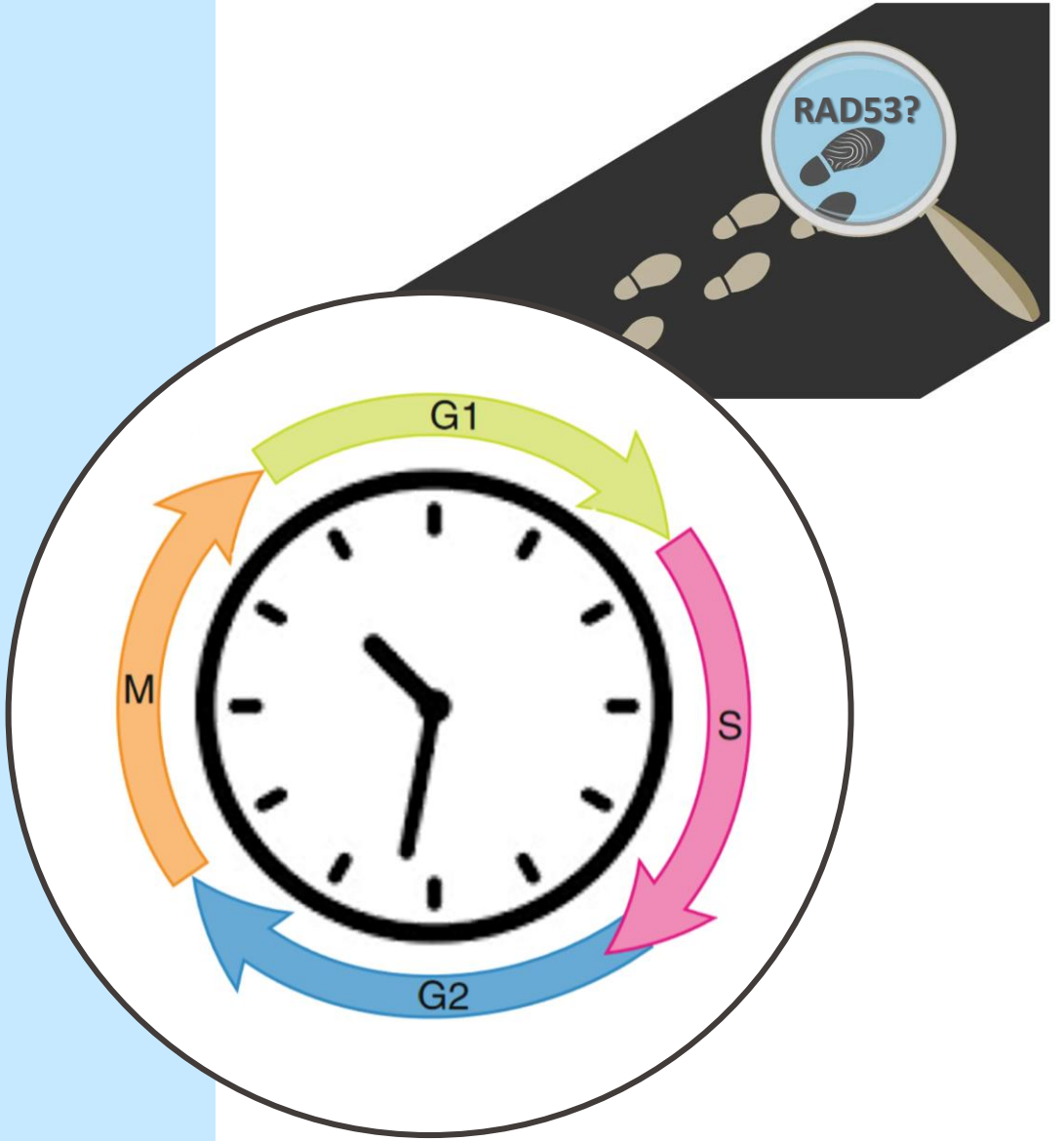
EPFL

# CHRONOBIOLOGY OF CHECKPOINT OVERRIDE

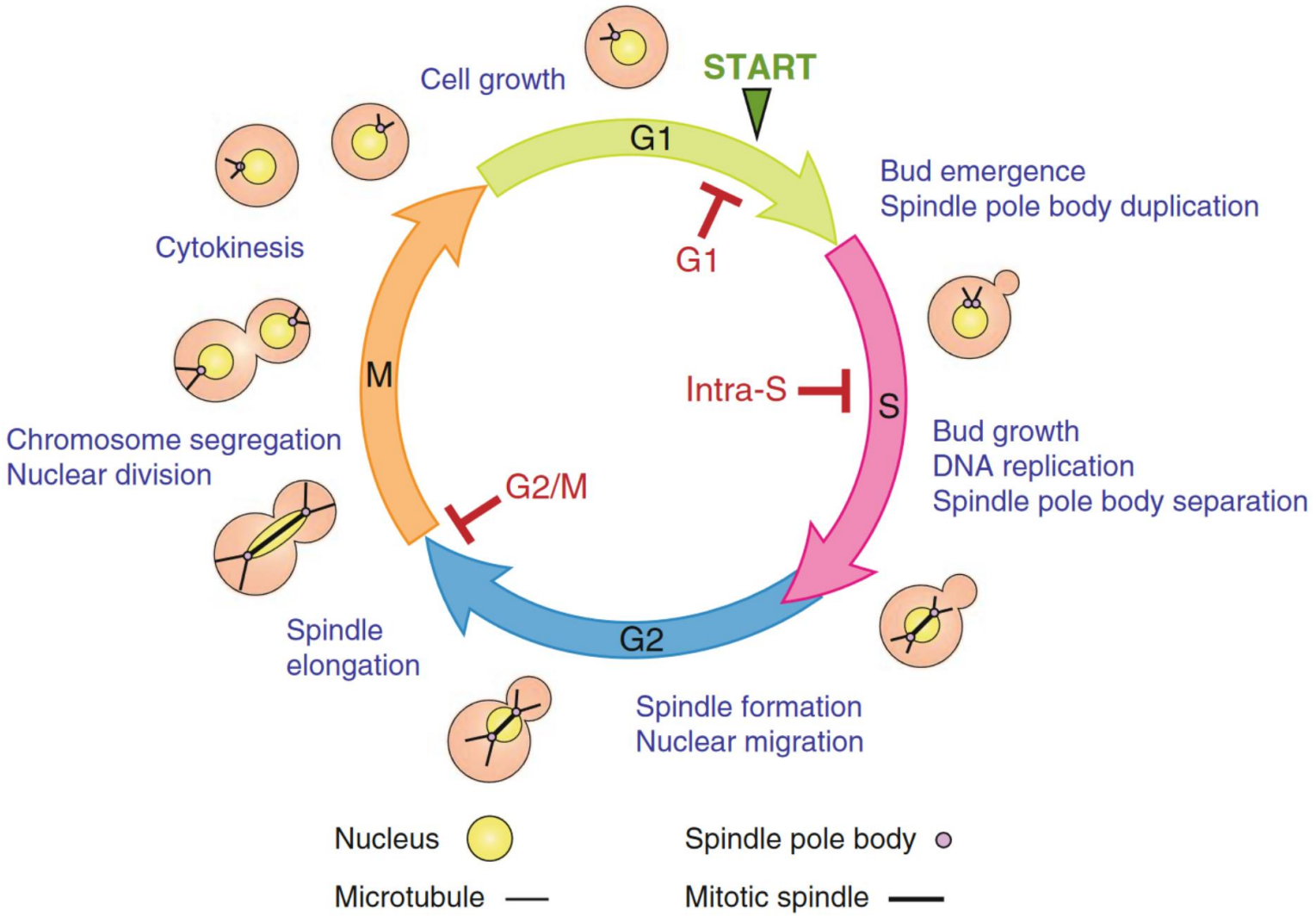



**Shedding light on the key regulator controlling DNA Damage Checkpoint override timing in yeast**

Lorenzo Scutteri (LPBS, EPFL)

