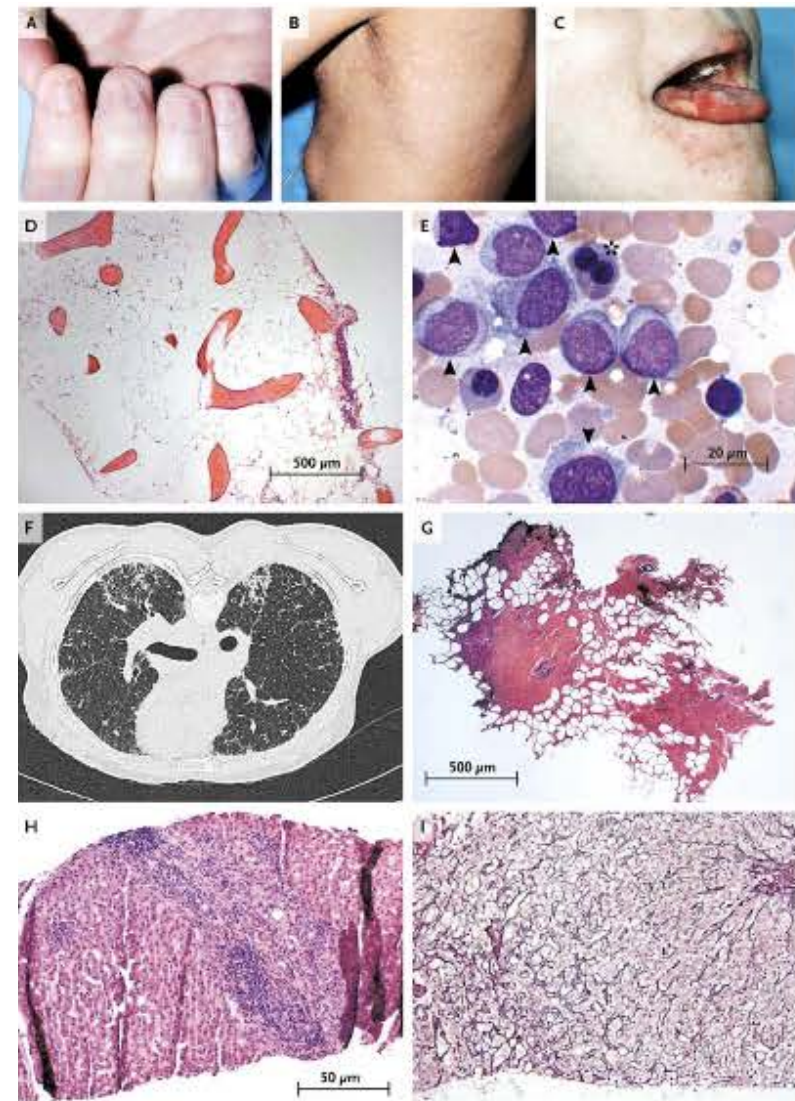
# hTERT expression is tightly regulated during human development



# Insufficient telomerase leads to human disease

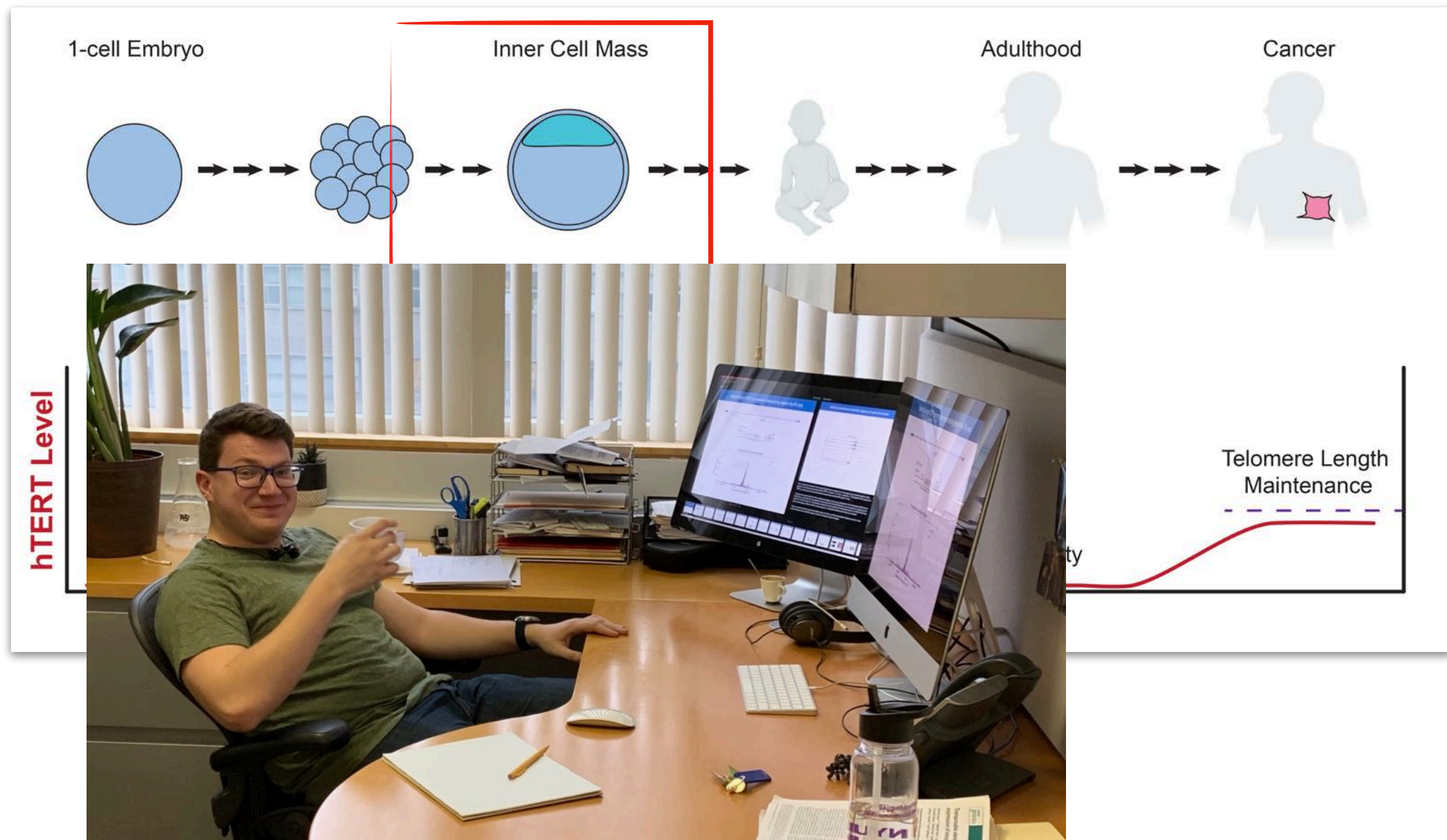


- Associated with mutations in telomerase pathway genes: DKC1, TERT, hTR, etc



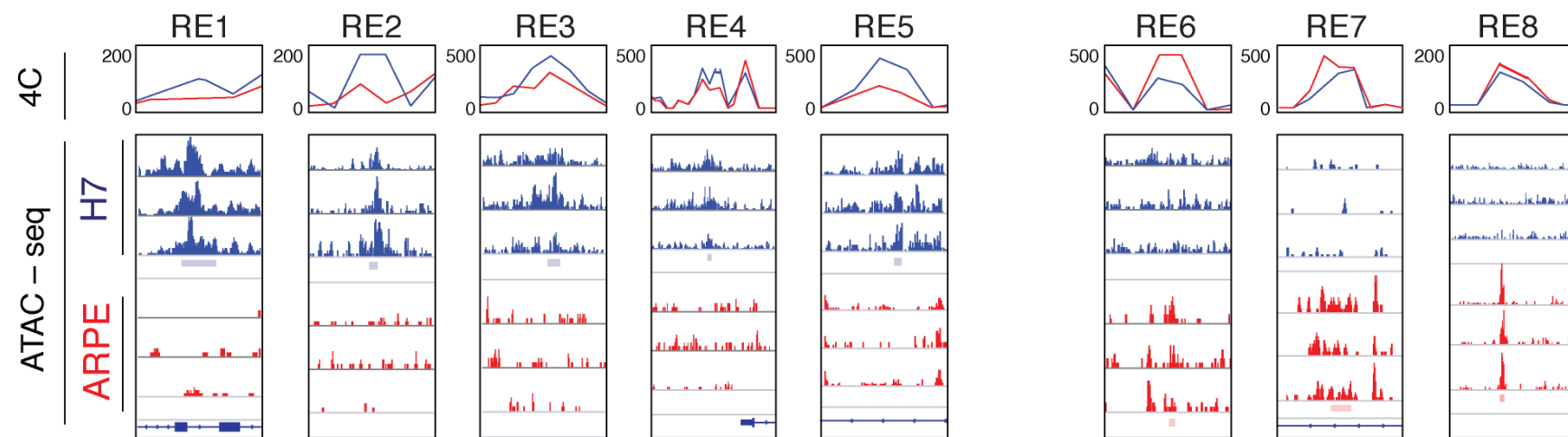


# Studying hTERT regulation as a function of pluripotency

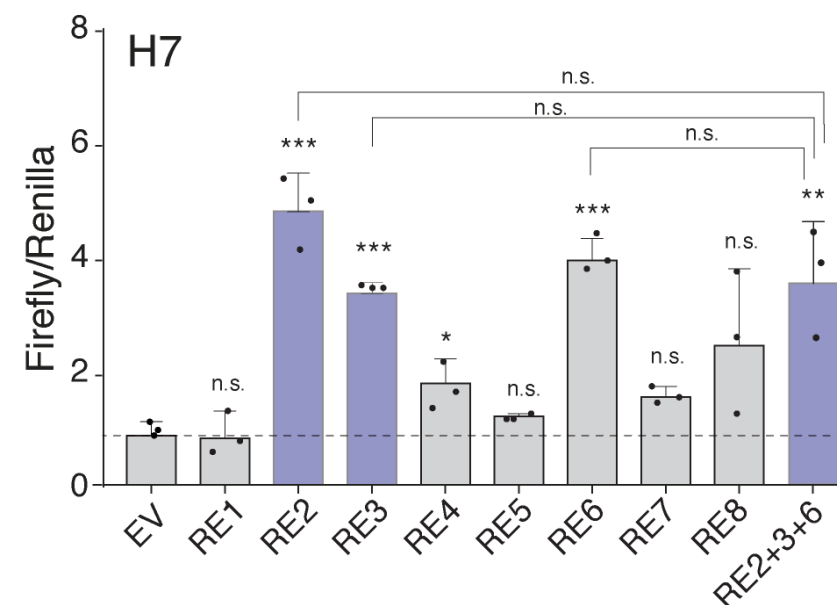


Alex Penev

# Mapping the landscape of hTERT enhancers

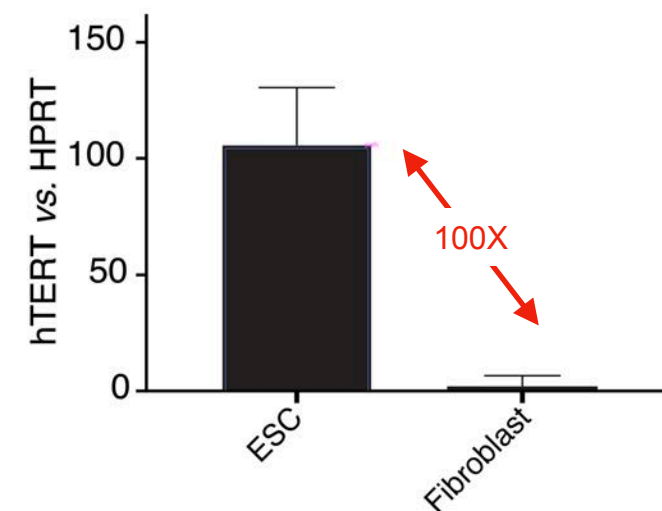
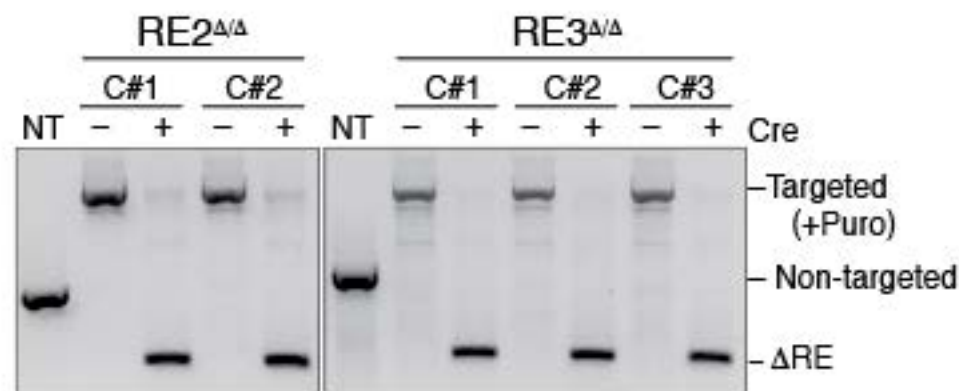
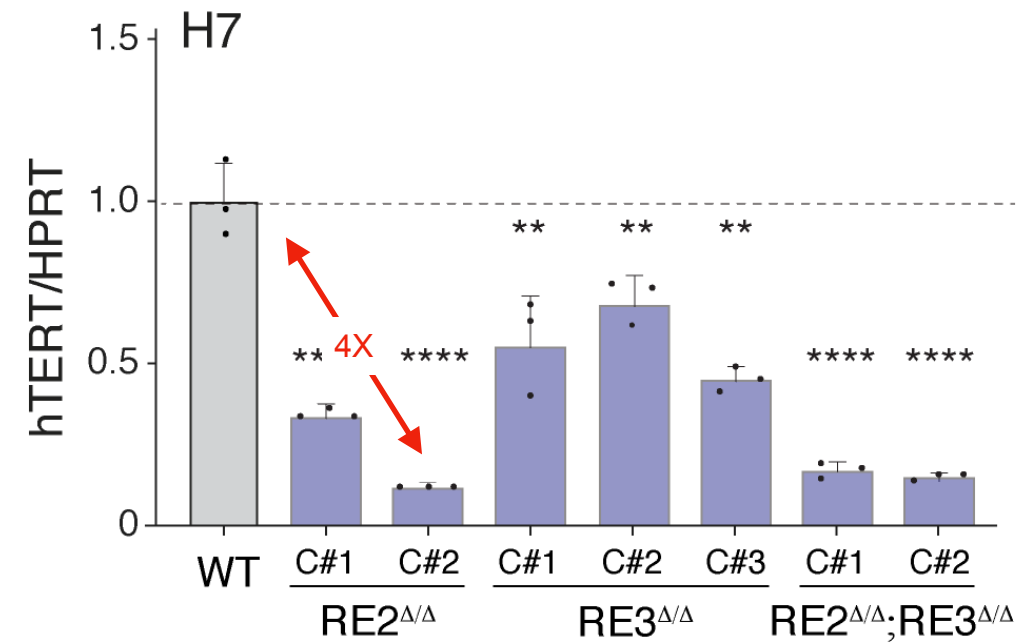
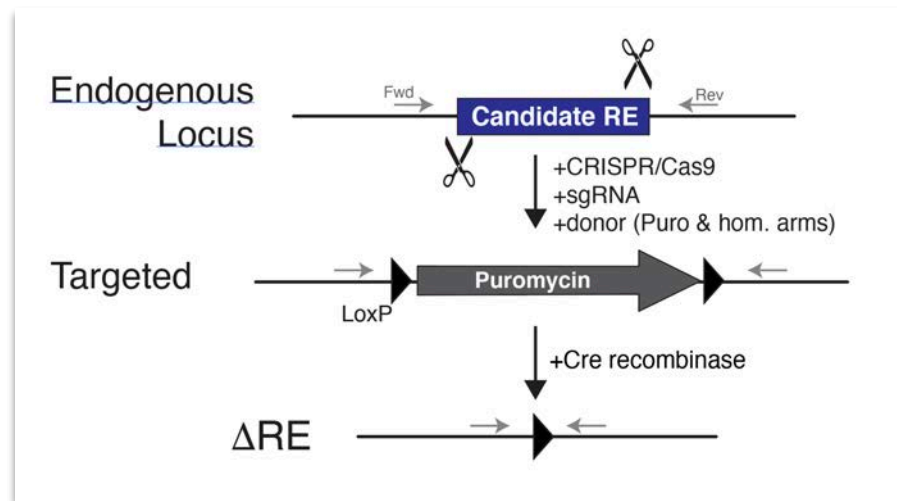


H7= ES cells  
ARPE= Epithelial cells

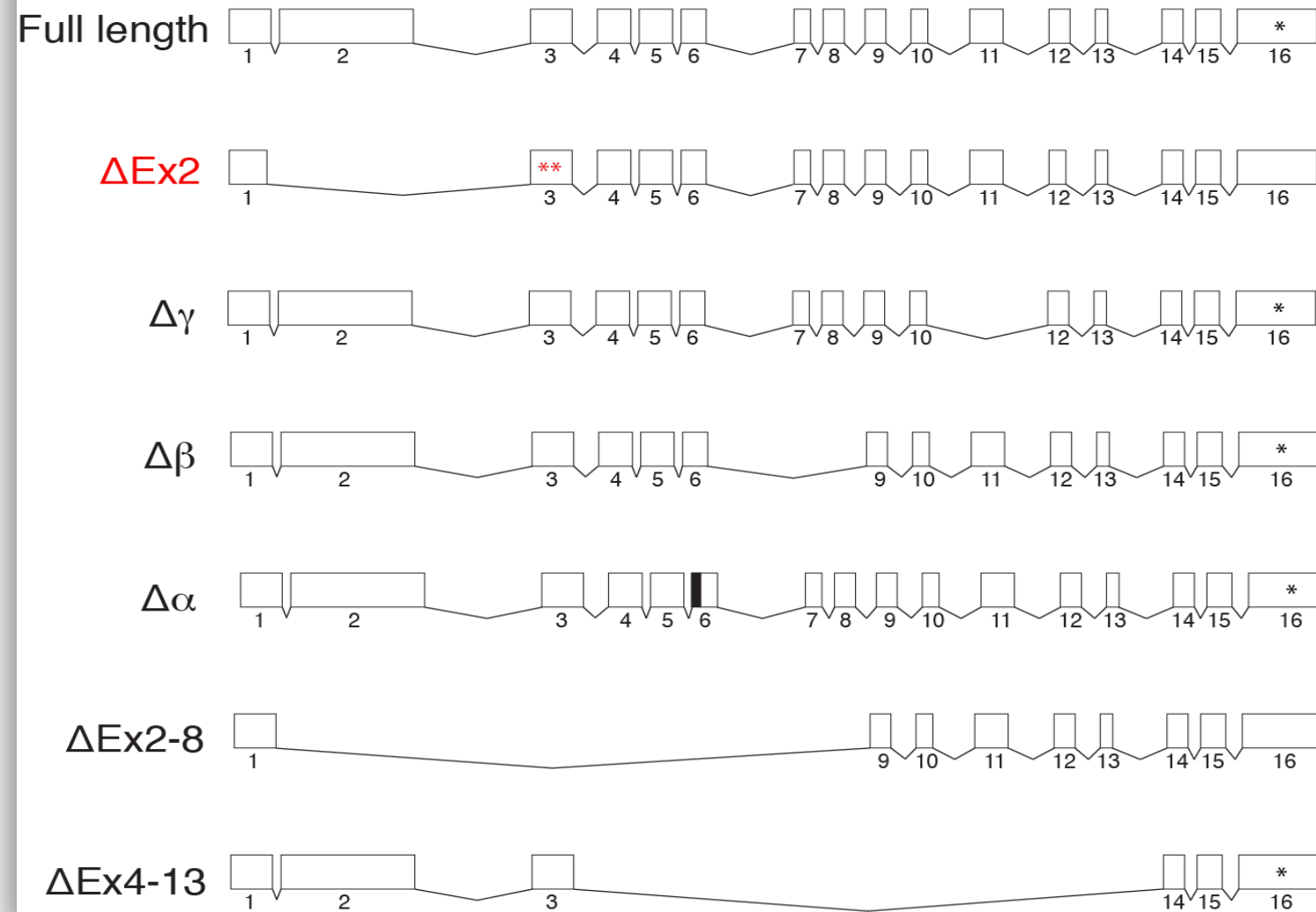




# Deletion of putative enhancers reduces hTERT mRNA



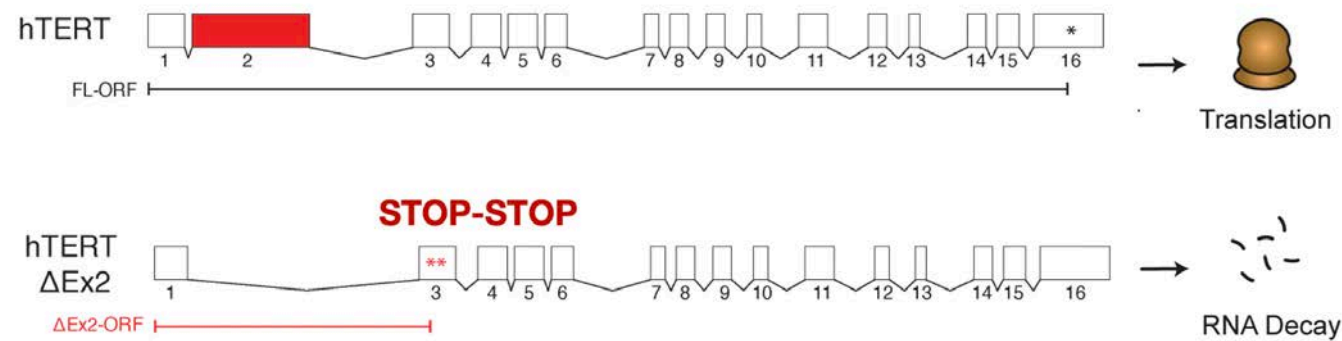
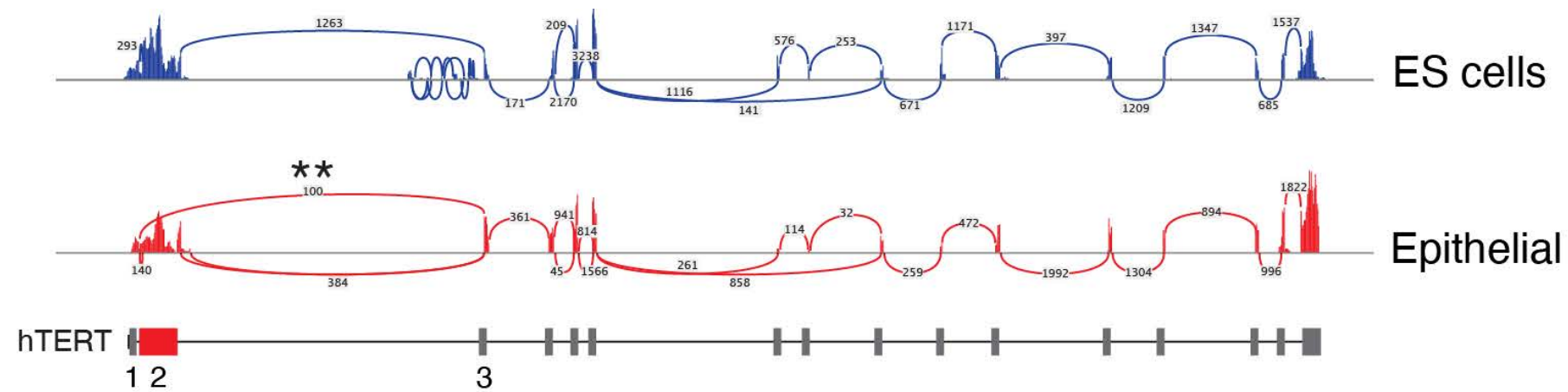
# hTERT mRNA is extensively alternatively spliced



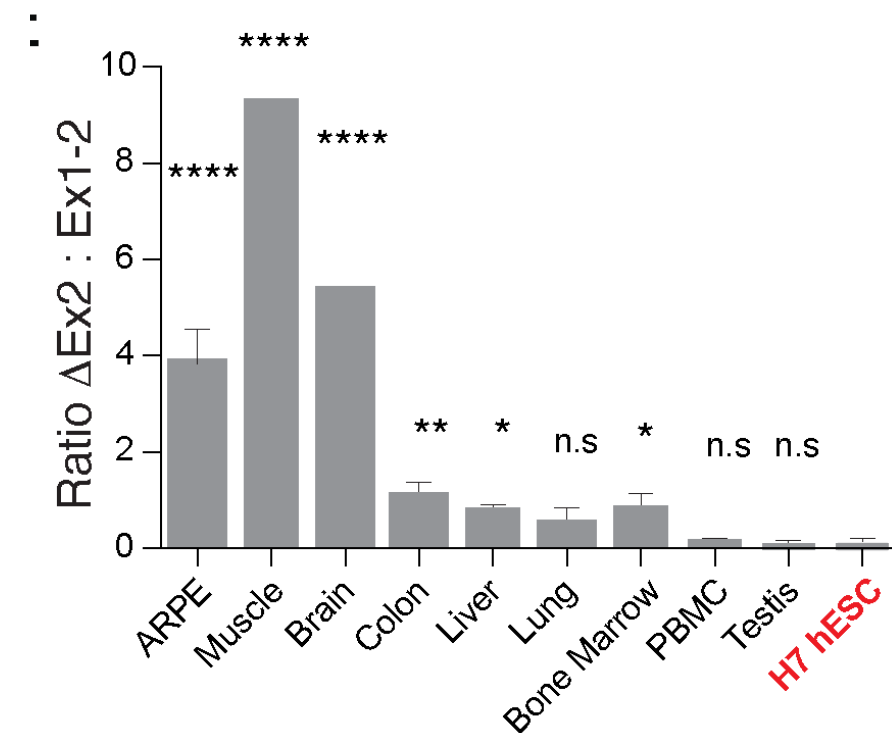
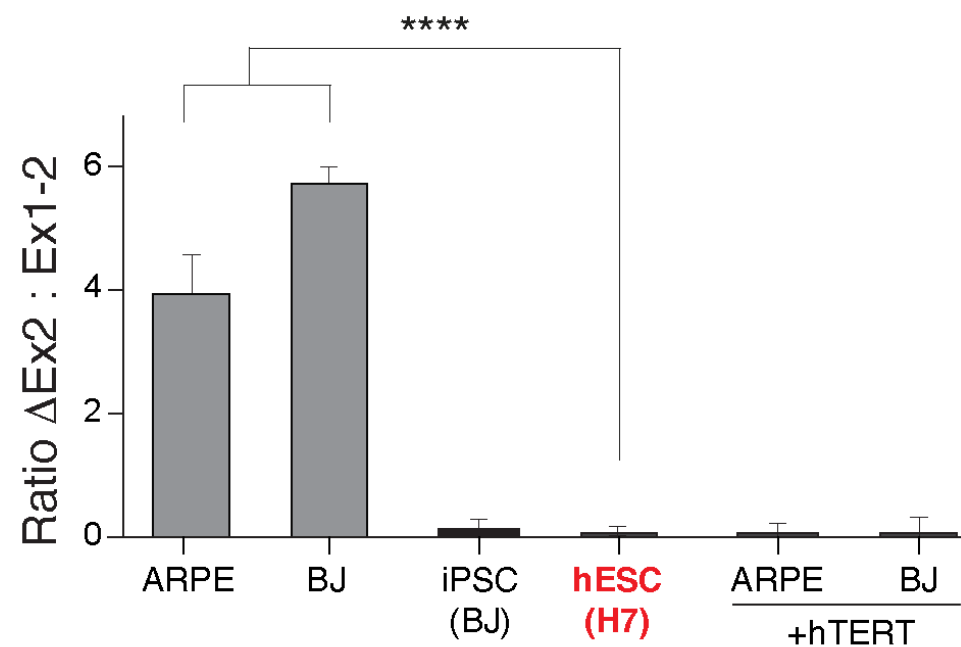
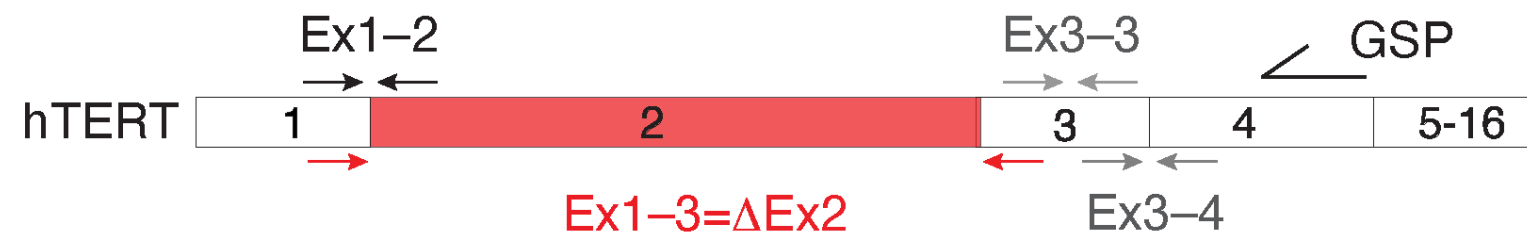


# Capture-Seq identifies the full spectrum of hTERT splice variants

## RNA Capture-seq

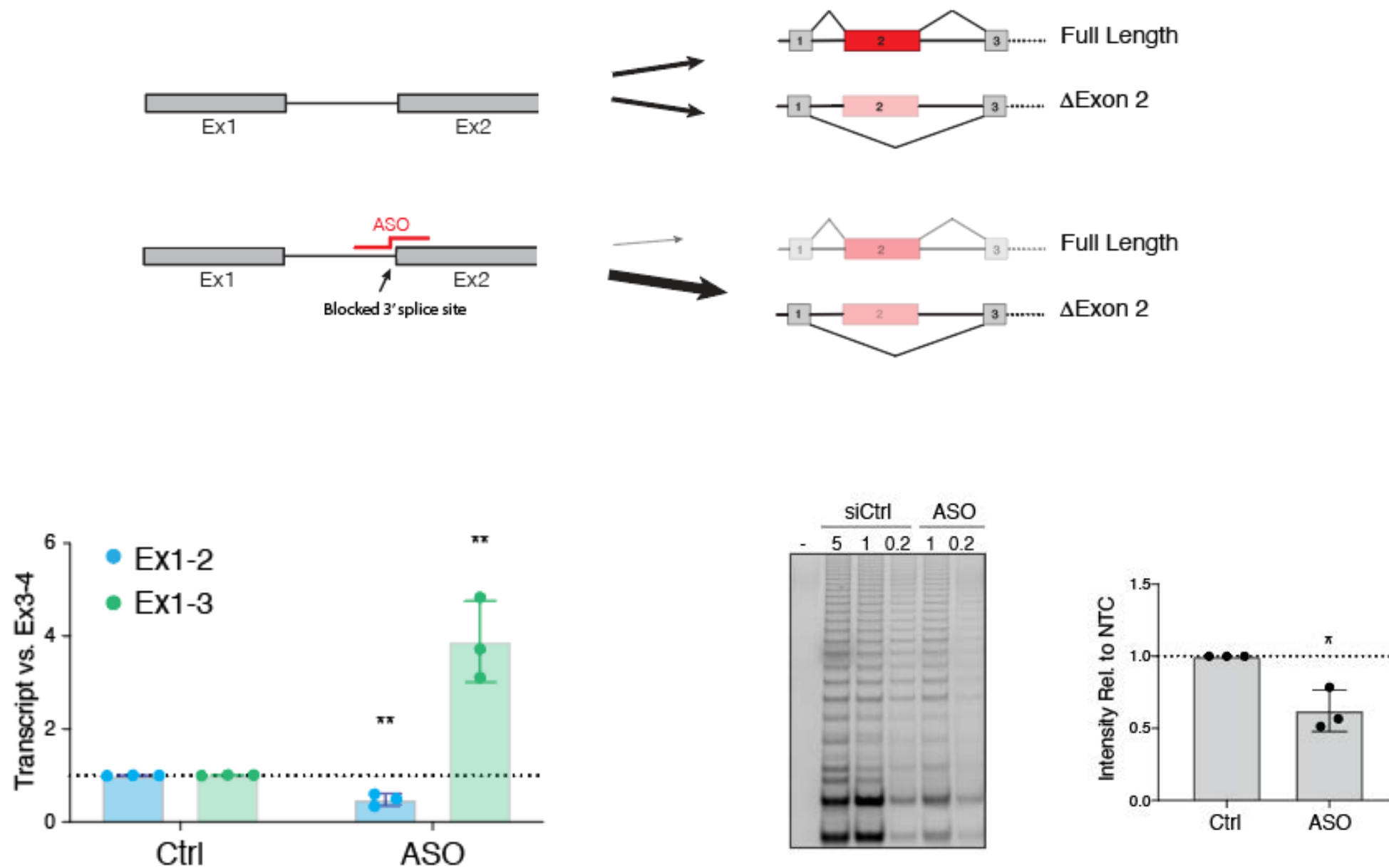


# hTERT exon-2 is differentially spliced in pluripotent vs. differentiated cells

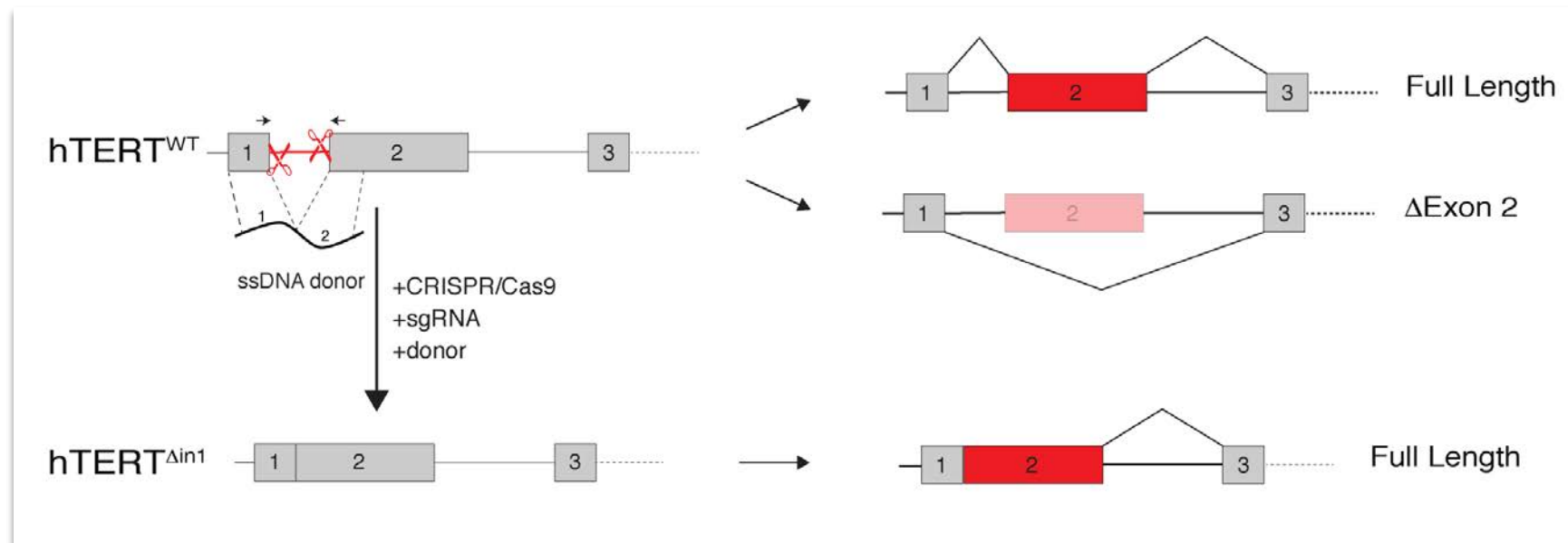




# hTERT exon-2 exclusion compromises telomerase activity in pluripotent cells

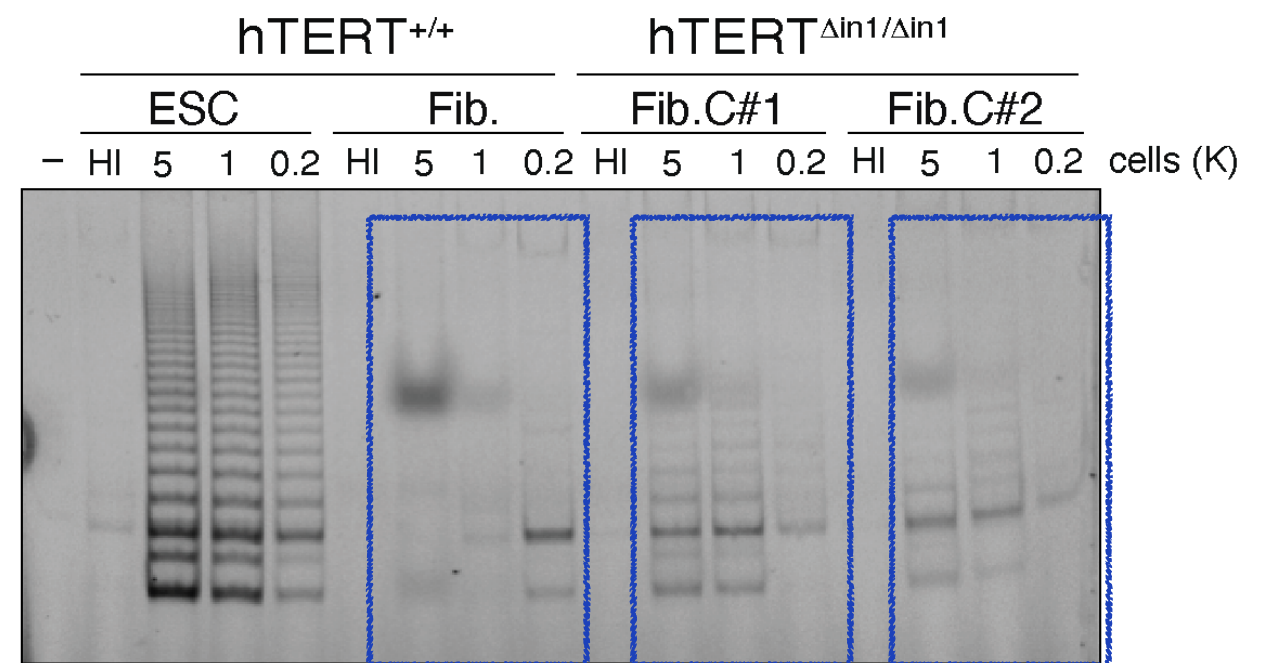
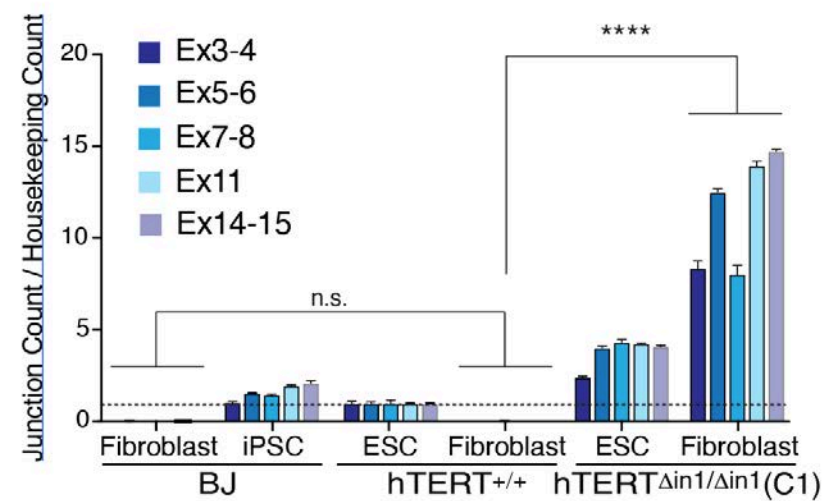
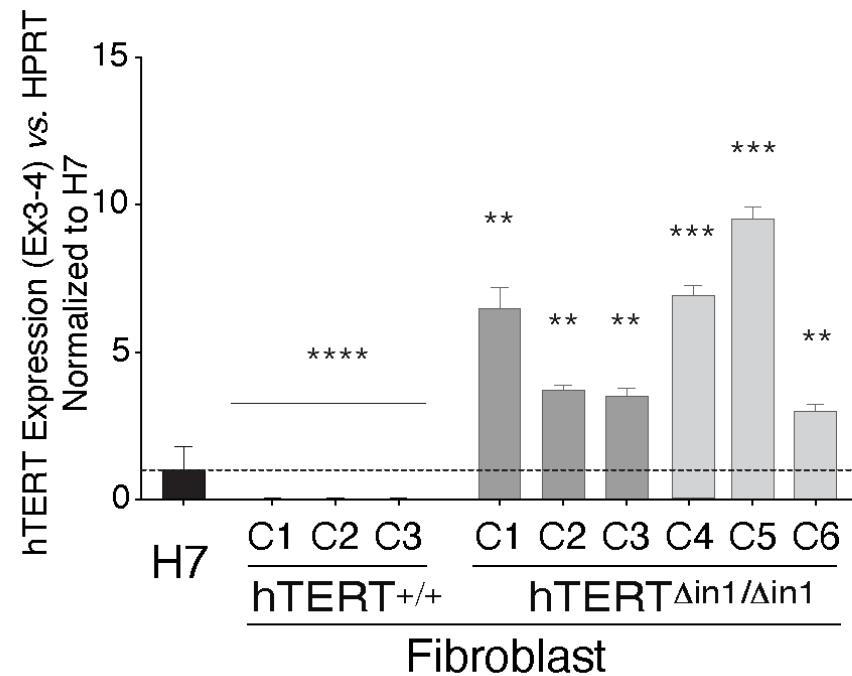


# Constitutive exon-2 retention by gene editing



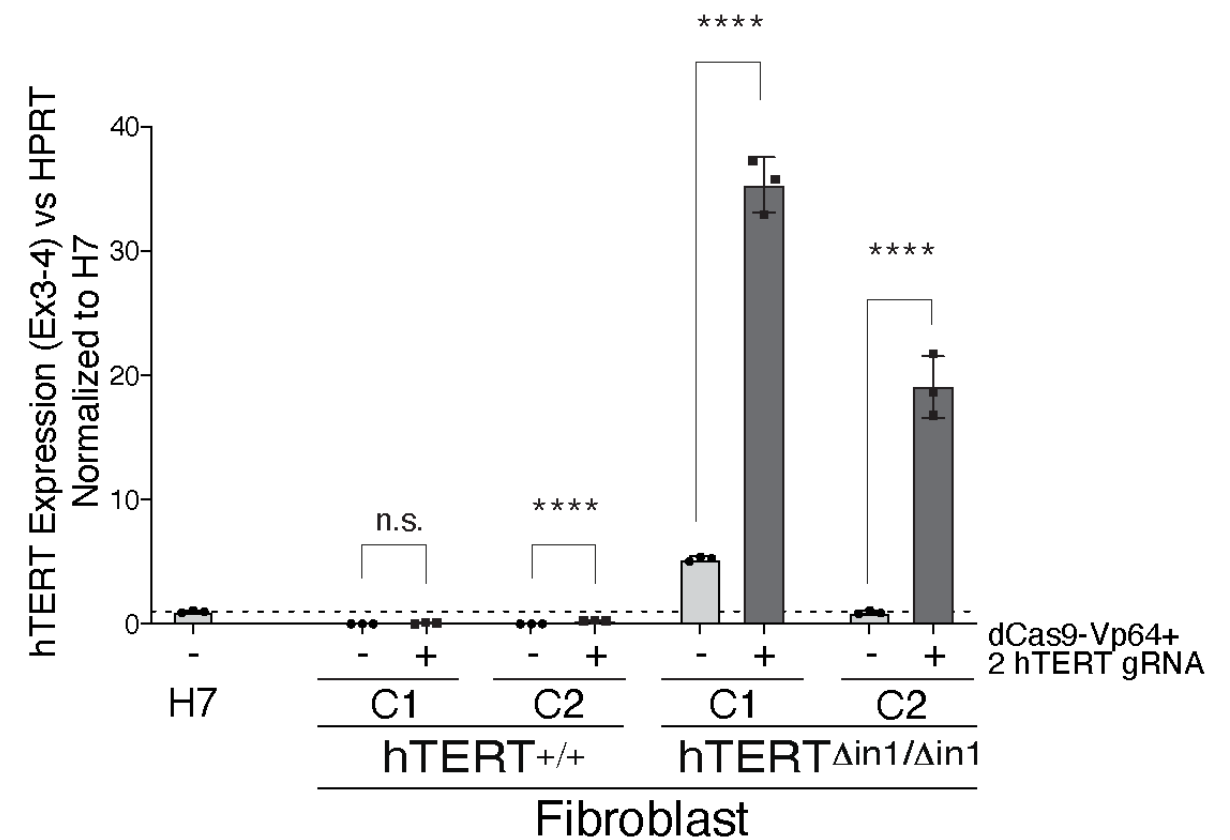
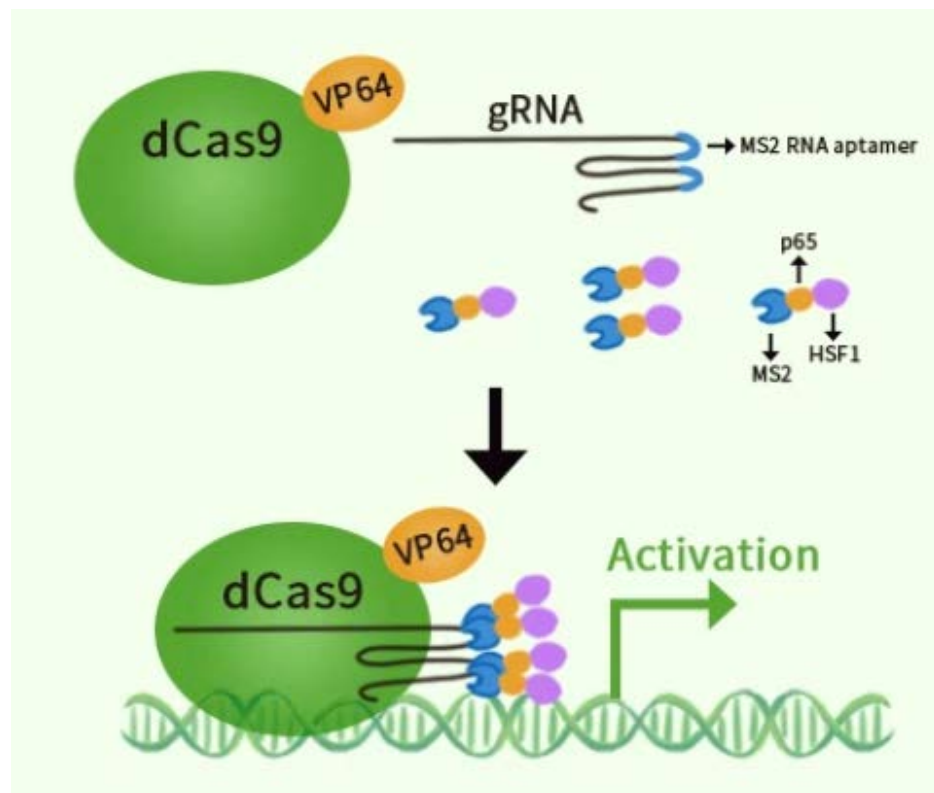