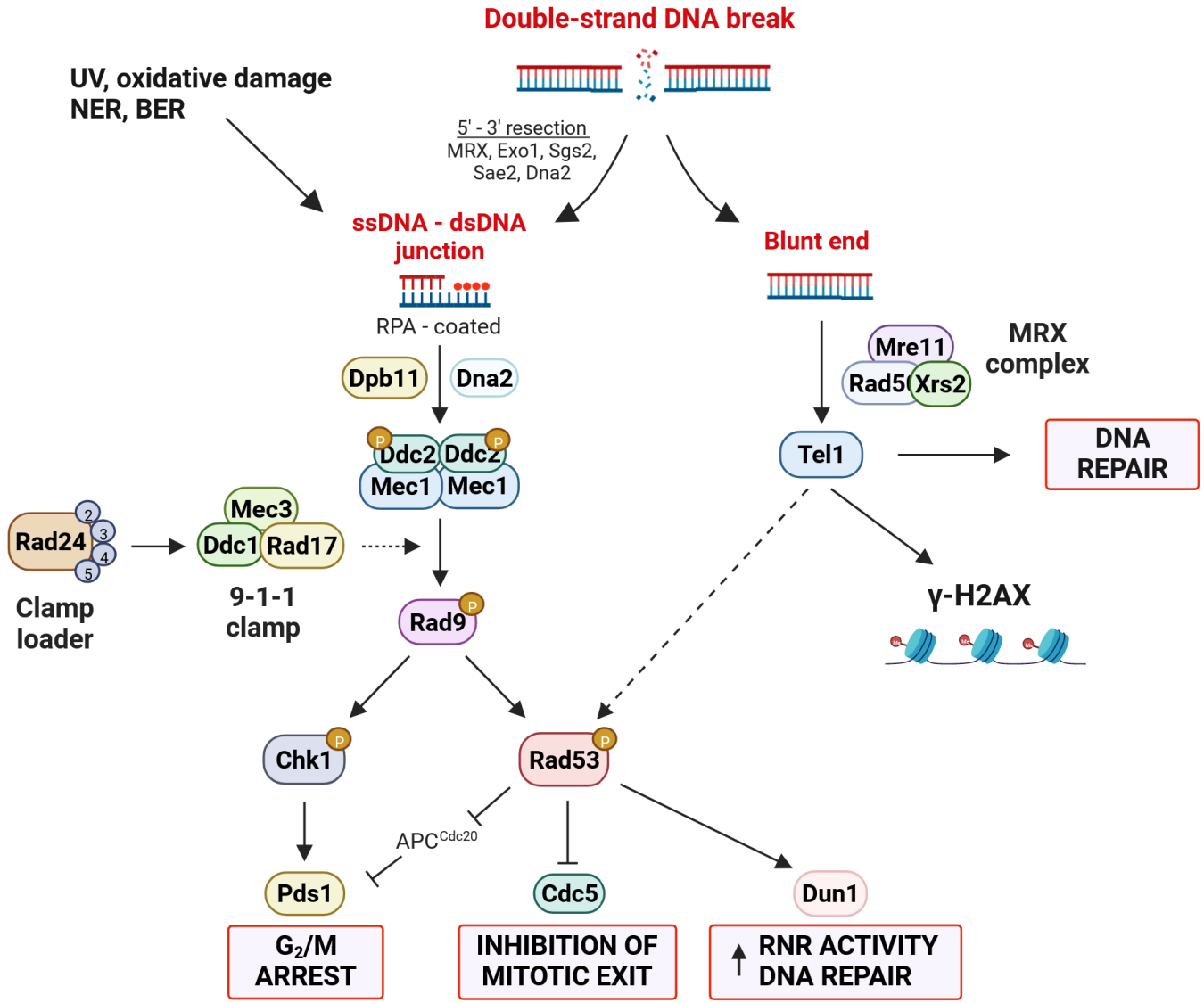
Swiss Physical Society Annual Meeting - 12.09.2024