# Forced exon-2 inclusion prevents hTERT silencing during differentiation

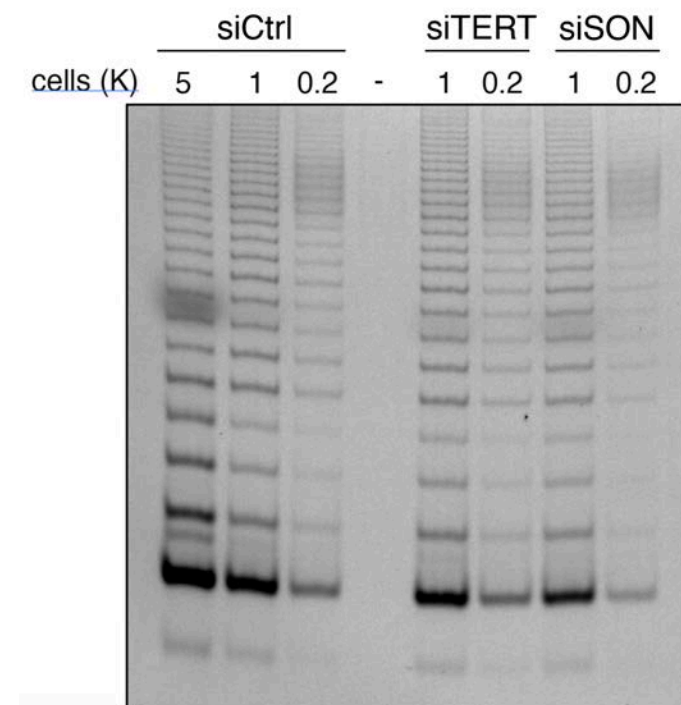
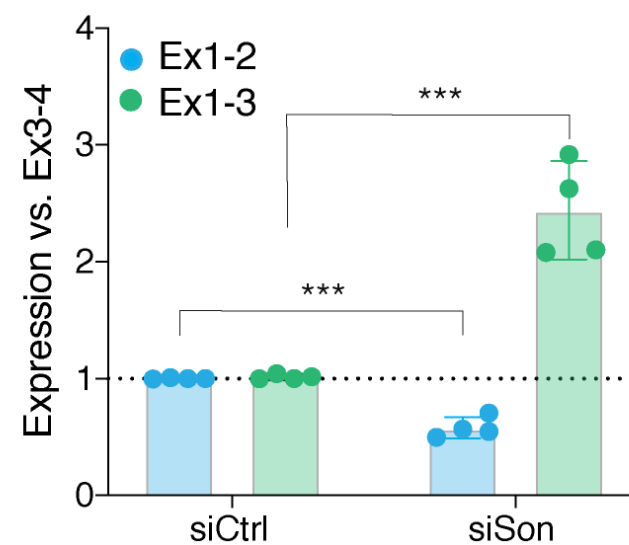
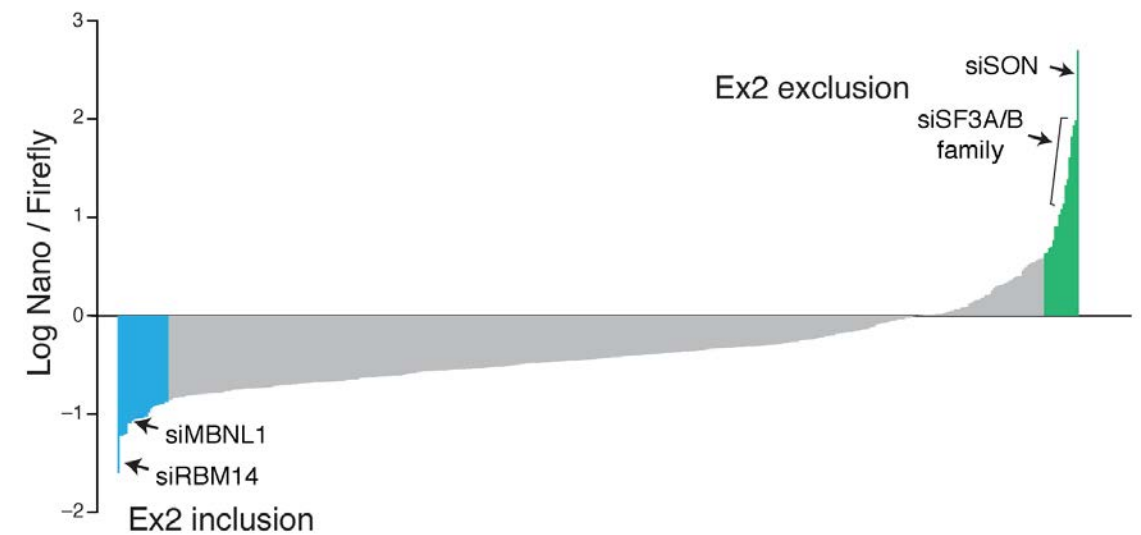
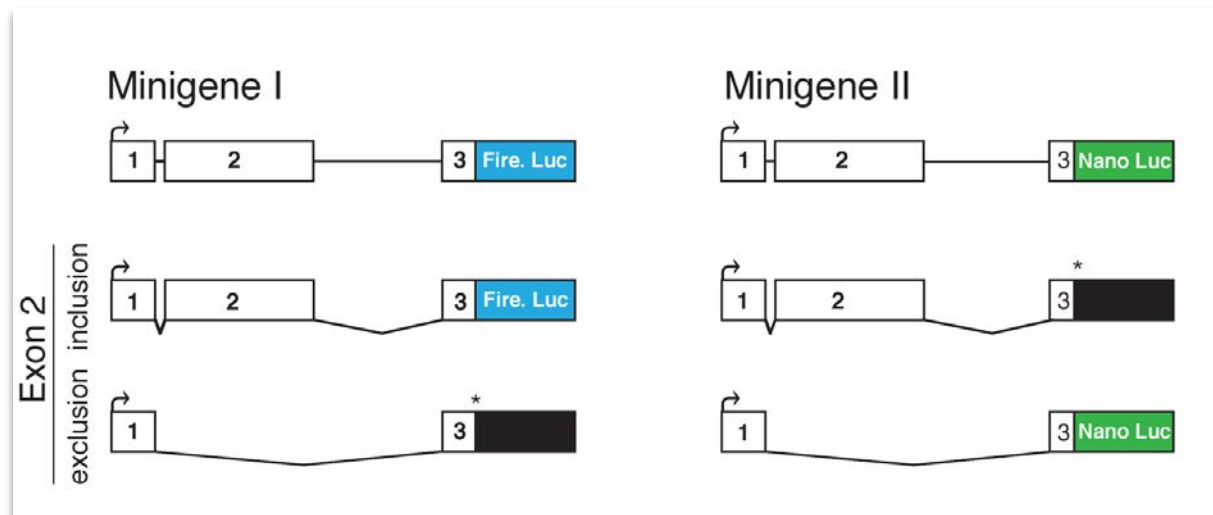


No enhancer element in intron 1  
Scrambling intron (keeping junctions) rescues phenotype

# Transcriptional and post-transcriptional processes synergize to regulate hTERT



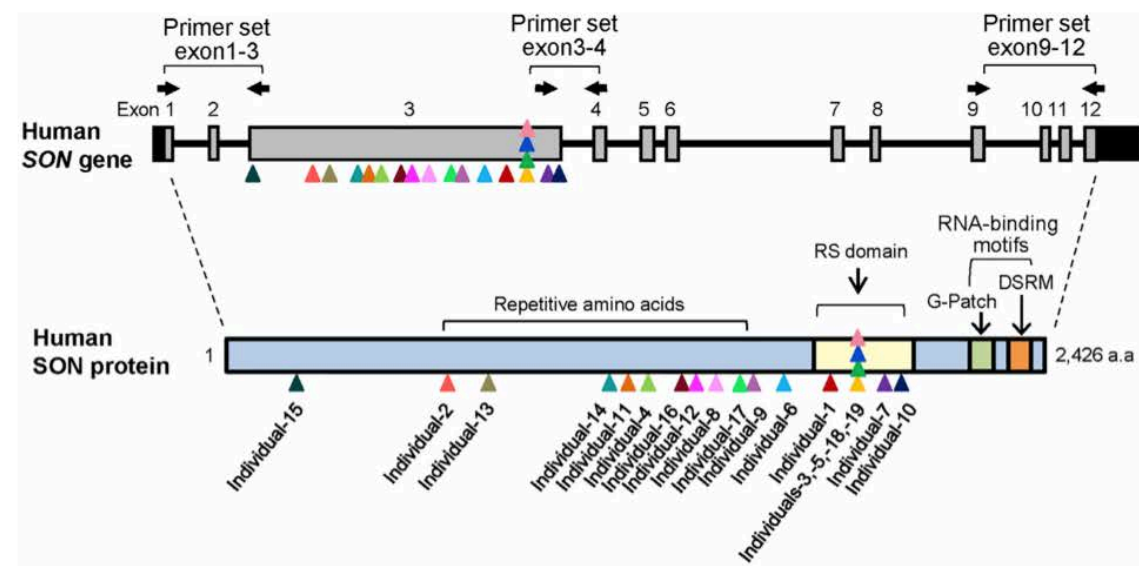
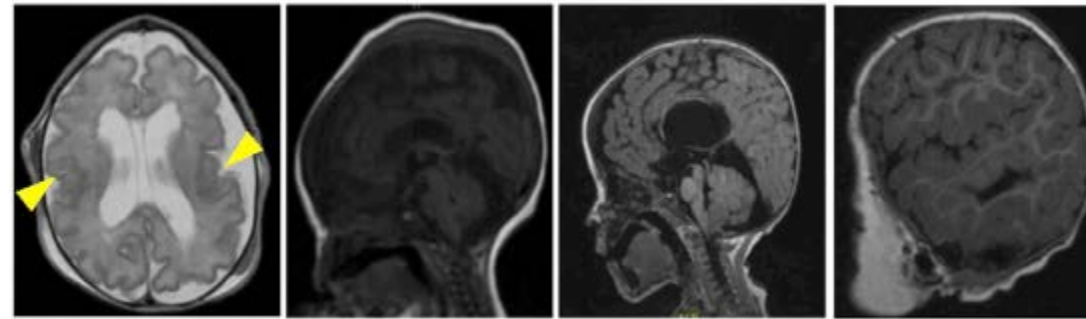
# Identification of hTERT splicing regulators using minigene reporters



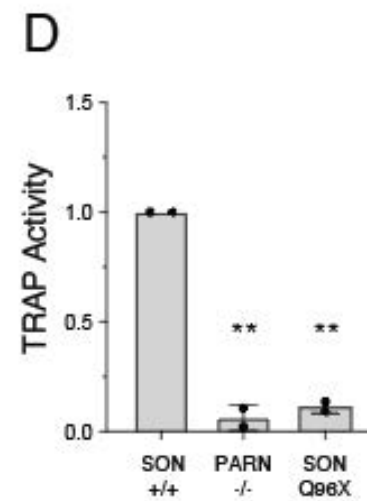
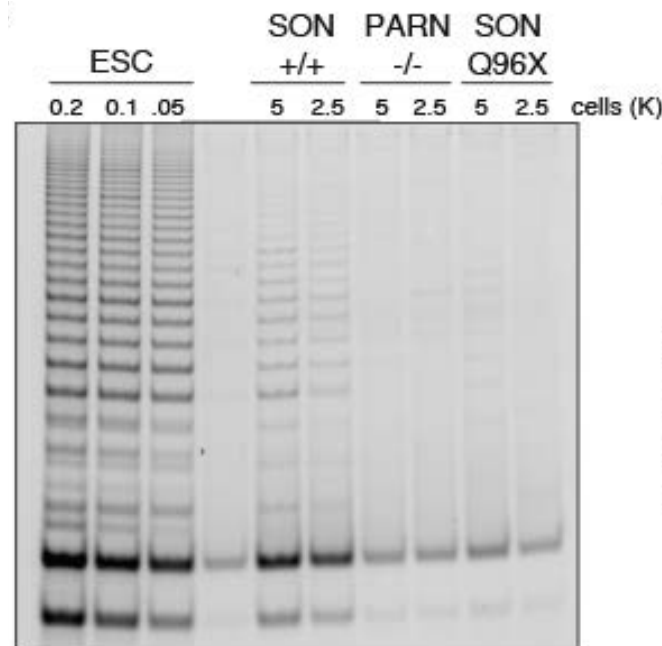
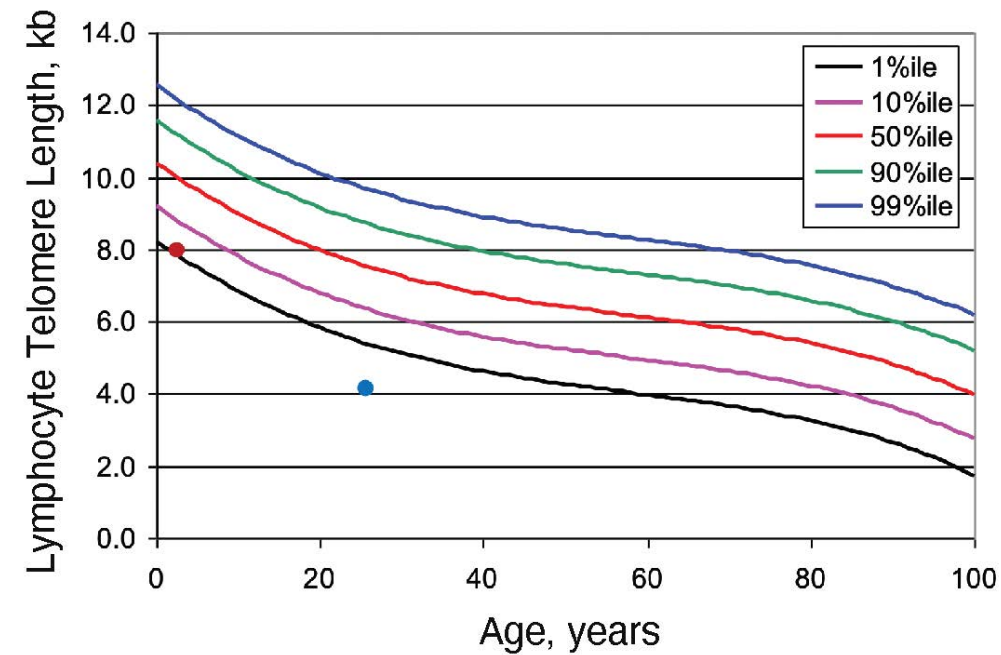
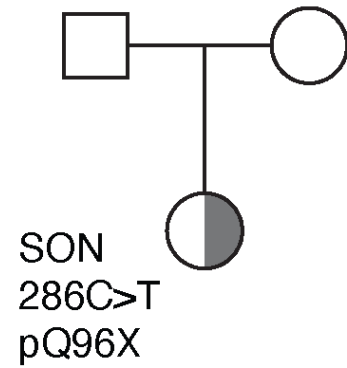


# De Novo Mutations in *SON* Disrupt RNA Splicing of Genes Essential for Brain Development and Metabolism, Causing an Intellectual-Disability Syndrome

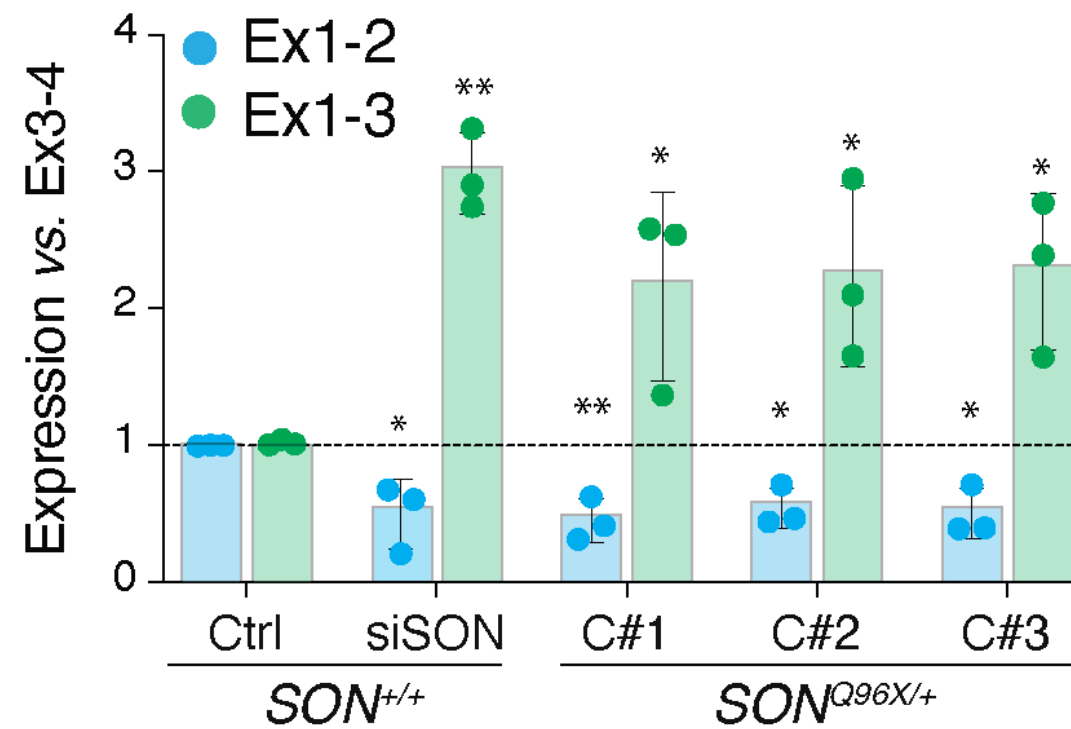
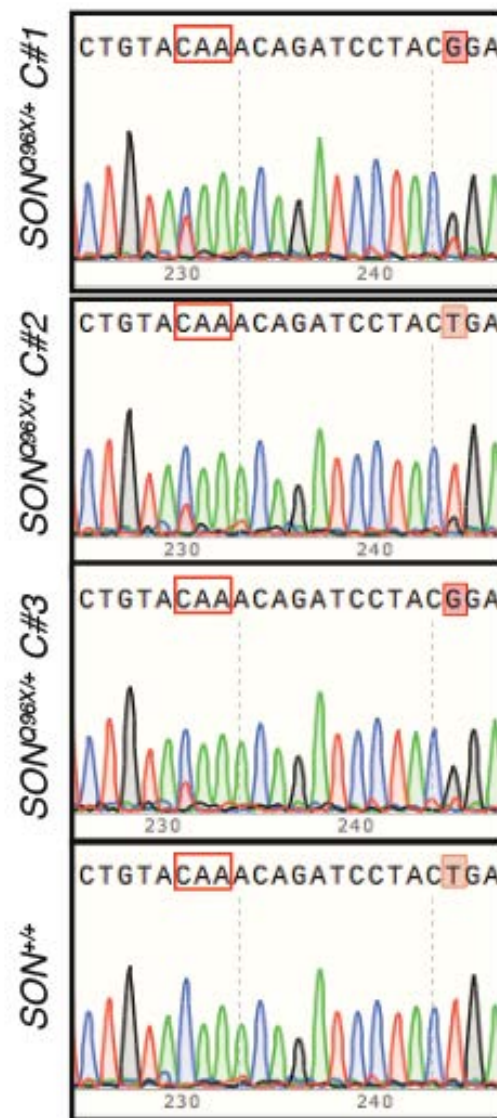
The American Journal of Human Genetics 99, 711–719, September 1, 2016 711



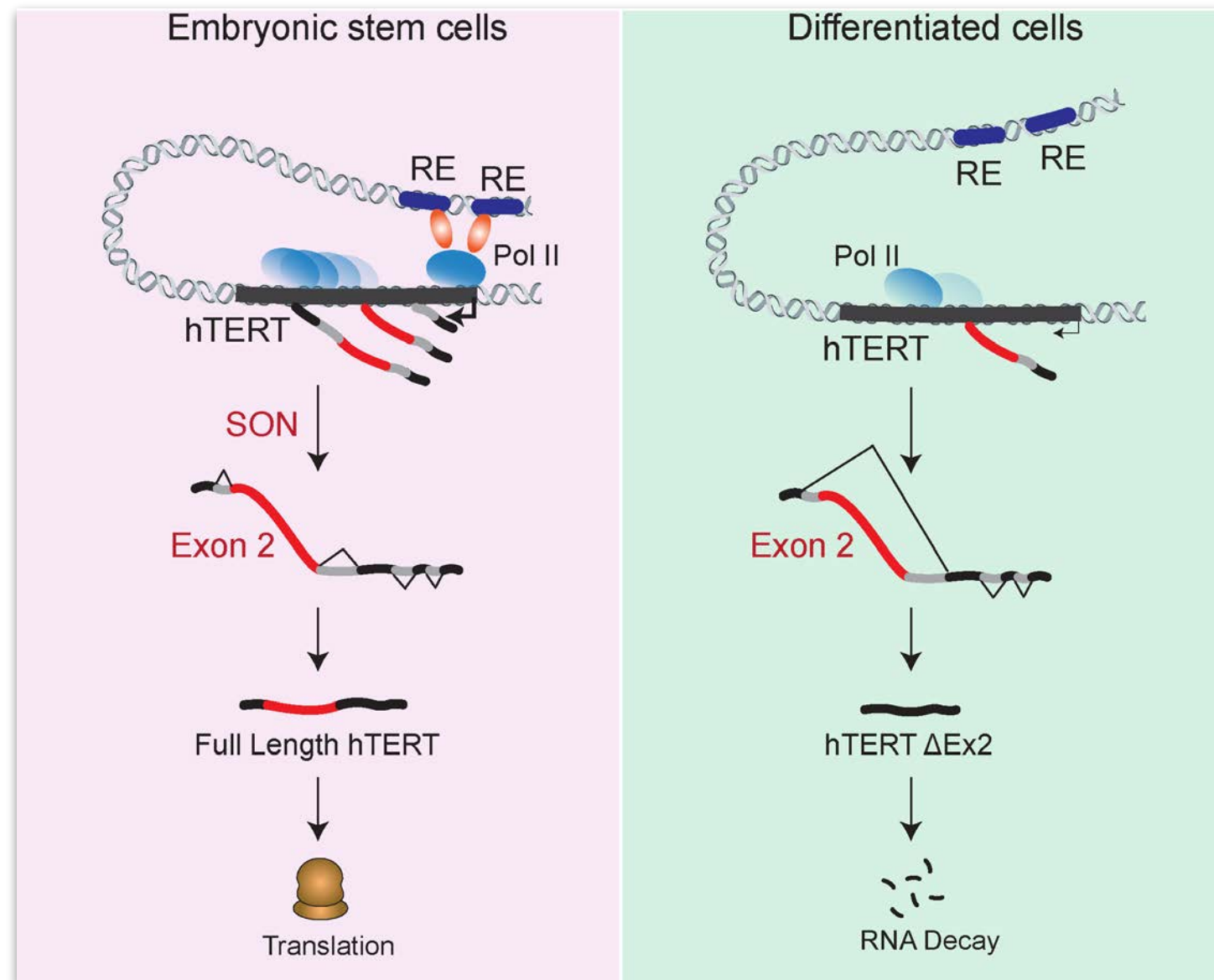
# Mutations in SON are linked to hTERT haploinsufficiency



# Mutations in SON are linked to hTERT haploinsufficiency

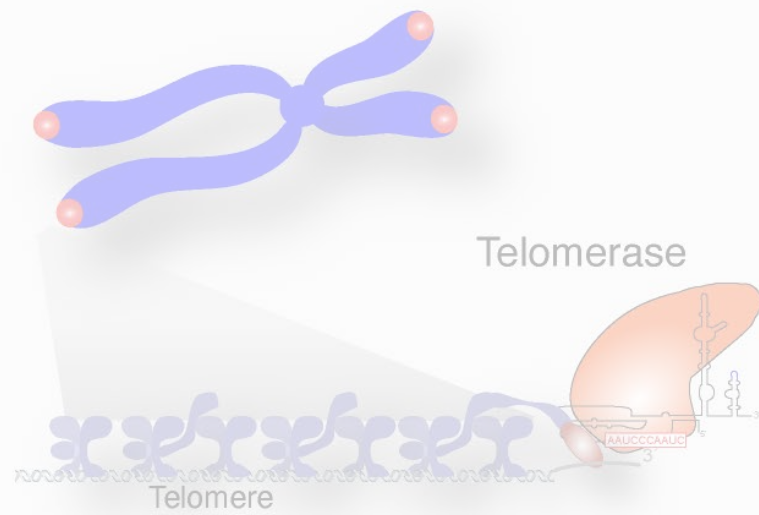


# Alternative splicing is a developmental switch for hTERT expression

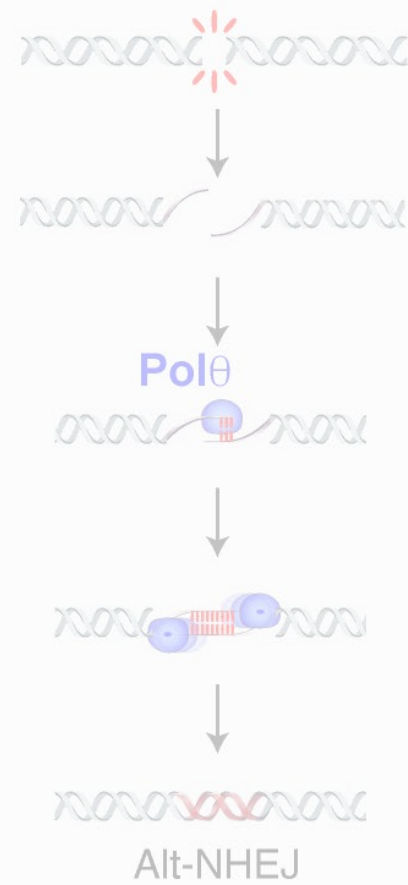




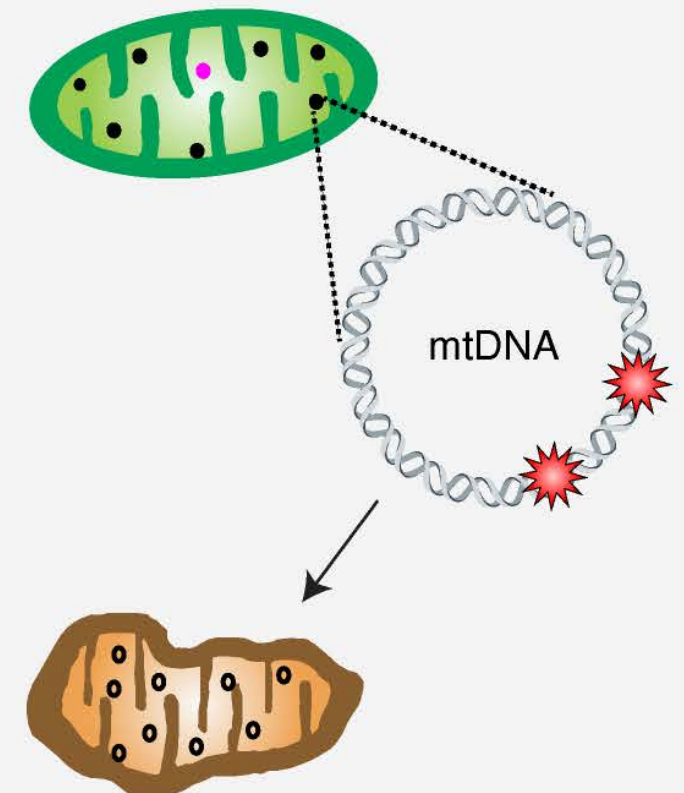
## Telomere maintenance



## DNA repair

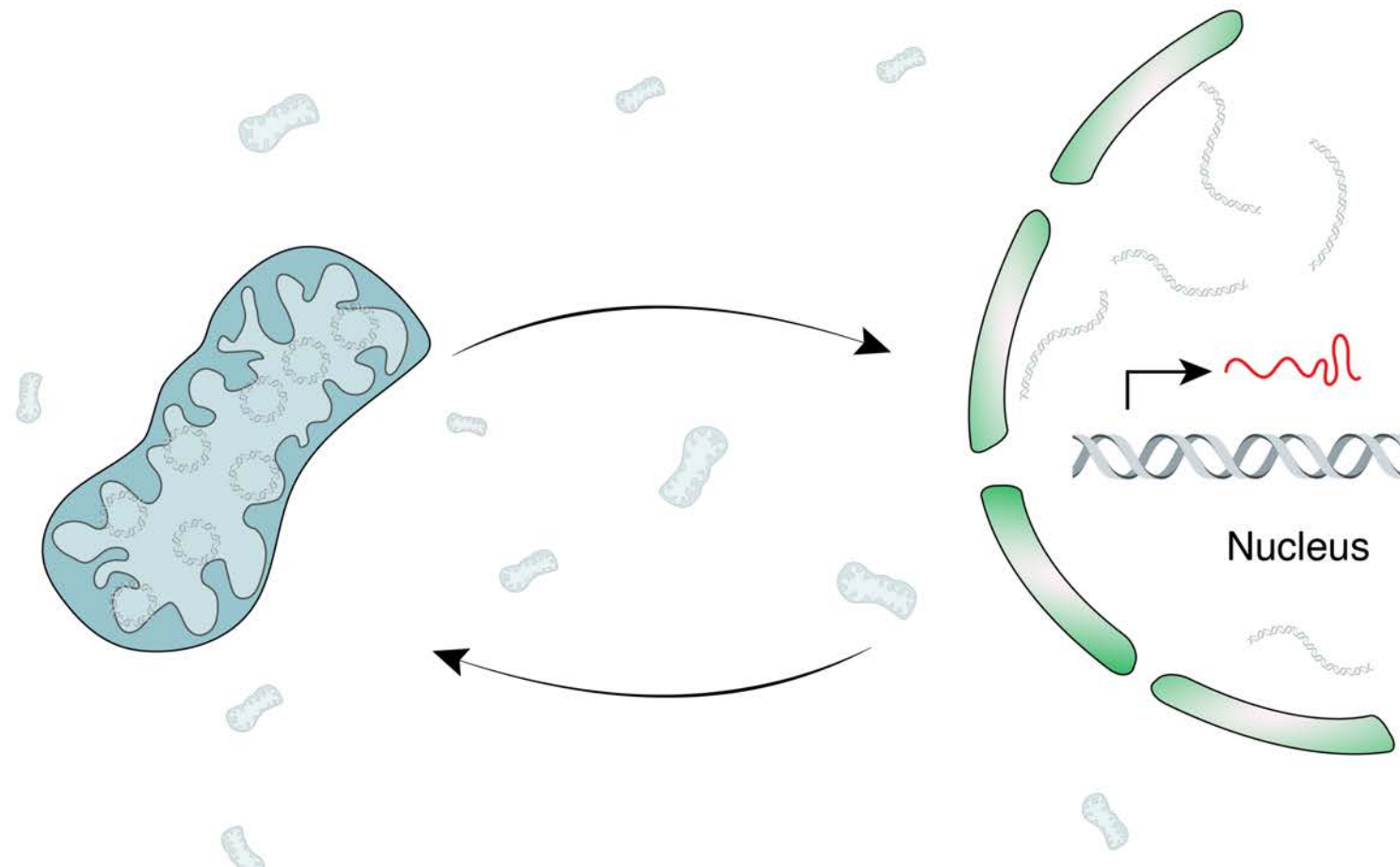


## Mitochondrial genome instability

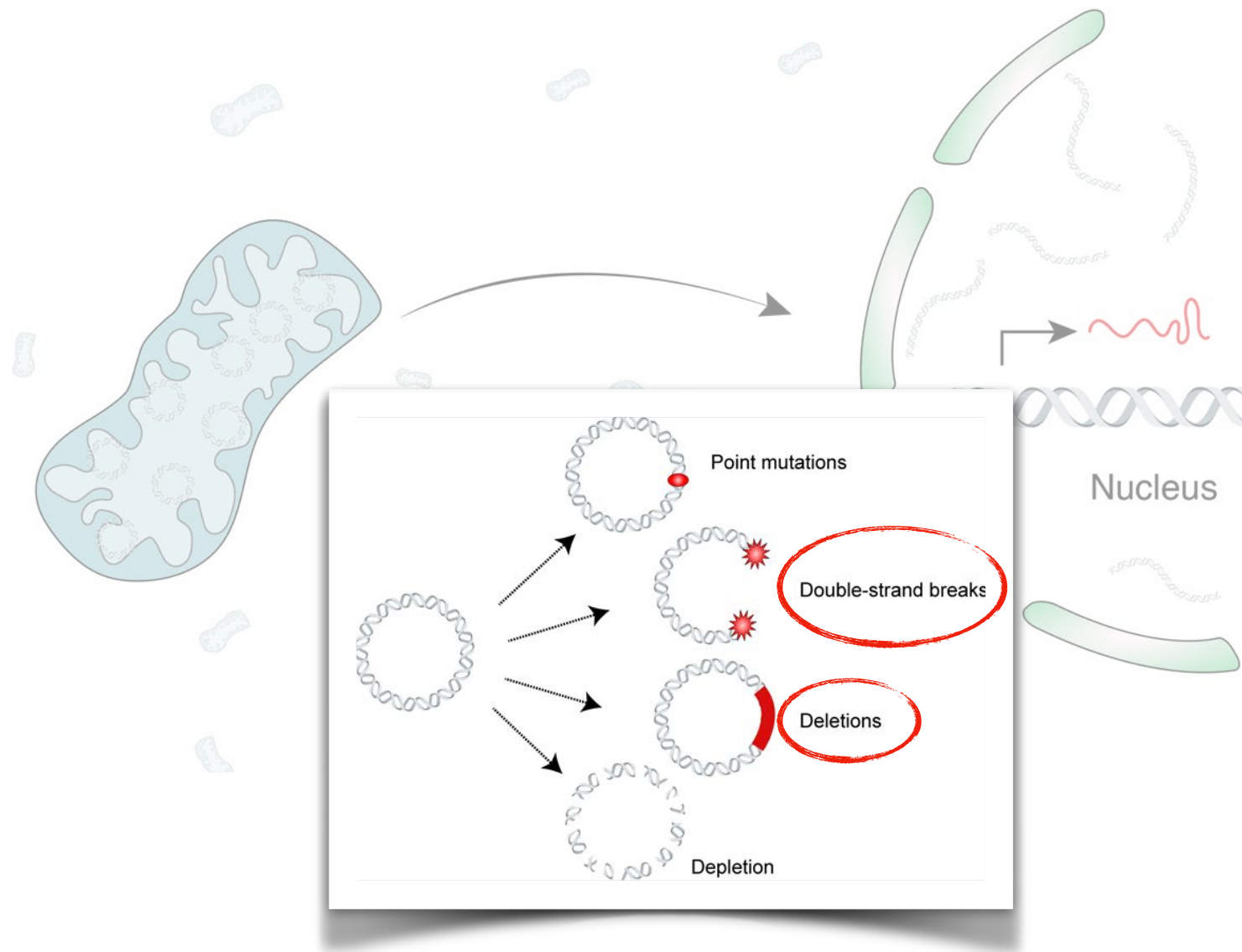


# Challenges inherent to the mitochondrial endosymbiosis

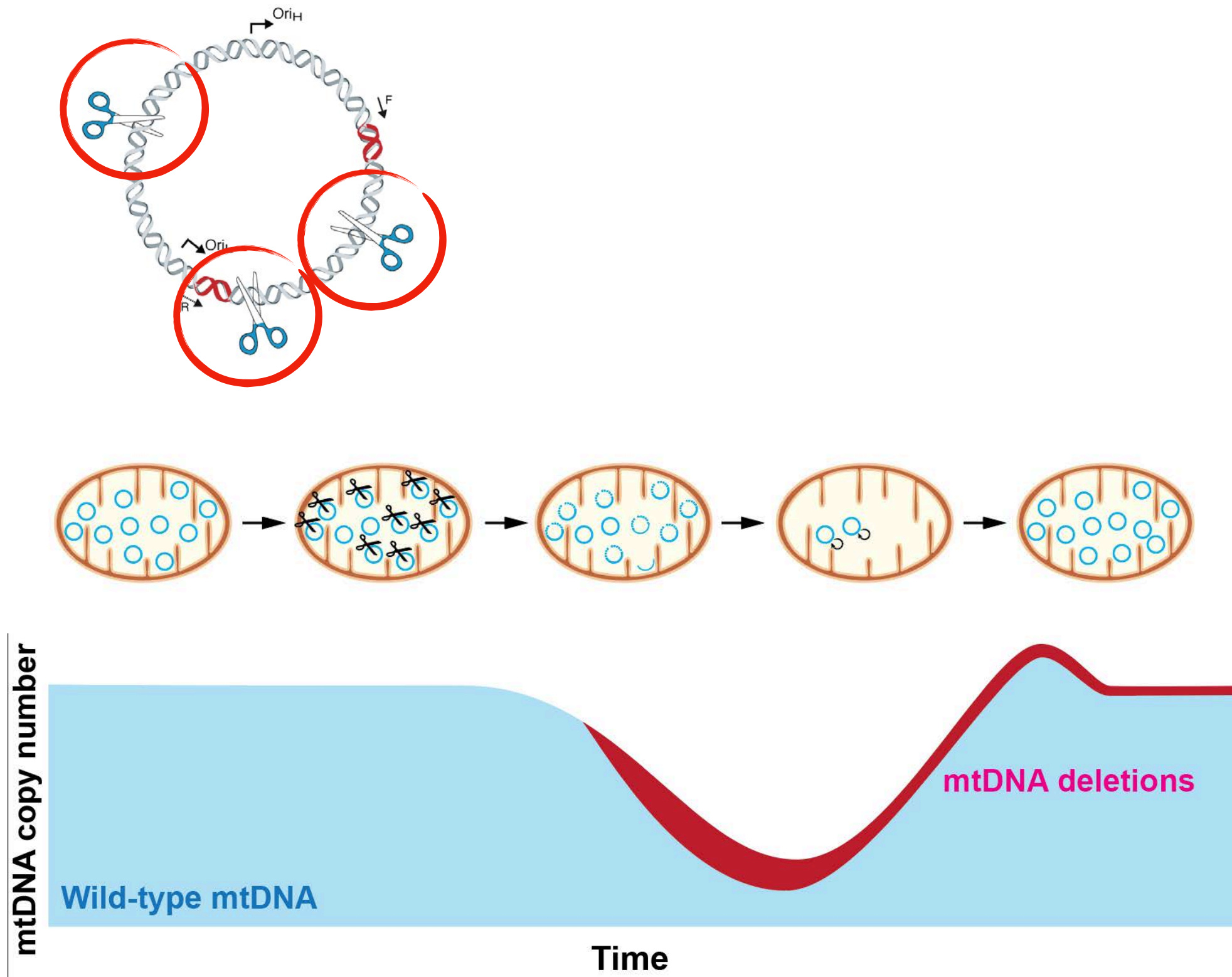
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# Mapping the nuclear response to mtDNA dysfunction

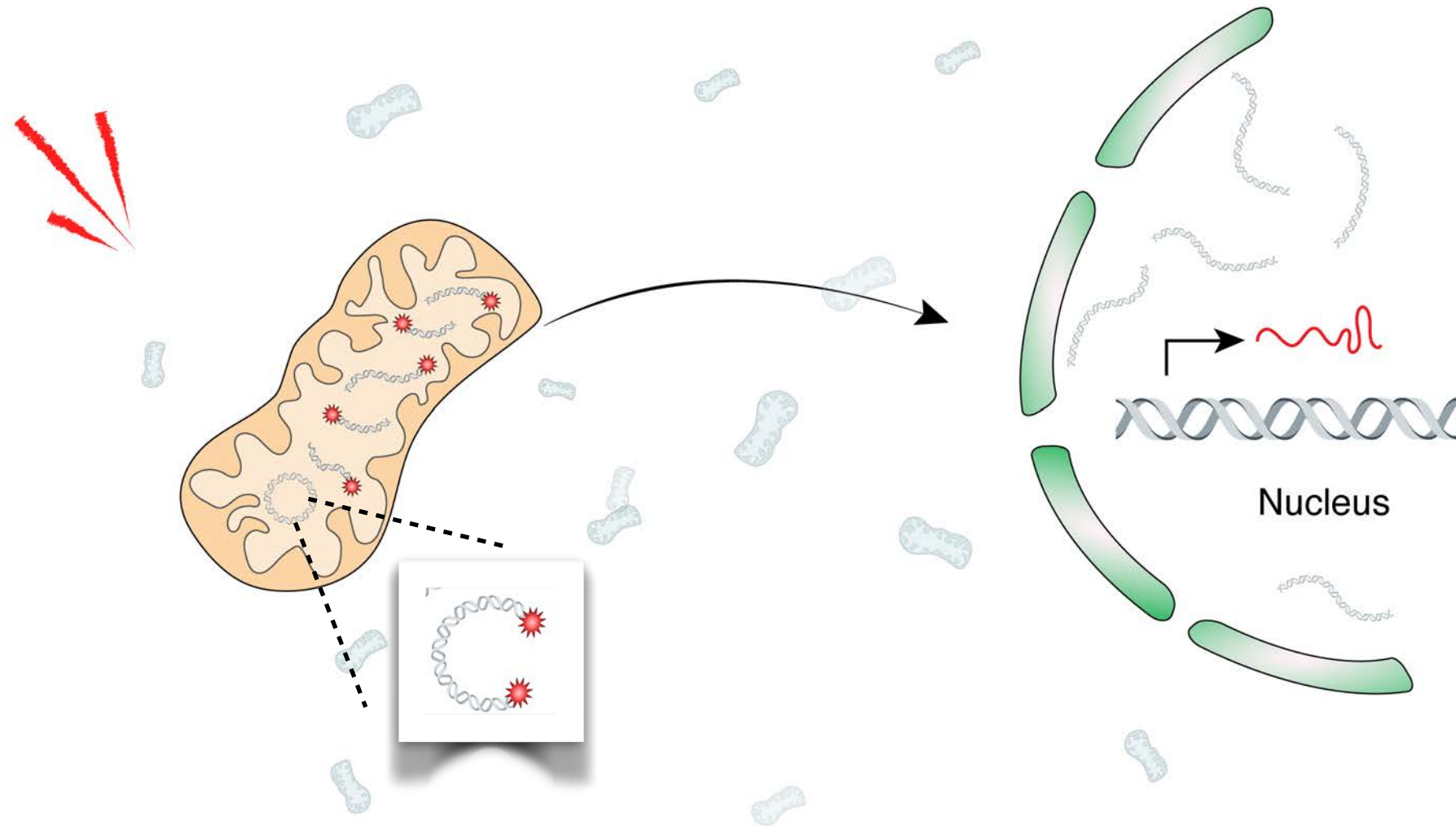


# mtDNA dynamics in response to mito-DSBs



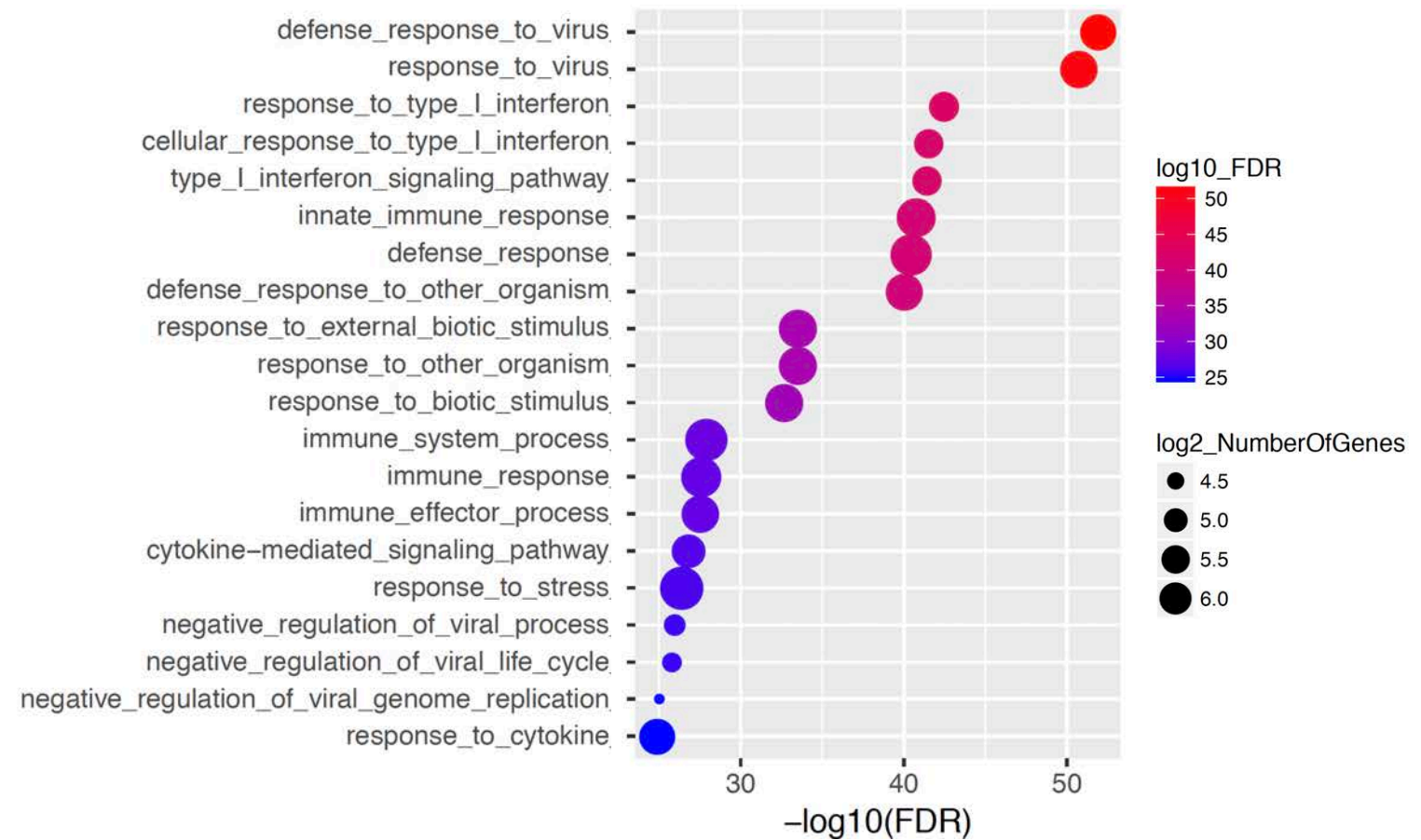
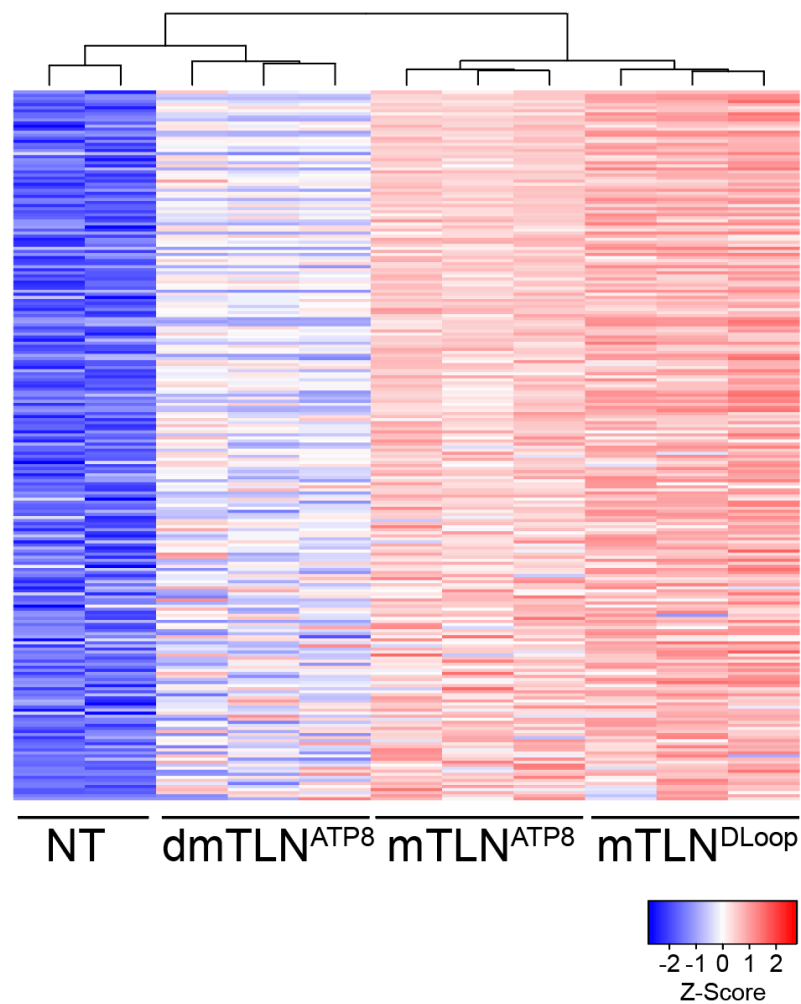