# CELL CYCLE CHECKPOINTS



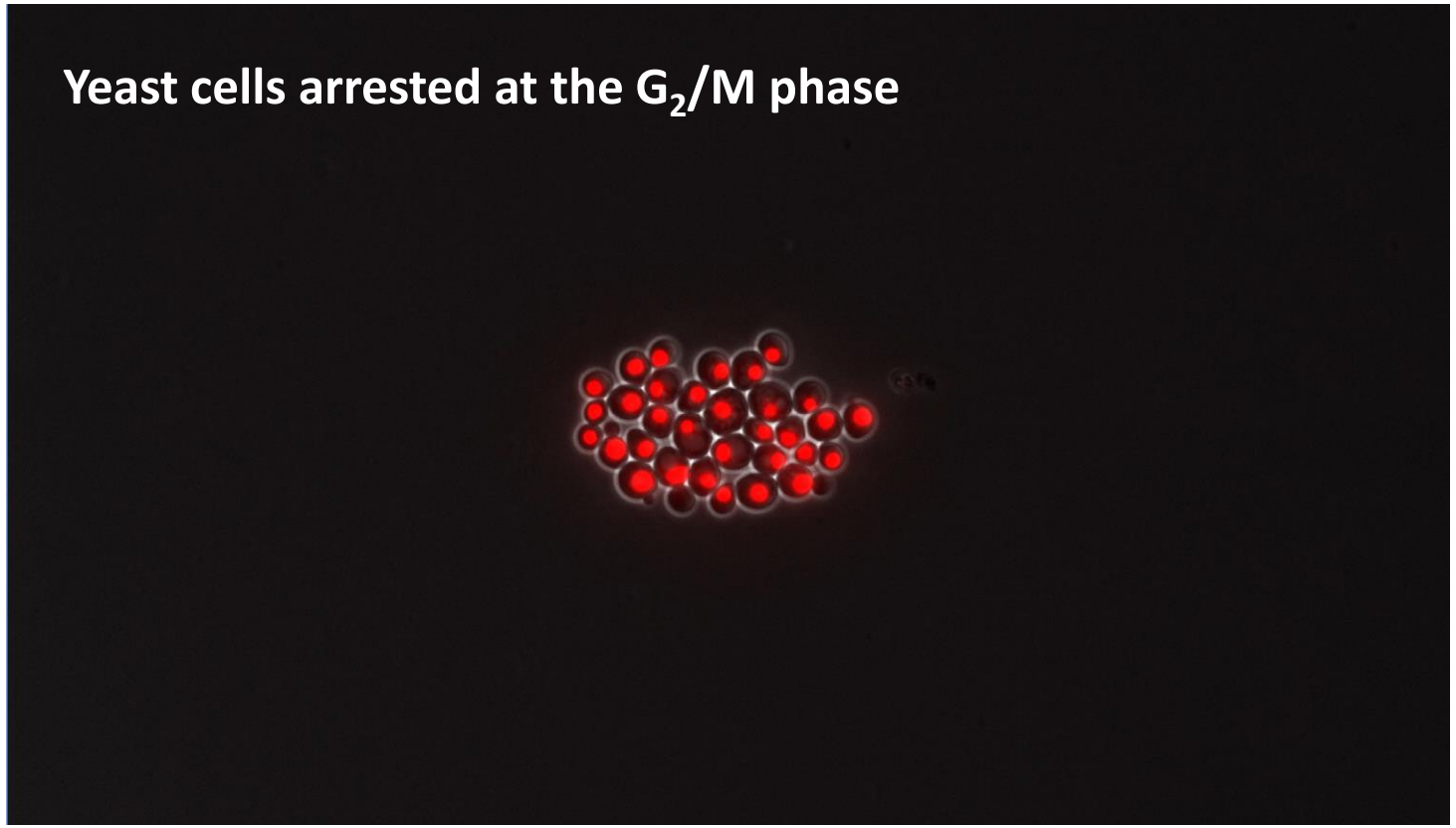

K. Finn et al, CMLS (2011)

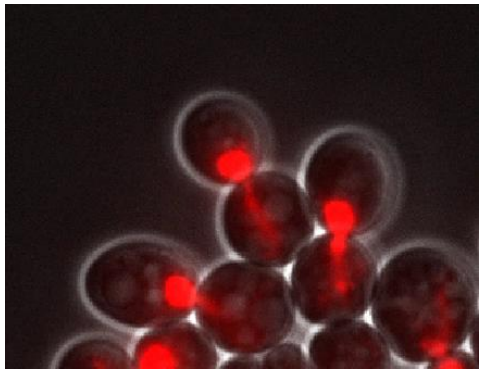
# DNA DAMAGE CHECKPOINT SIGNALING PATHWAY

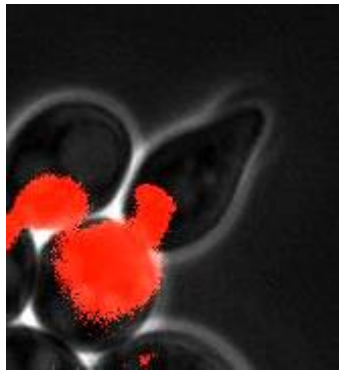


# DNA DAMAGE CHECKPOINT OVERRIDE

Checkpoint override represents a trade-off between risk and speed for offspring maximization