# Nuclear sensing of mitochondrial DSBs

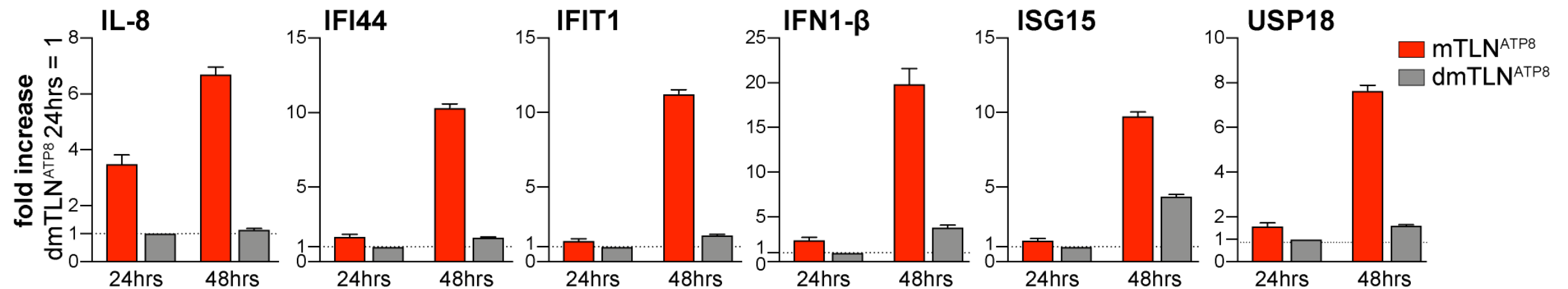


Marco Tígano

# Activation of innate immune response to mito-DSB

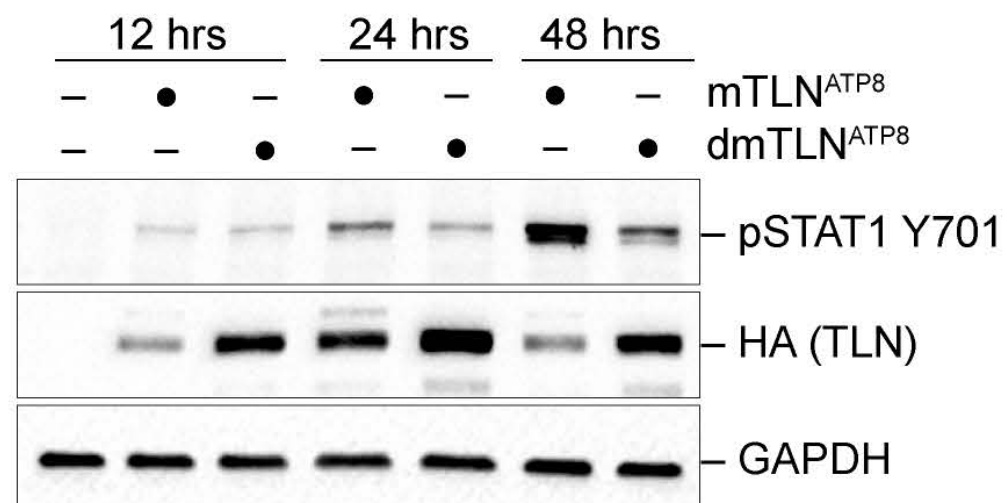


# Activation of ISG and p-STAT1 upon mtDNA cleavage

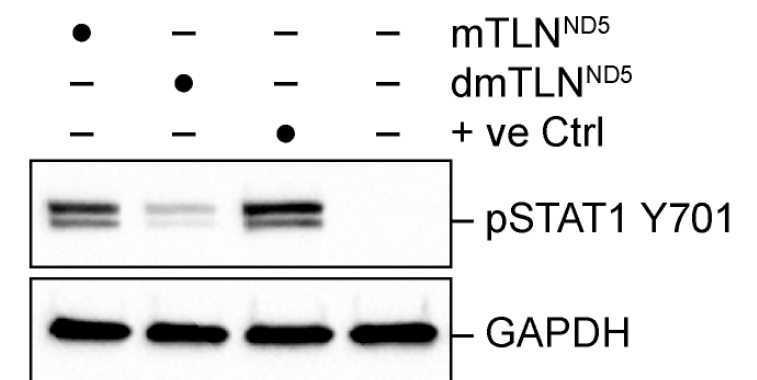


Conditioned media

Treated Cells

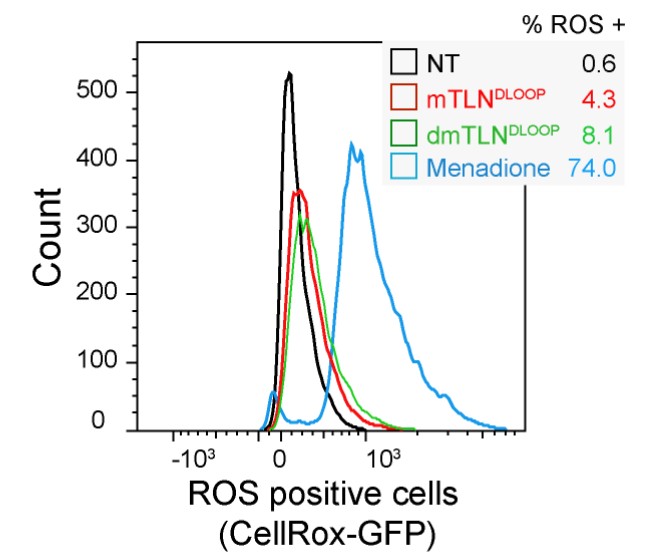
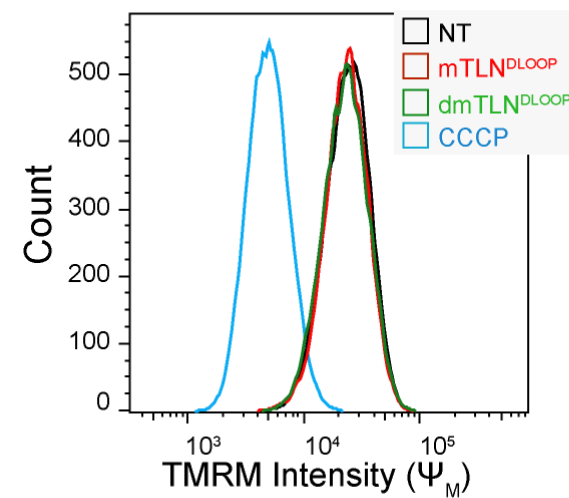
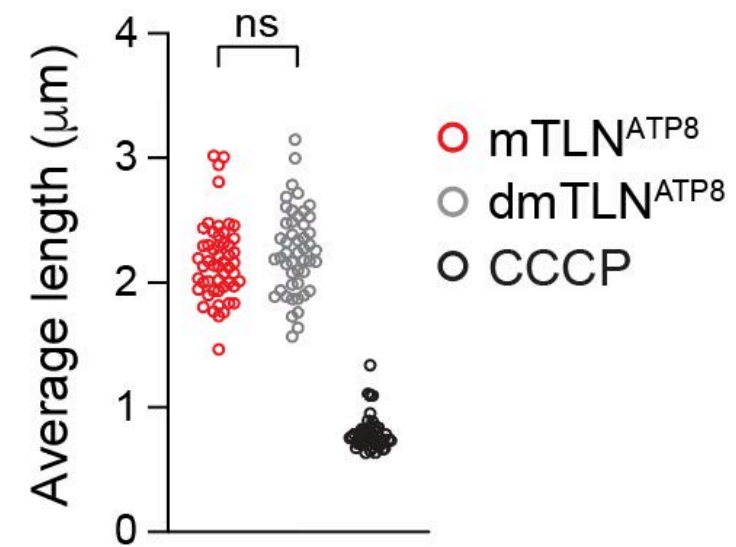
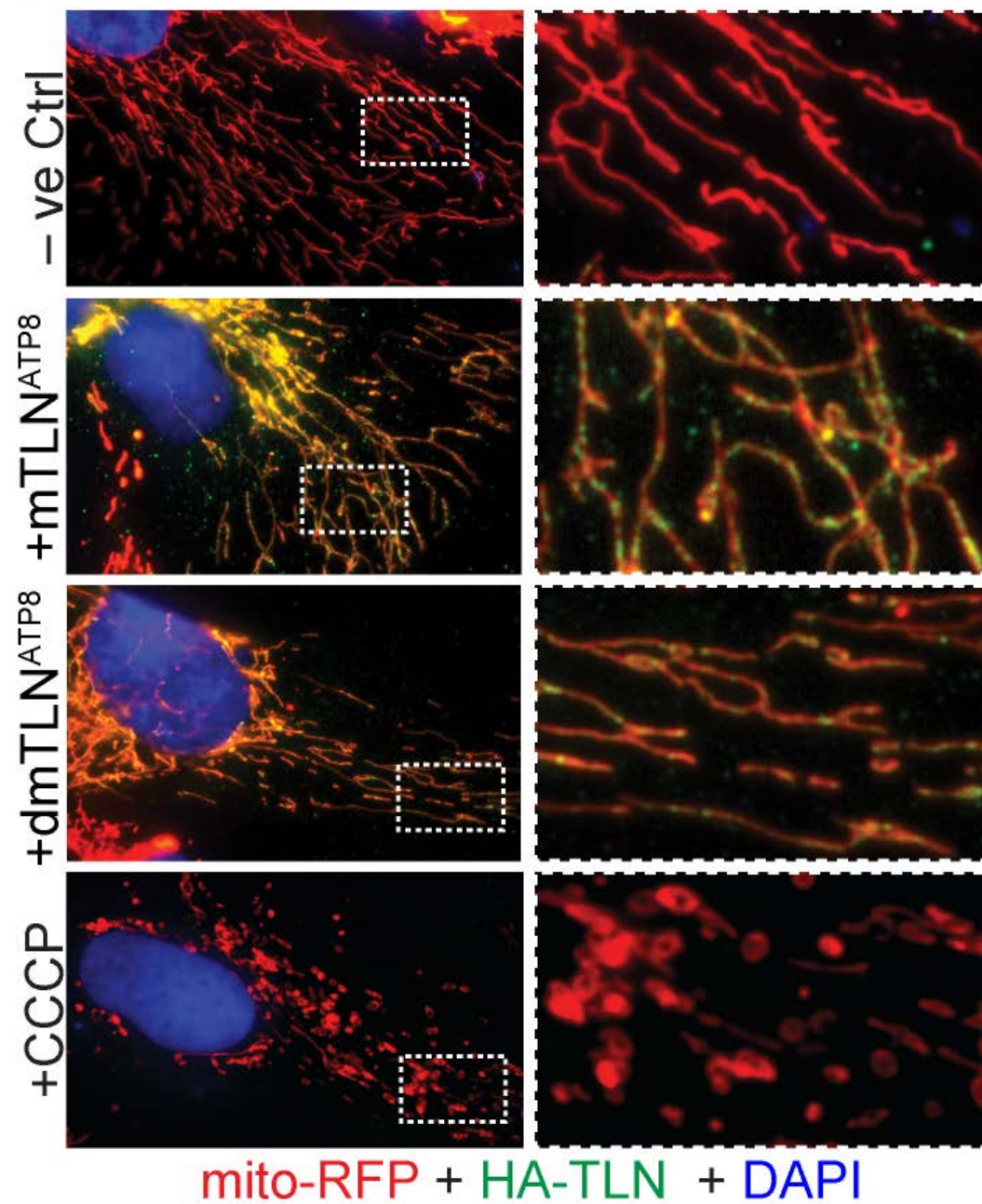


Naive Cells



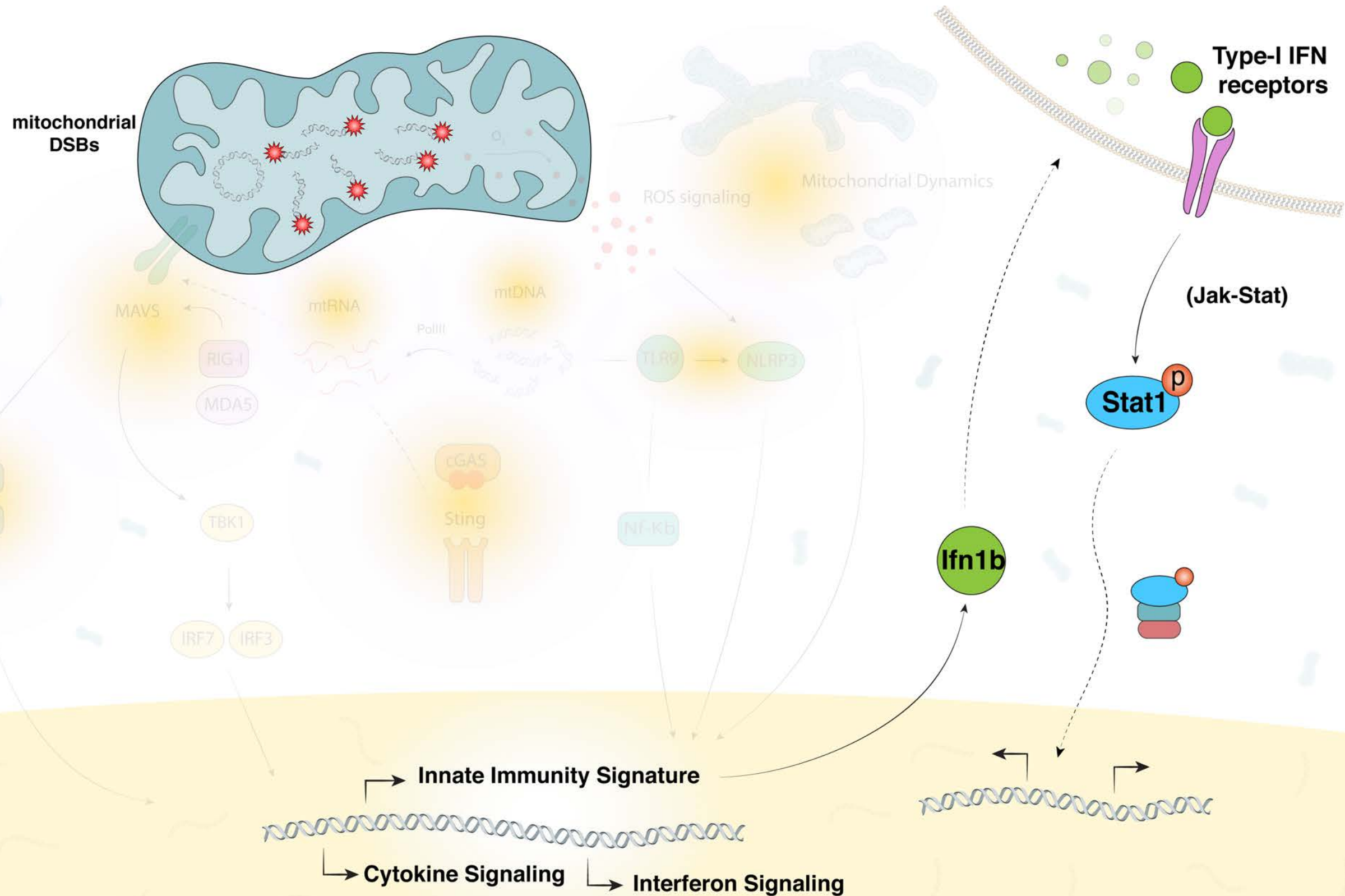


# No overt mitochondrial dysfunction upon mito-DSB

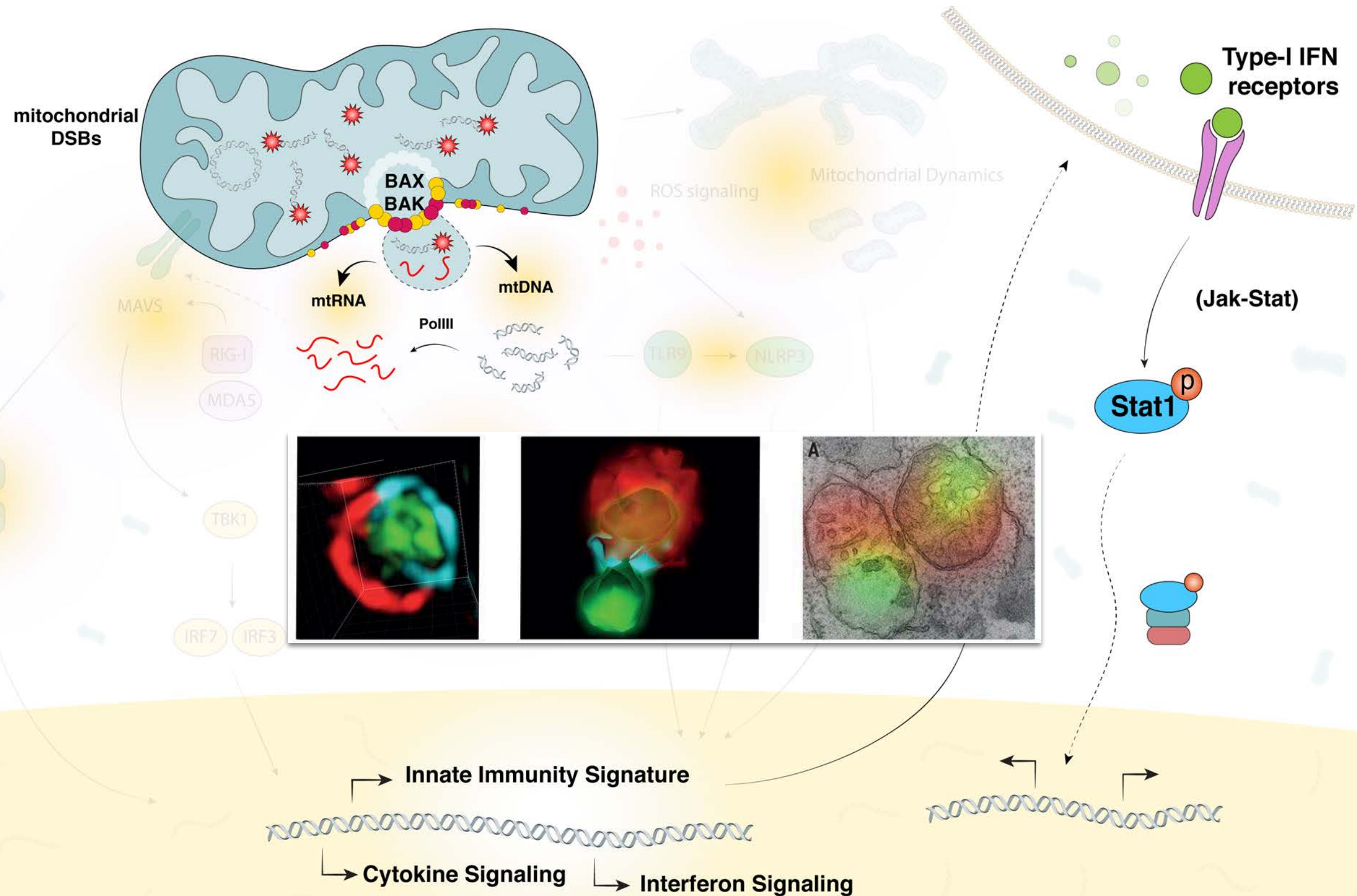




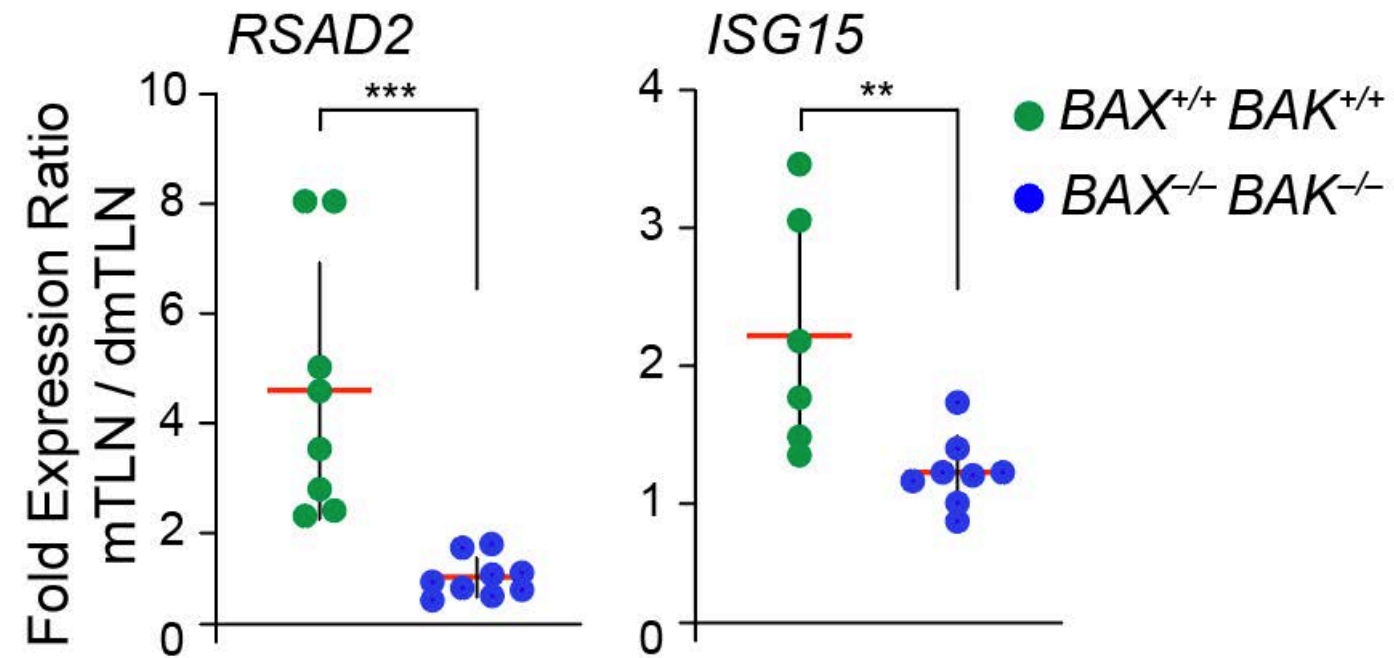
# Mitochondria are a signaling hub for innate immunity



# Mitochondria herniation releases matrix contents to the cytosol

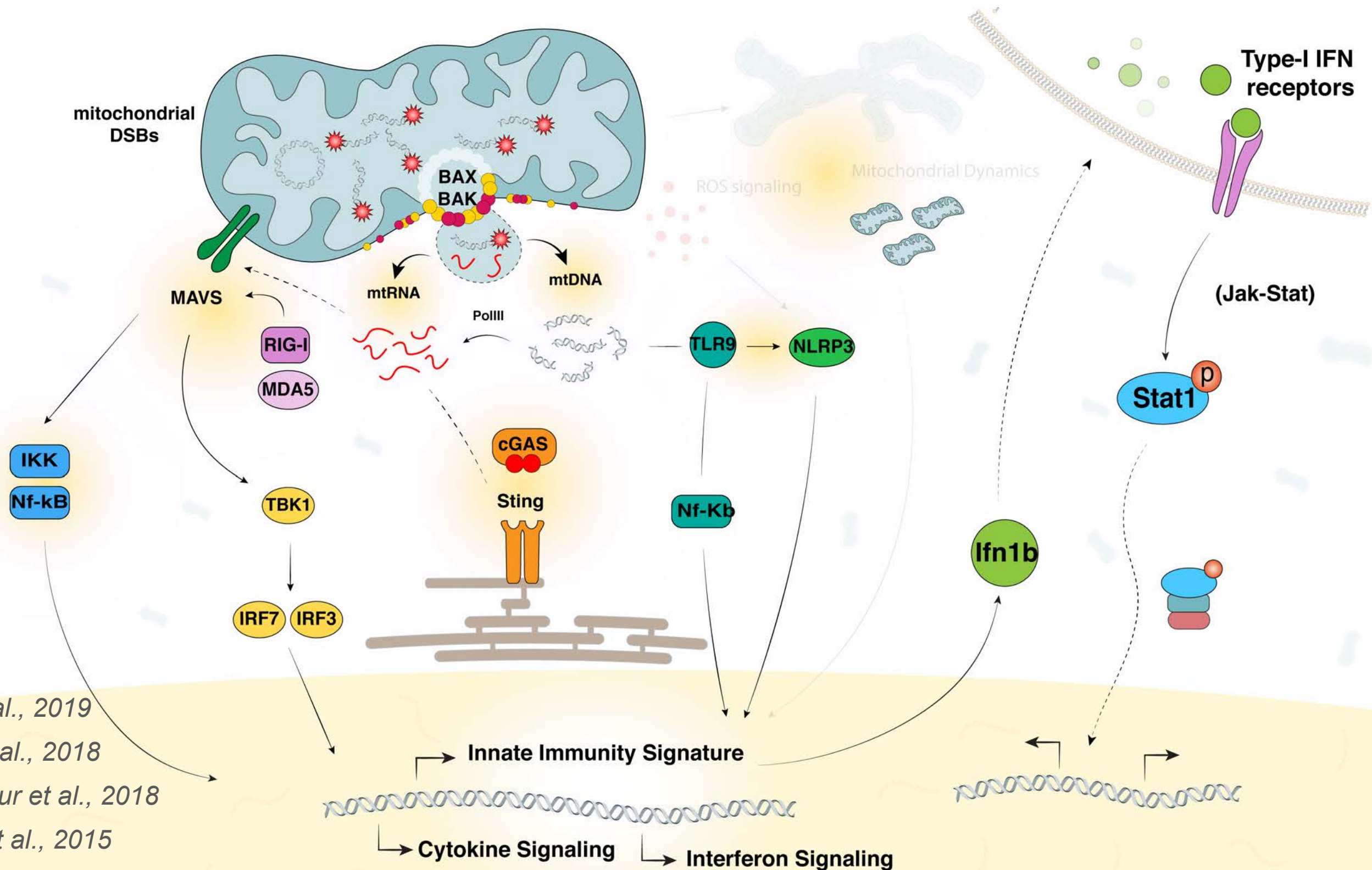


# Loss of BAK/BAX inhibits immune sensing in response to mito-DSBs





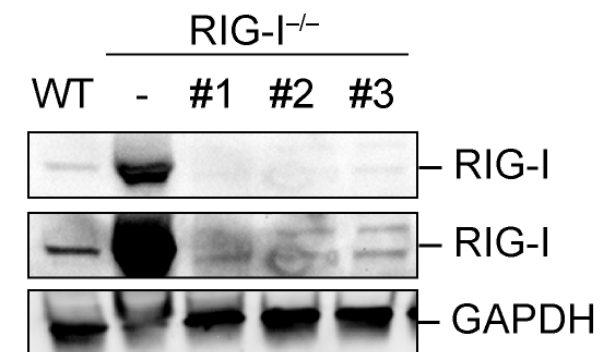
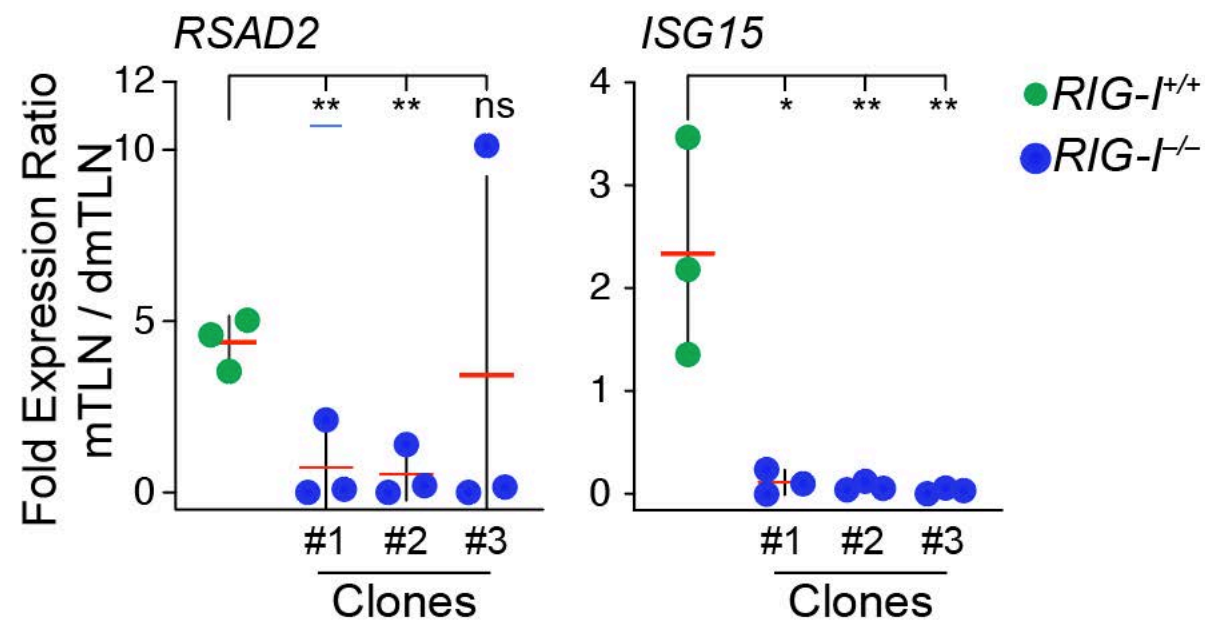
# What cytoplasmic sensors are involved in the response?

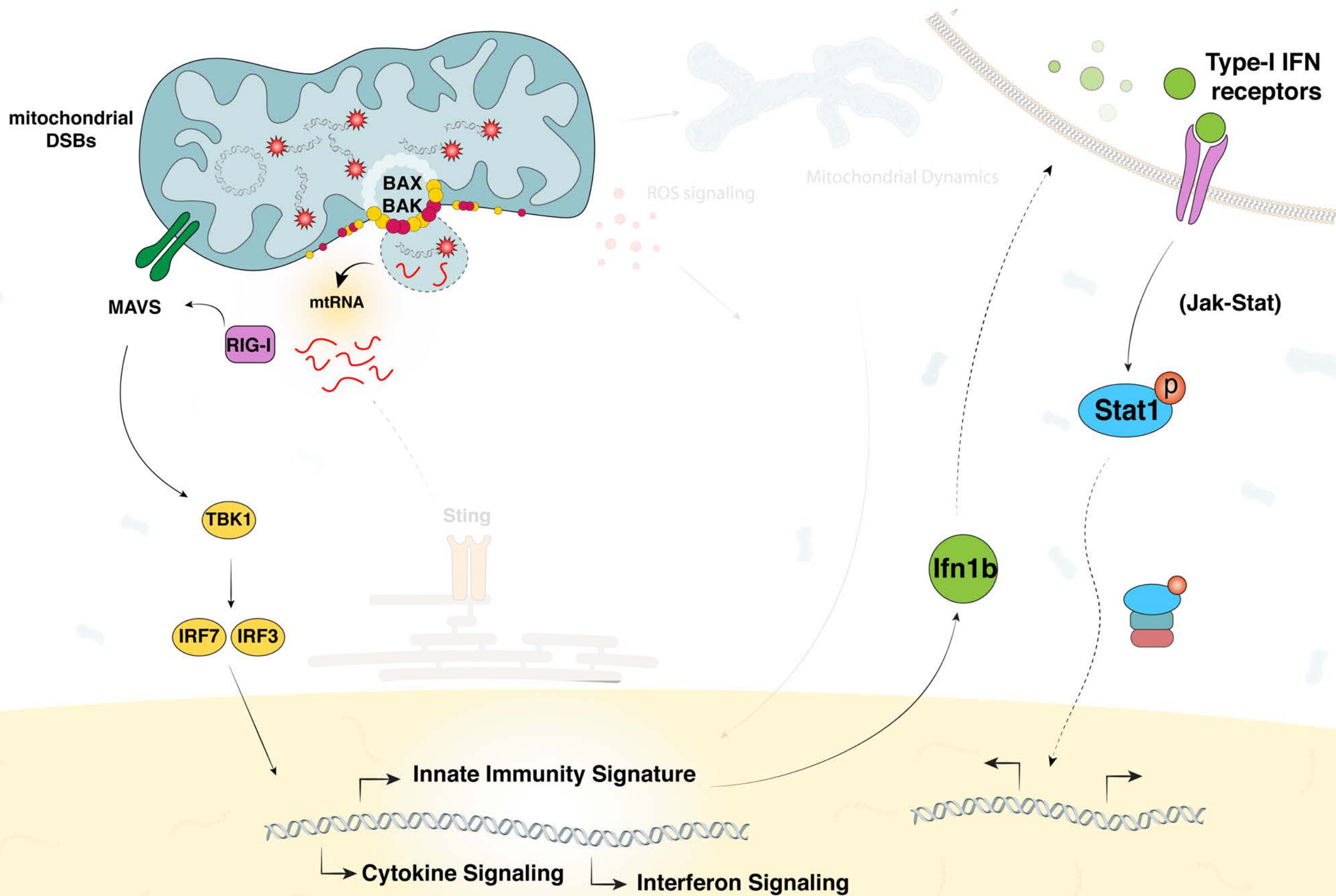


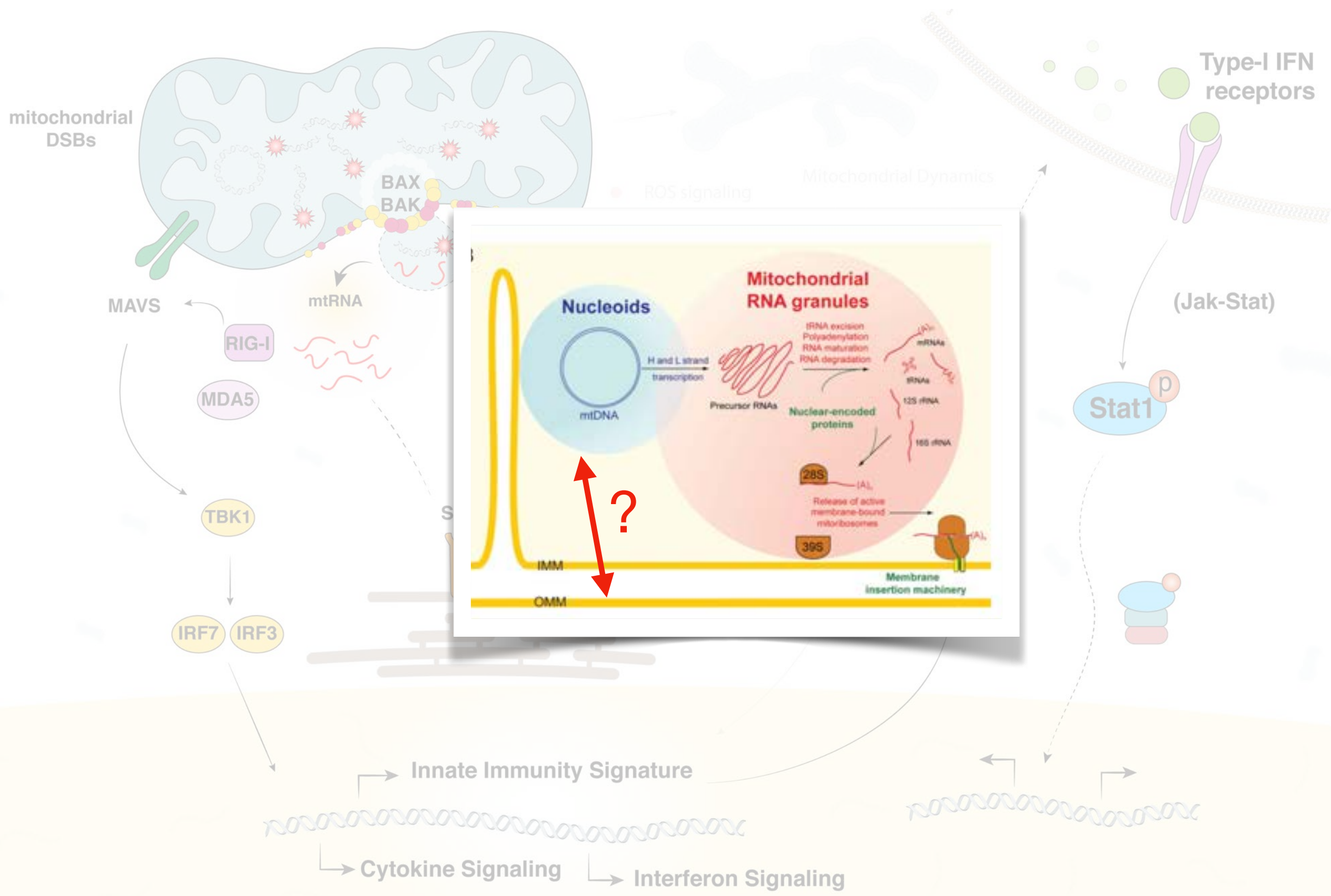
Wu et al., 2019  
Dhir et al., 2018  
McArthur et al., 2018  
West et al., 2015  
Castanier et al., 2010



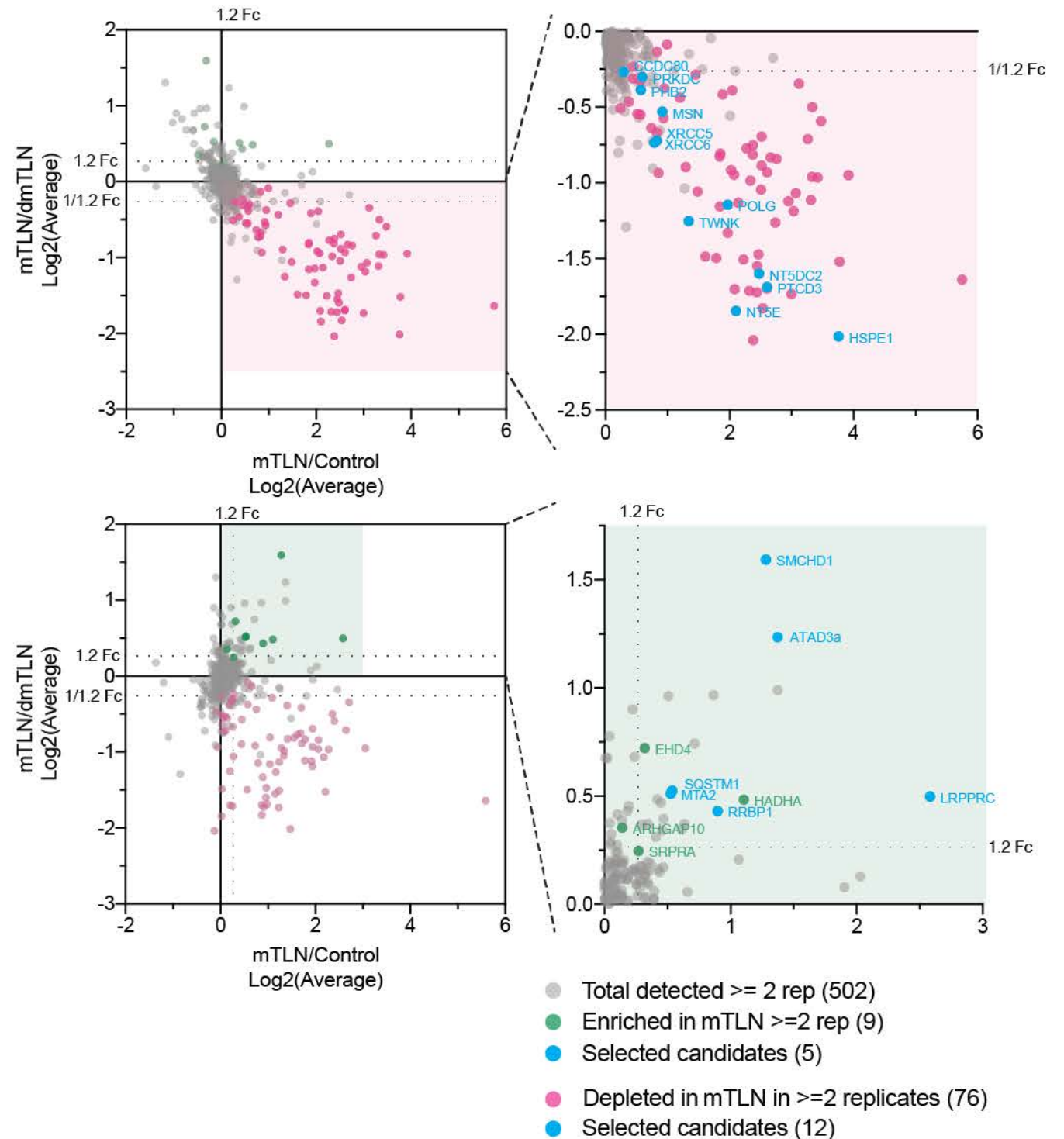
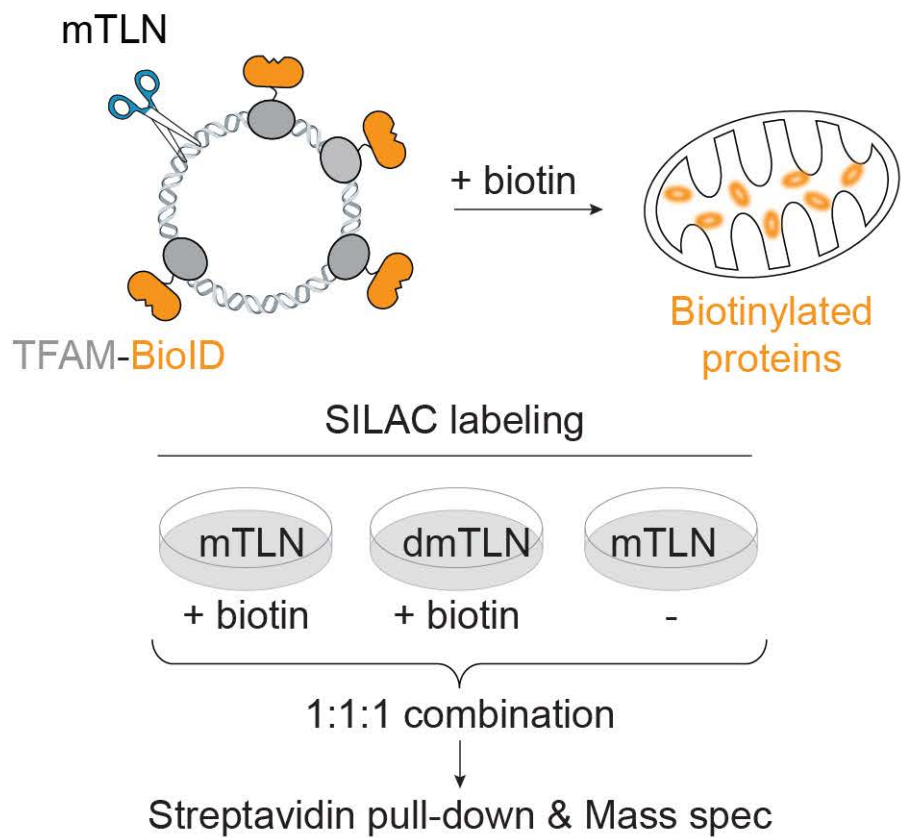
# RNA sensing by RIG-I mediates immune response to mito-DSBs



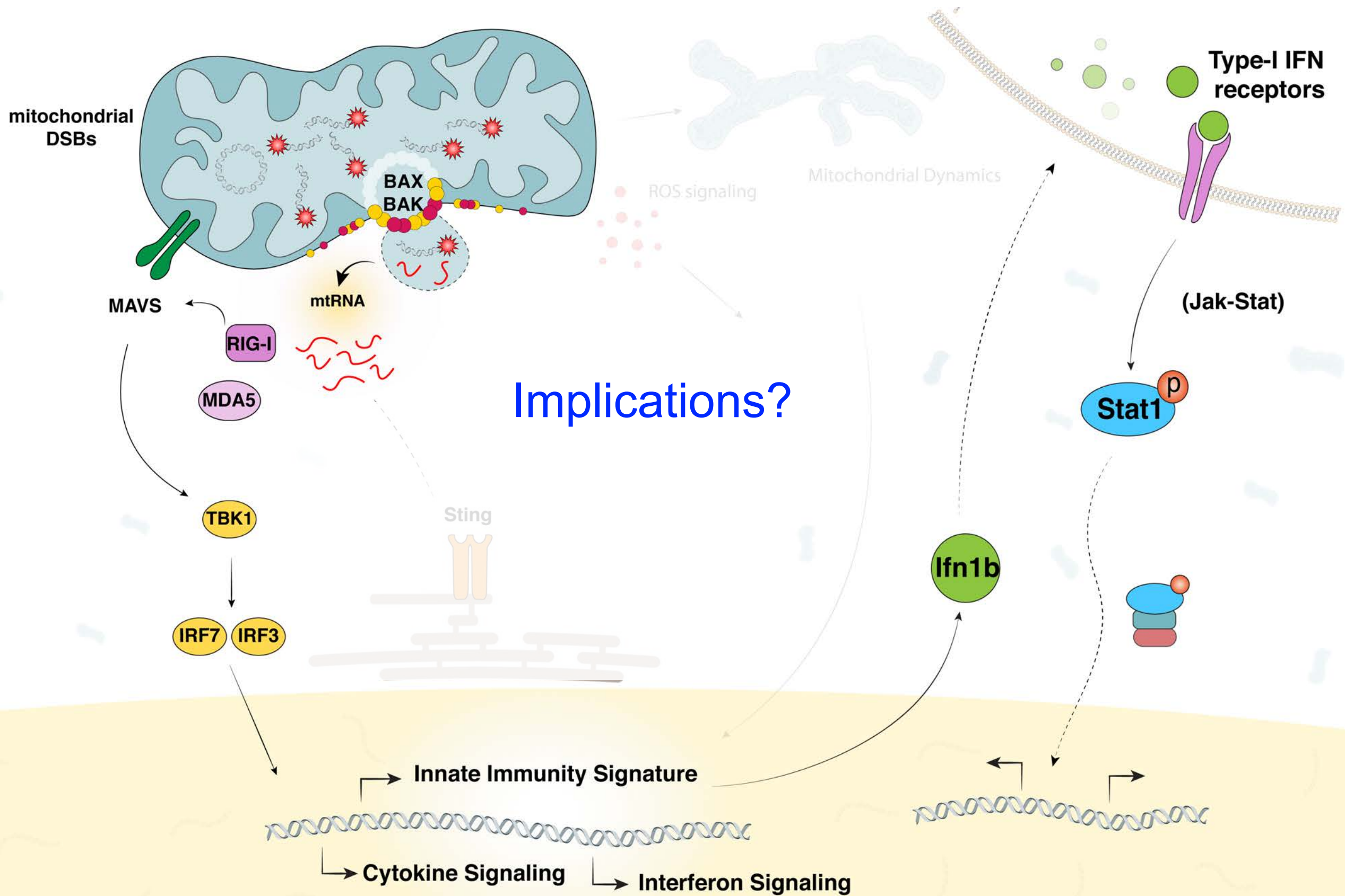




# A proteomic approach to uncover the direct outcome of mtDNA breaks



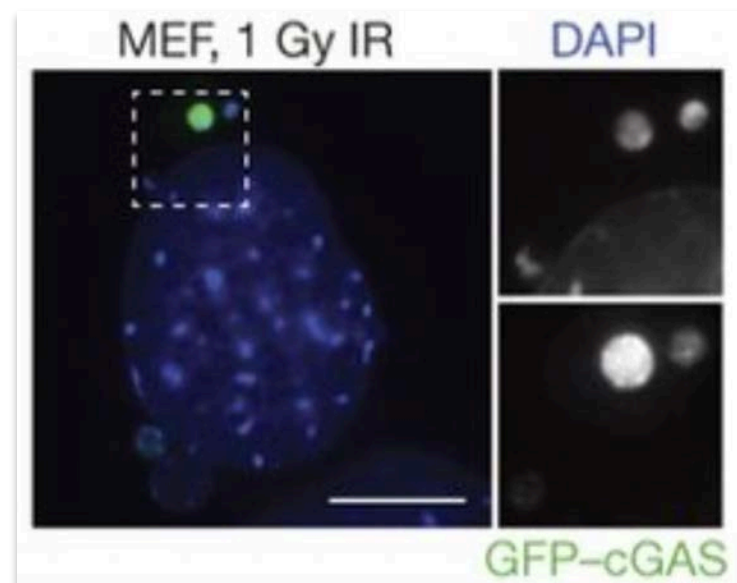
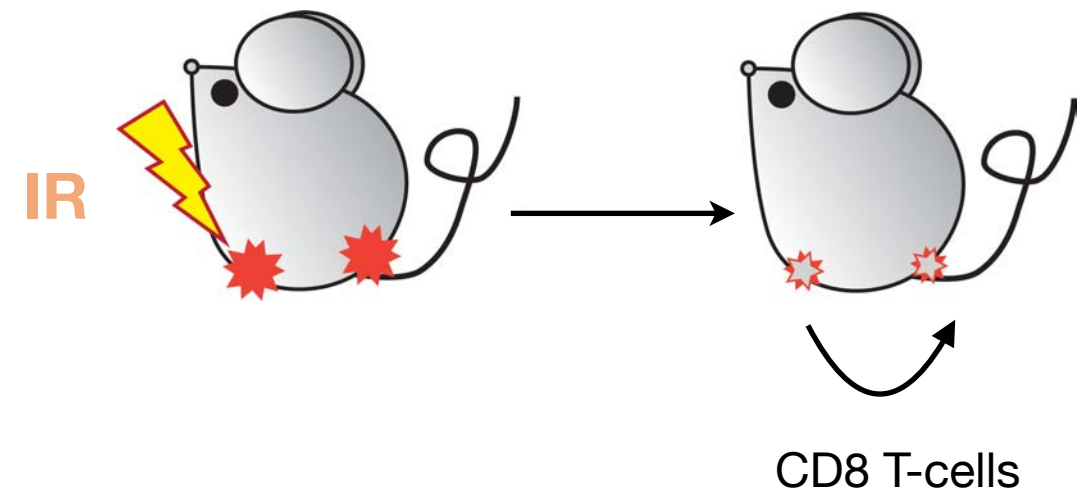




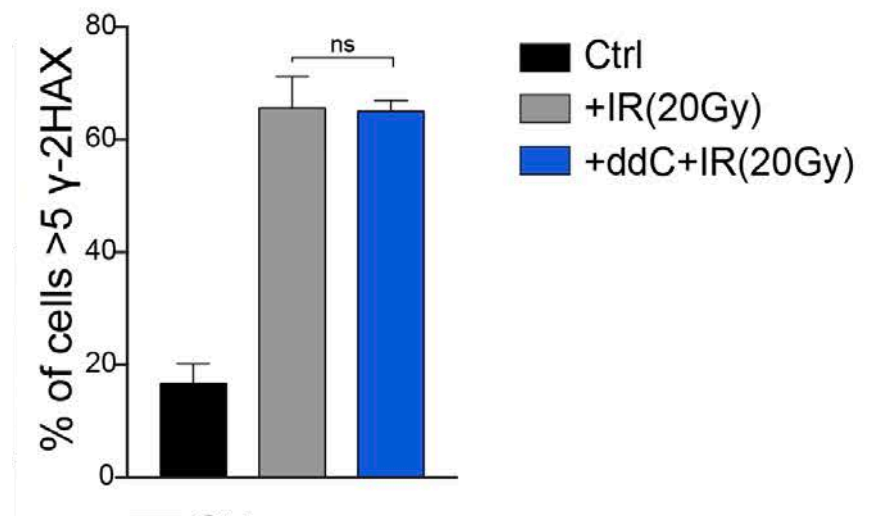
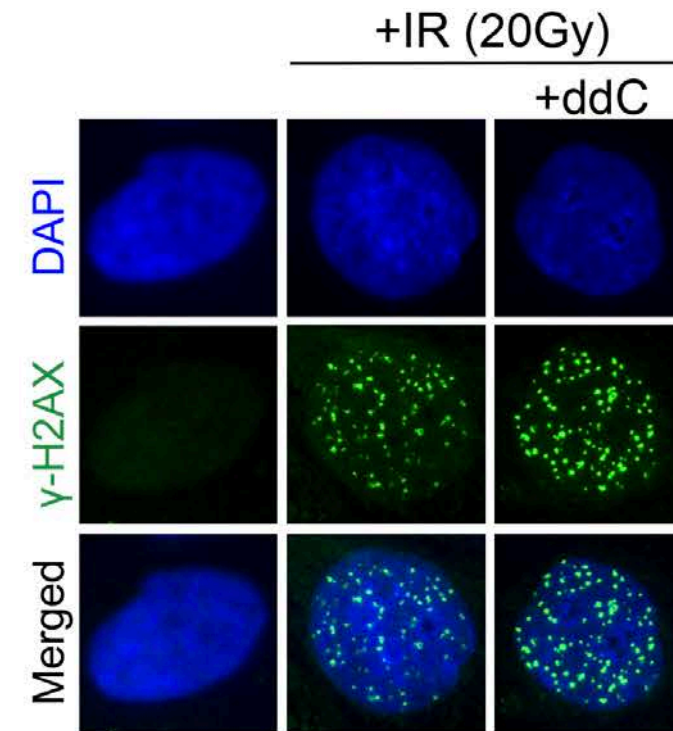
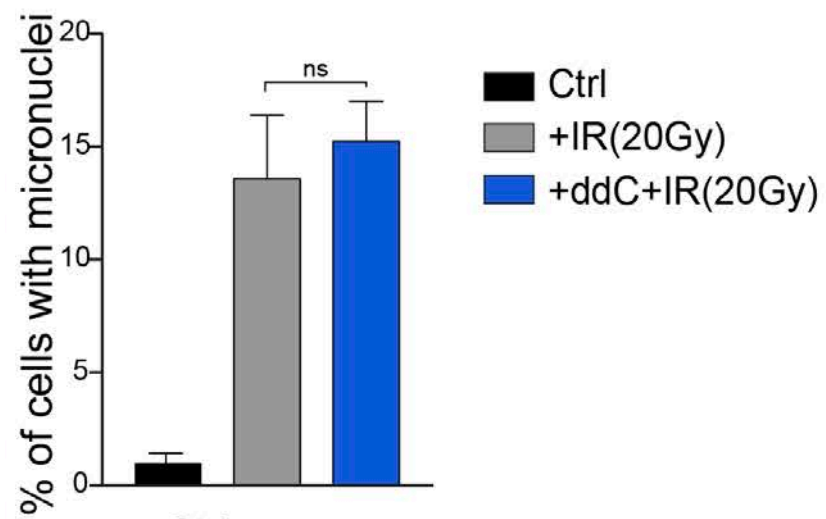
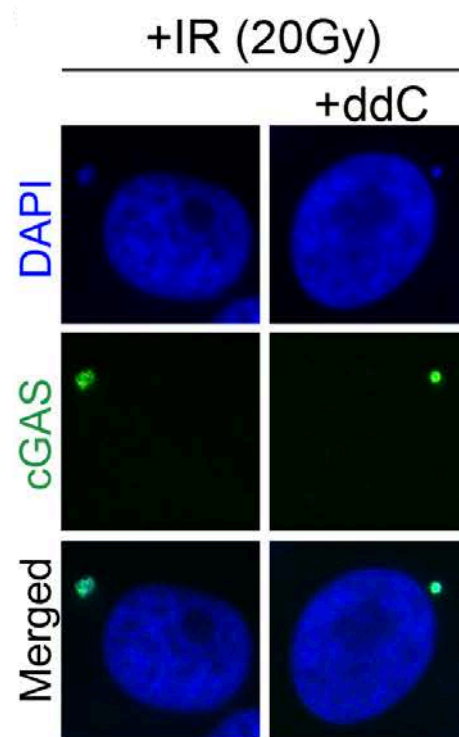
# Innate immune activation in response to gamma-irradiation



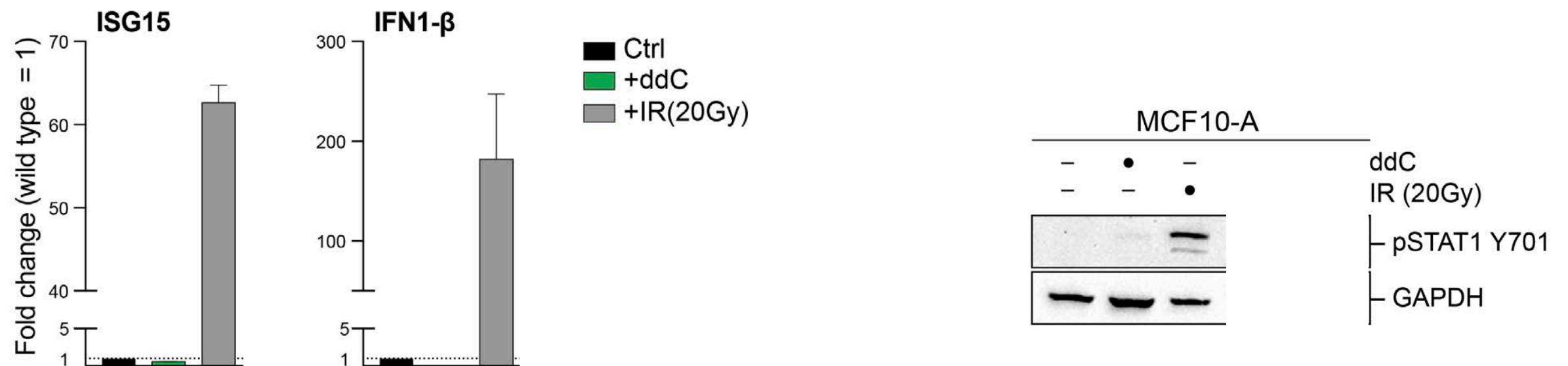
## Abscopal Effect



# Assessing the immune response in cells devoid of mtDNA

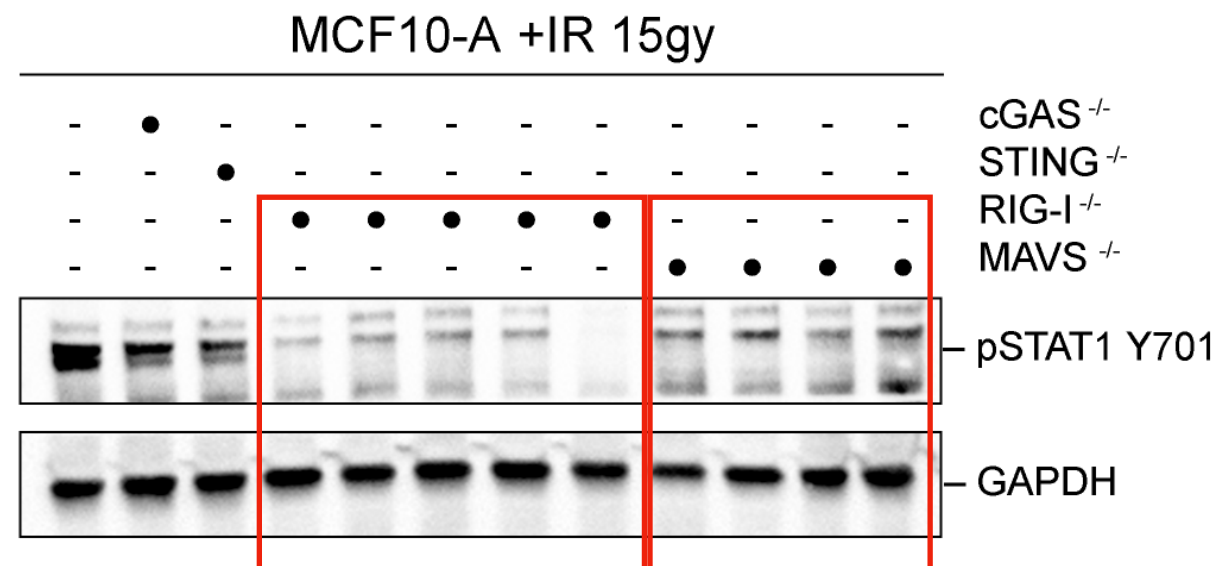
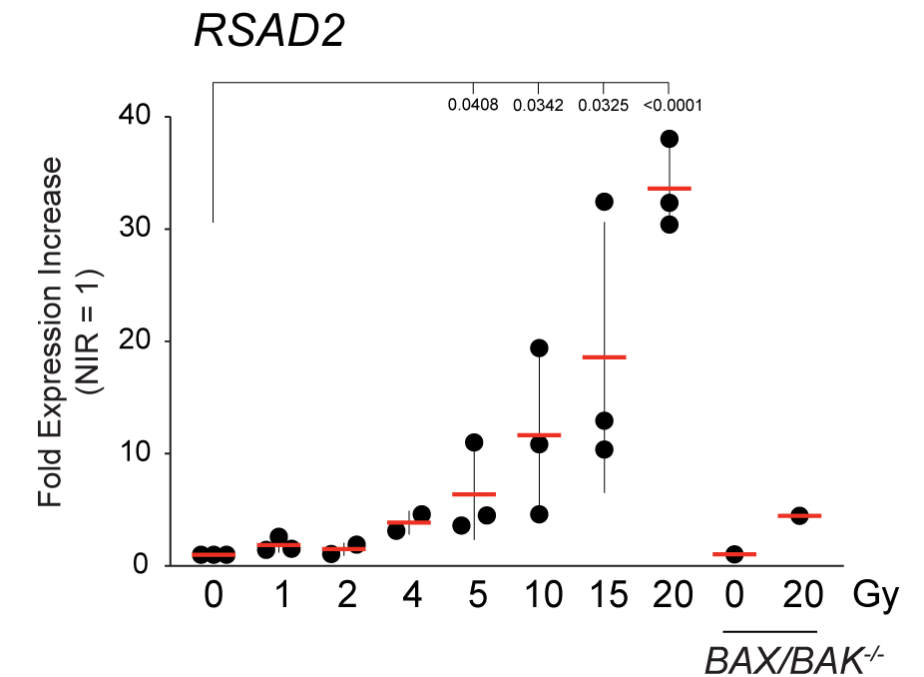
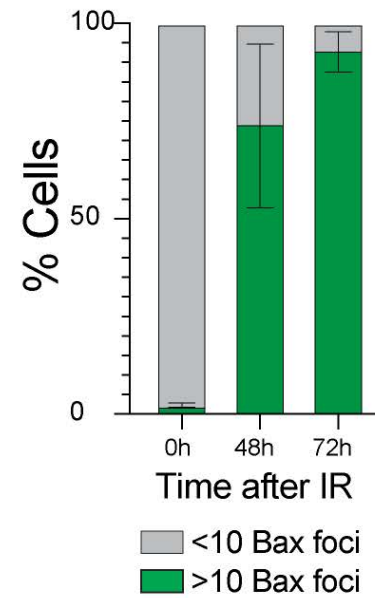
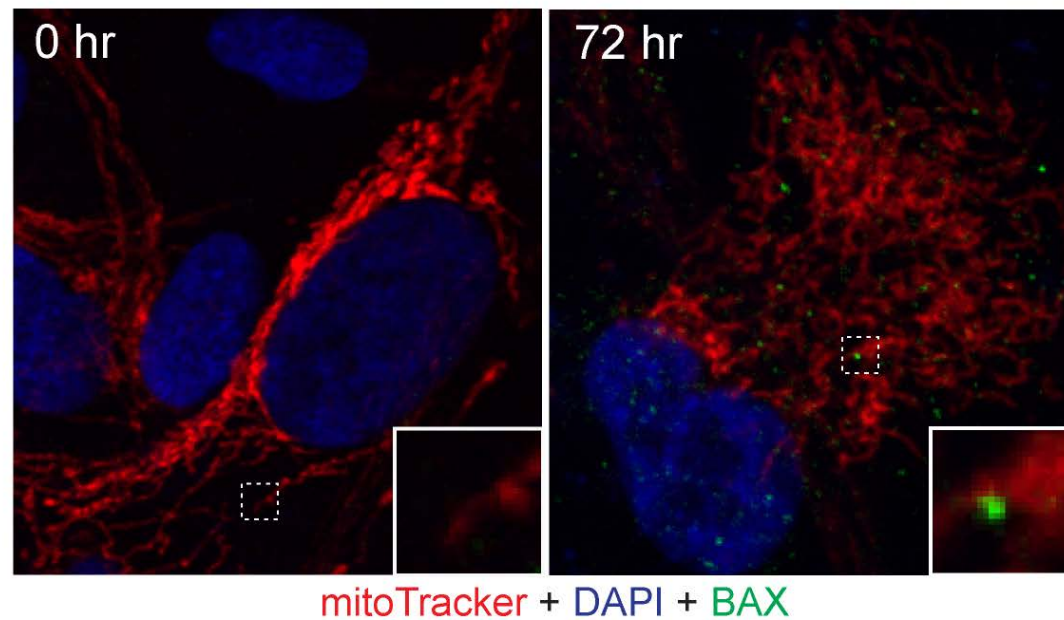


# Diminished innate immunity upon irradiation of *rho0* cells

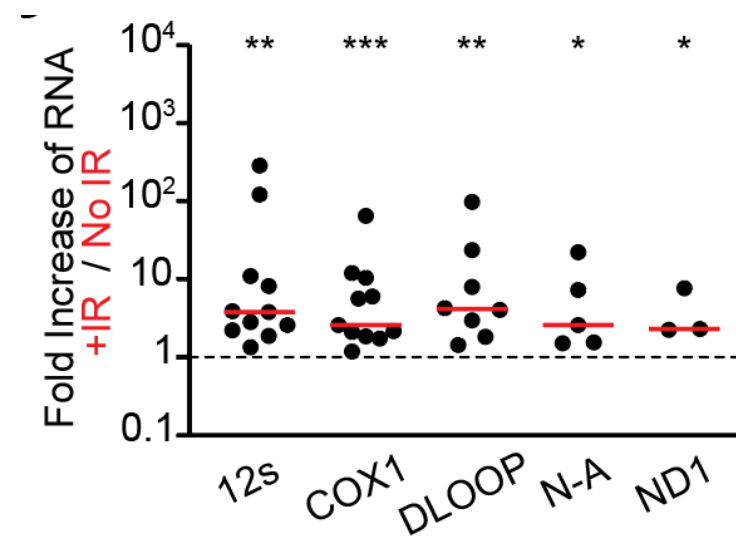
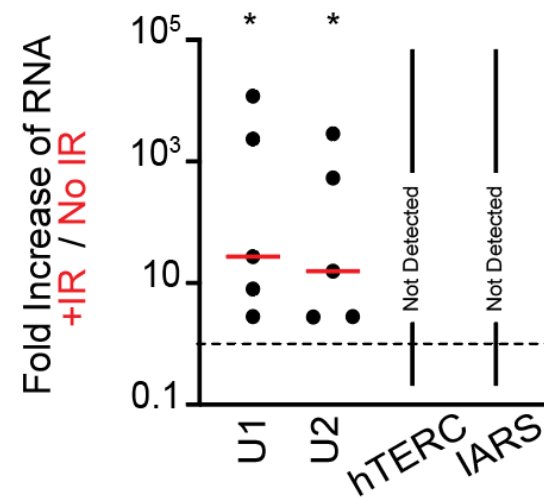
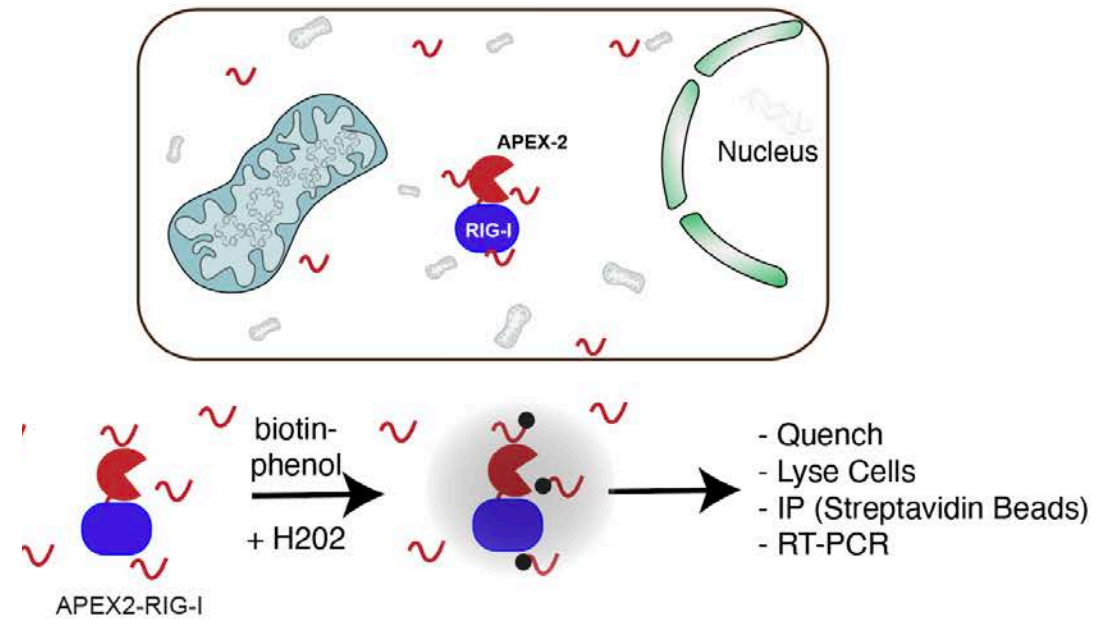




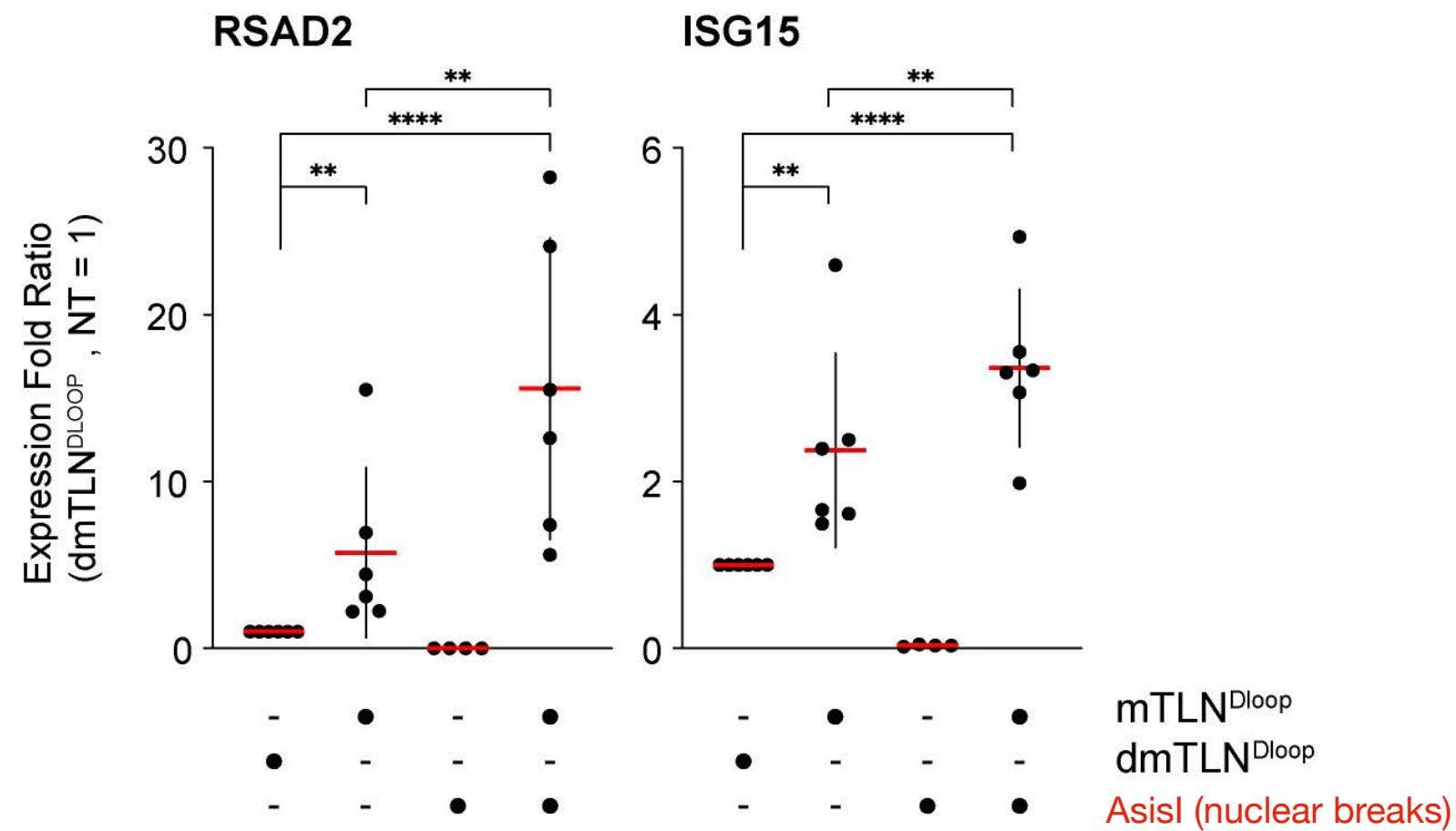
# Mitochondrial herniation and RIG-I activation in response to irradiation



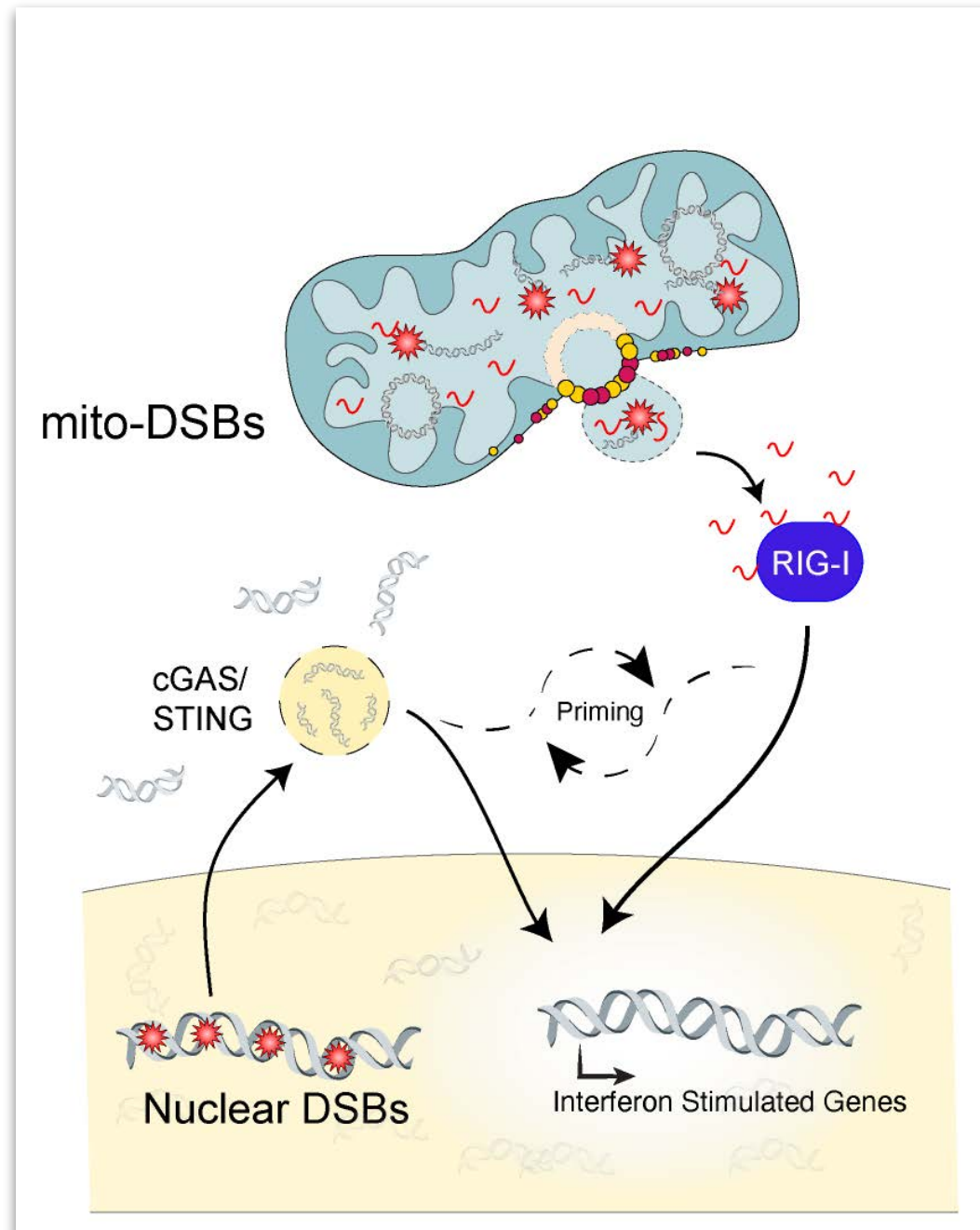
# Cytoplasmic RNA sensing by RIG-I in response to IR



# mtDNA breaks synergize with nuclear DNA damage to induce ISGs



# Nuclear sensing of mtDNA breaks primes immune surveillance





# Acknowledgements



## Funding

- NYSTEM
- NIH-DP2
- NIH-NCI
- David and Lucille Packard Foundation
- Damon Runyon-Rachleff
- Pew Innovation Funds
- Human Frontier Science Program
- Mallinkrodt Scholar
- Pew-Stewart Scholar
- Pershing Square Sohn Cancer foundation
- V-foundation
- Shifrin Myers

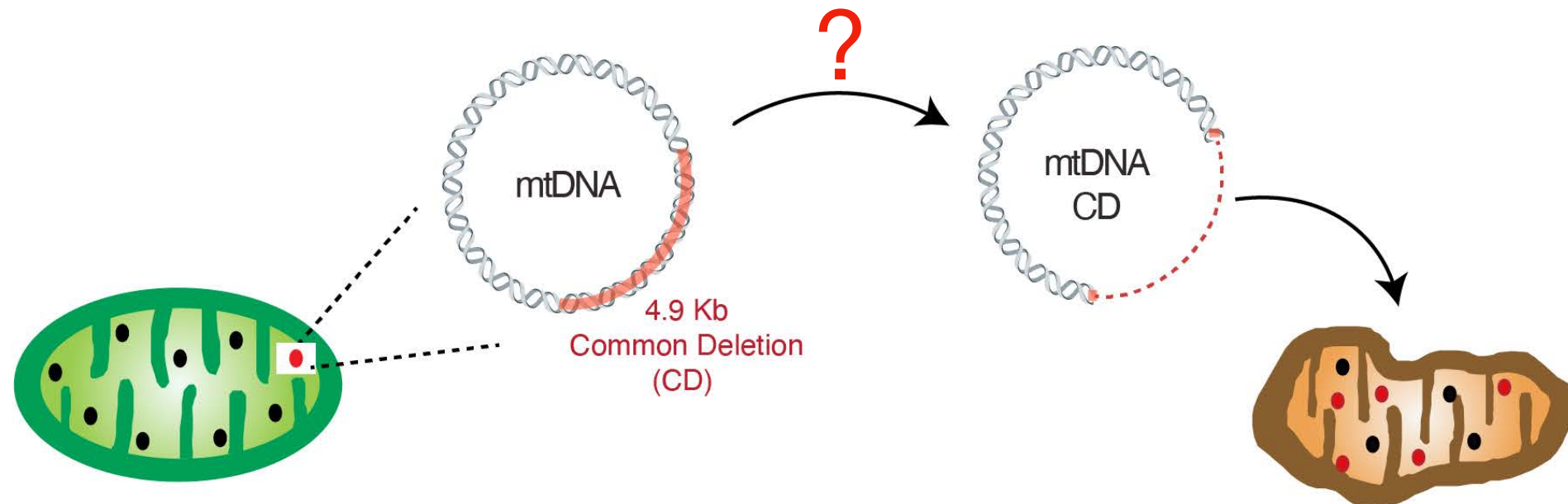
## Collaborators and Reagent Providers

- Sharon Savage (NIH)
- Jef Boeke (NYU)
- Roger Greenberg (UPenn)

Post-doc positions available

[www.sfeirlab.com](http://www.sfeirlab.com)

# The mitochondrial common deletion



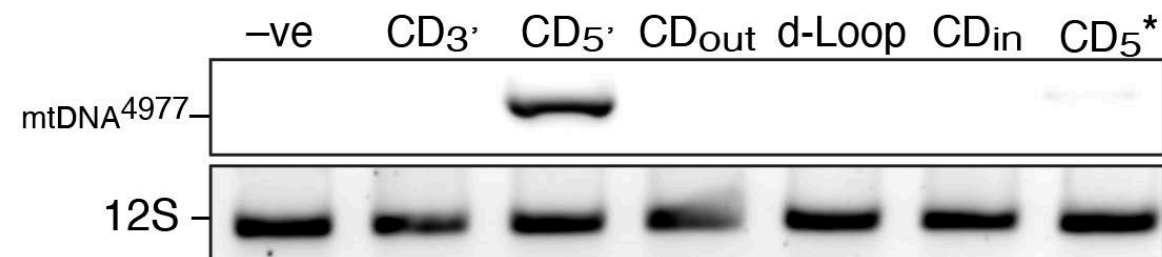
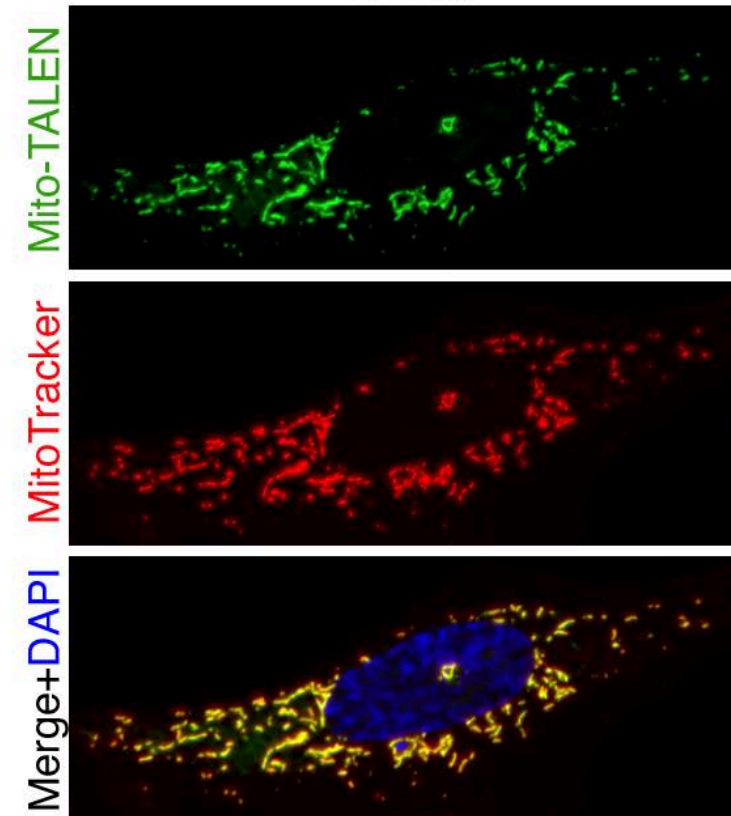
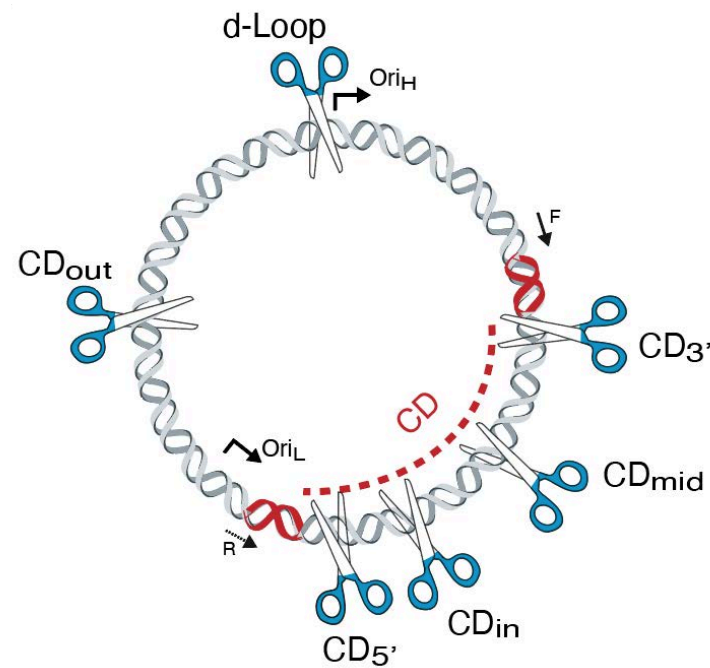
## Disease manifestation:

- Kearns-Sayre syndrome
- Progressive external ophthalmoplegia
- Pearson marrow syndrome
- Aging

## Characteristic features:

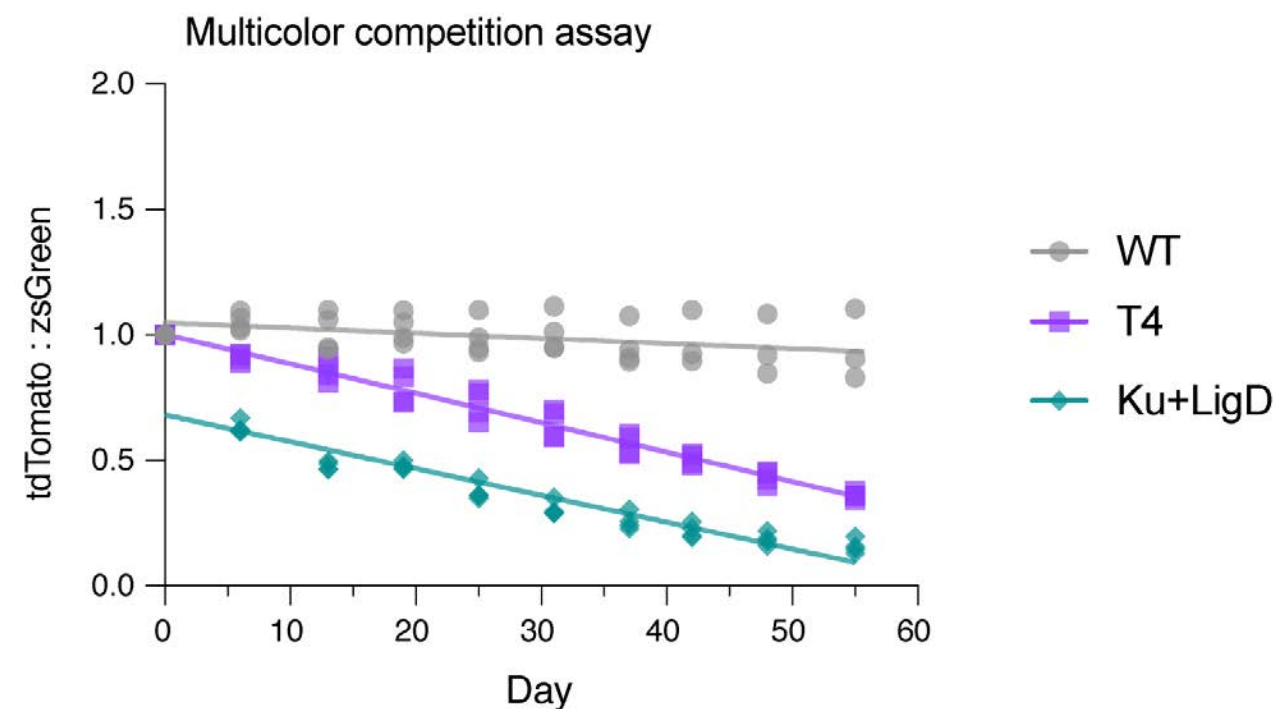
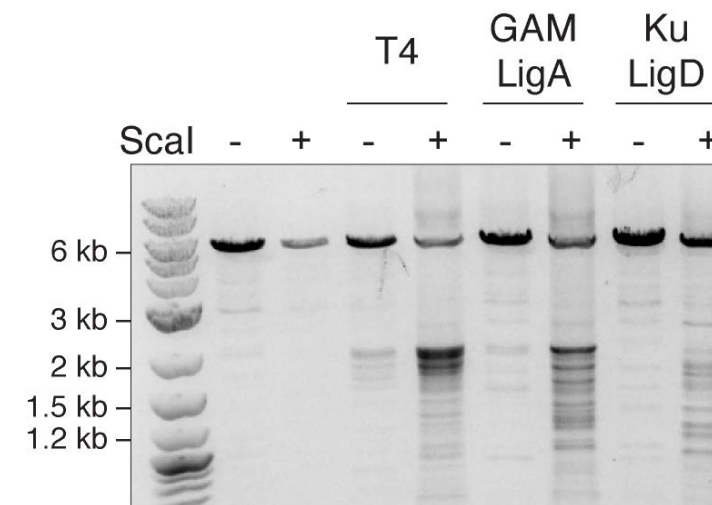
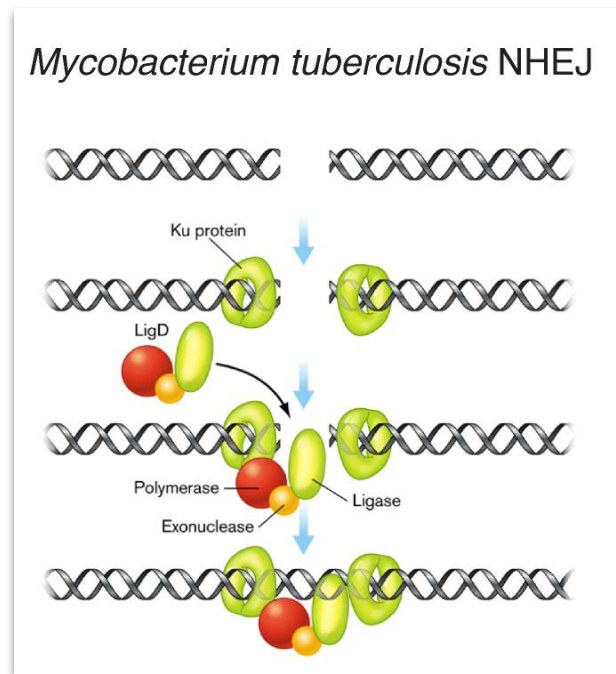
- Flanked by repeats
- Primarily impacts muscle and brain
- Heteroplasmic in nature

# mito-TALENs (mTLNs) induced breaks trigger the common deletion



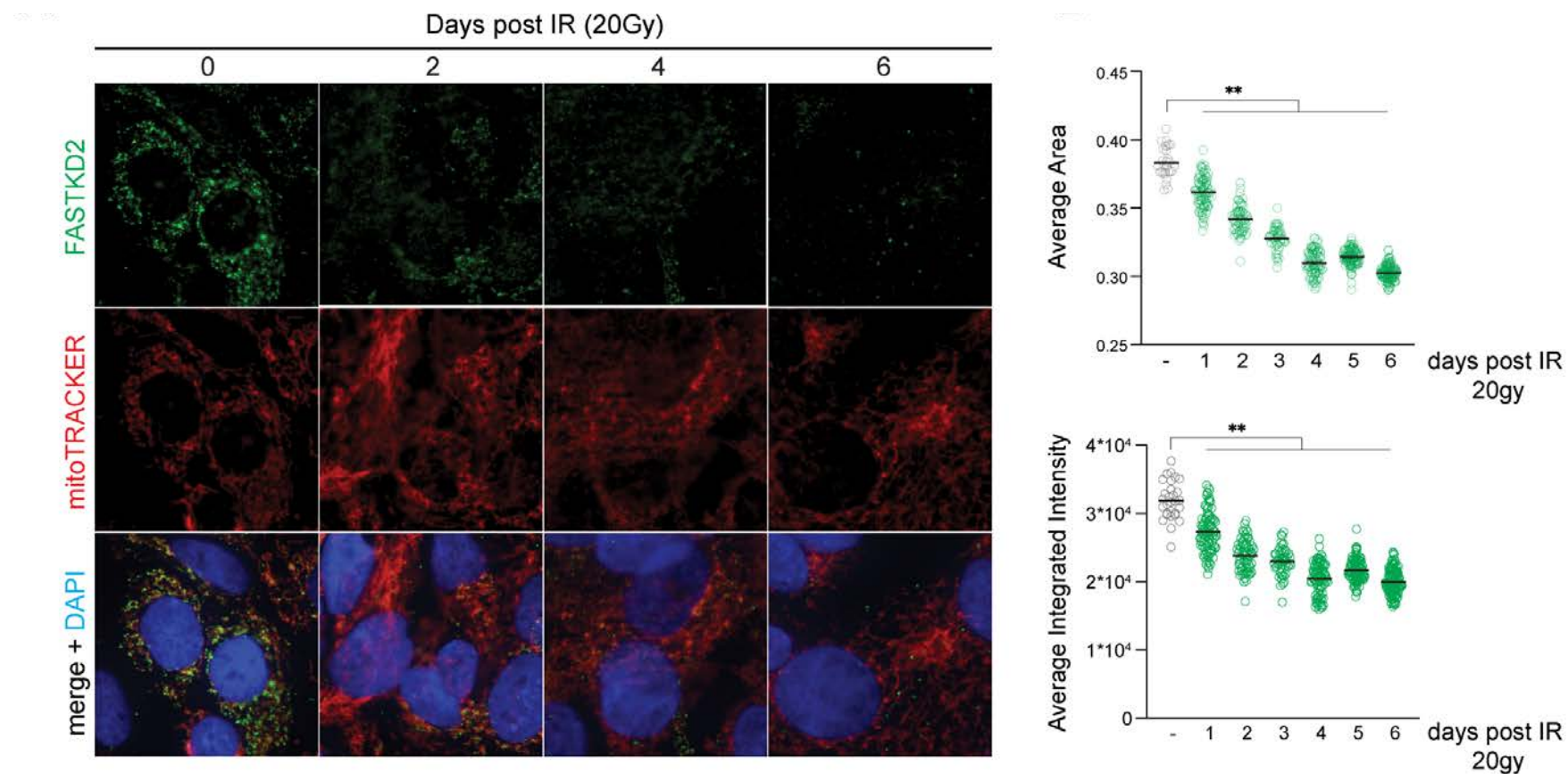
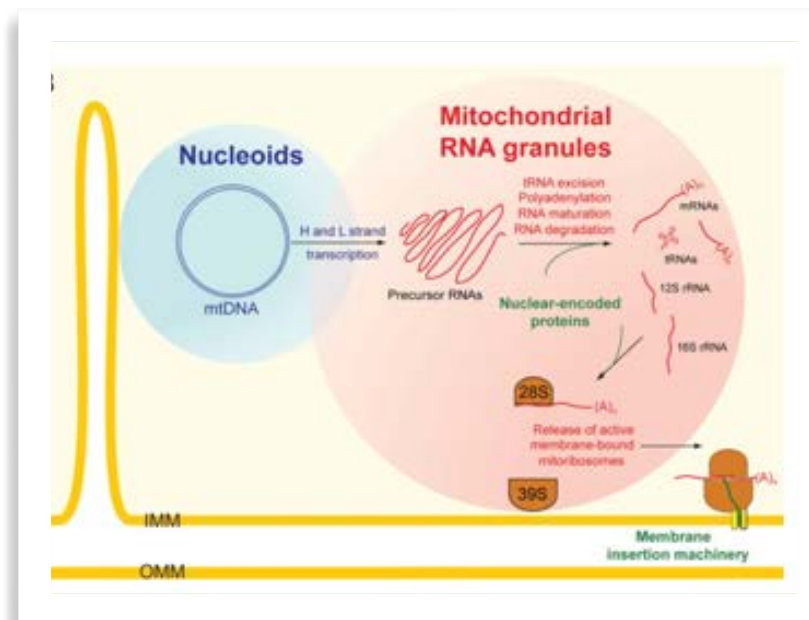


# Cross-kingdom approach to reconstitute DSB repair in mitochondria



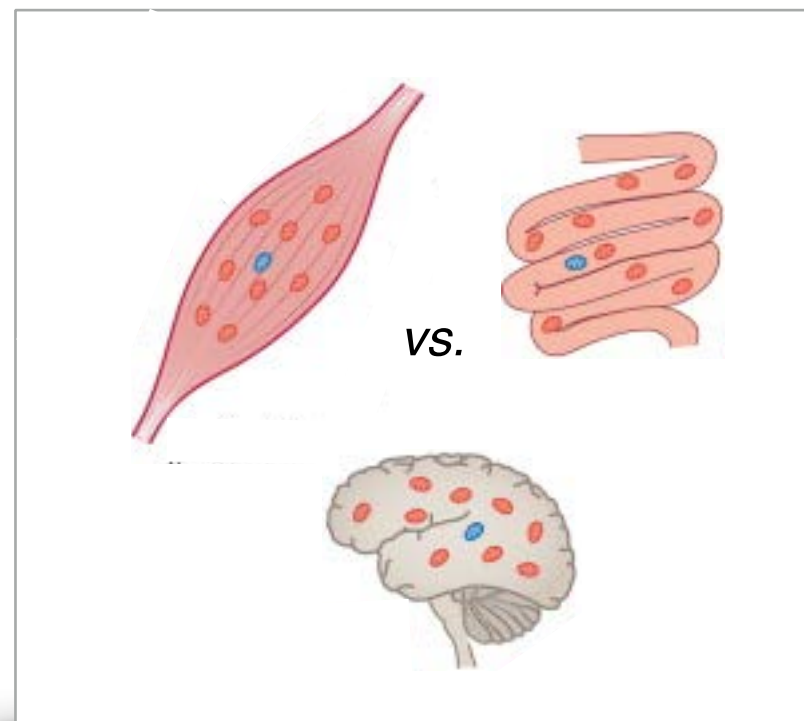
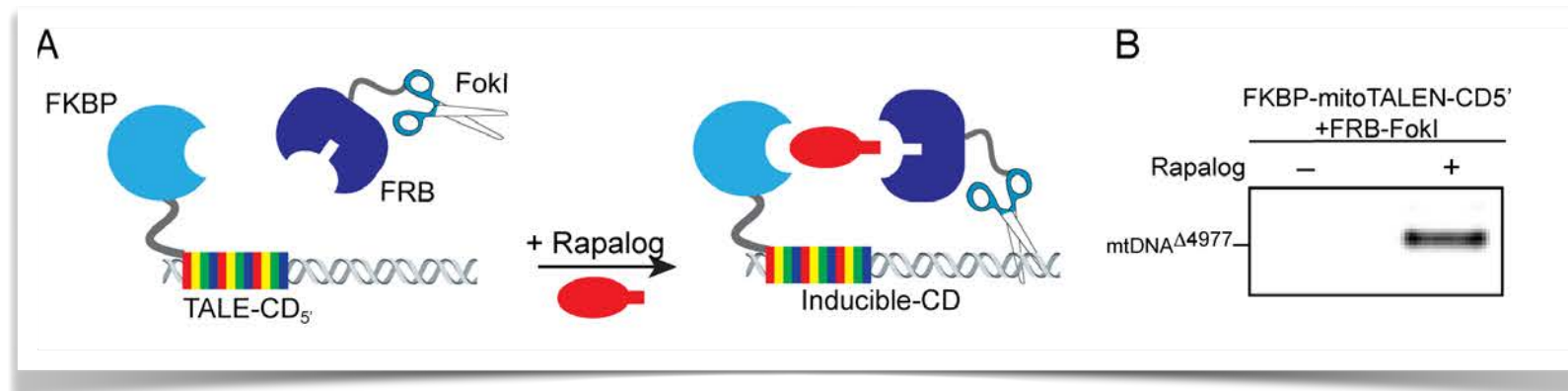


# Mitochondrial RNA granules, a source of cytoplasmic RNA following IR



# Investigating the outcome of the common deletion

## Inducible quasi-dimeric mTLN



- Deletion propagation
- Mitochondrial function
- Cellular/tissue function



# Acknowledgements



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**Positions available**

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