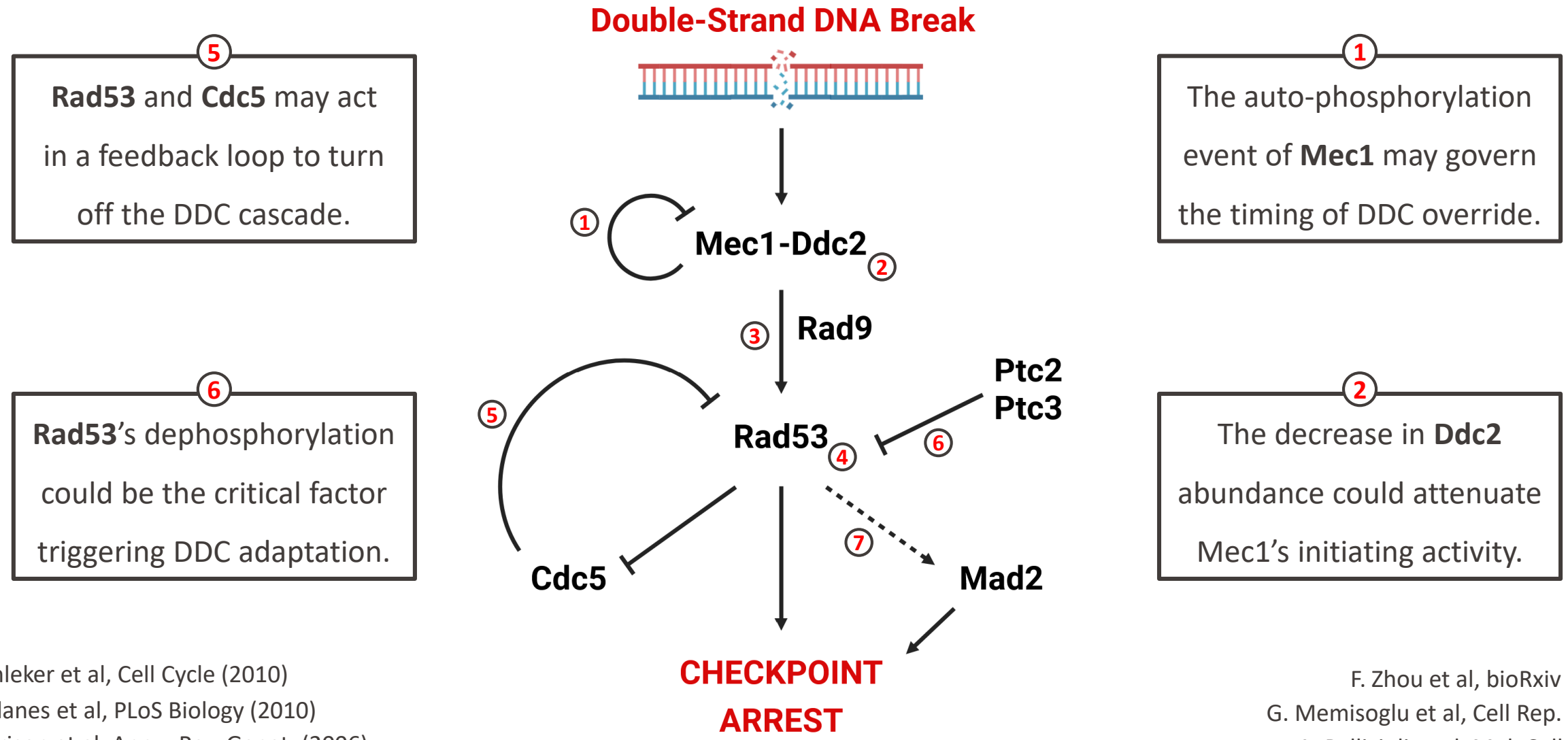




**Examples of DNA Damage Checkpoint override events**

# HOW DOES THE DDC DETERMINE WHEN OVERRIDE OCCURS?




5 **Rad53** and **Cdc5** may act in a feedback loop to turn off the DDC cascade.

1 The auto-phosphorylation event of **Mec1** may govern the timing of DDC override.

6 **Rad53**'s dephosphorylation could be the critical factor triggering DDC adaptation.

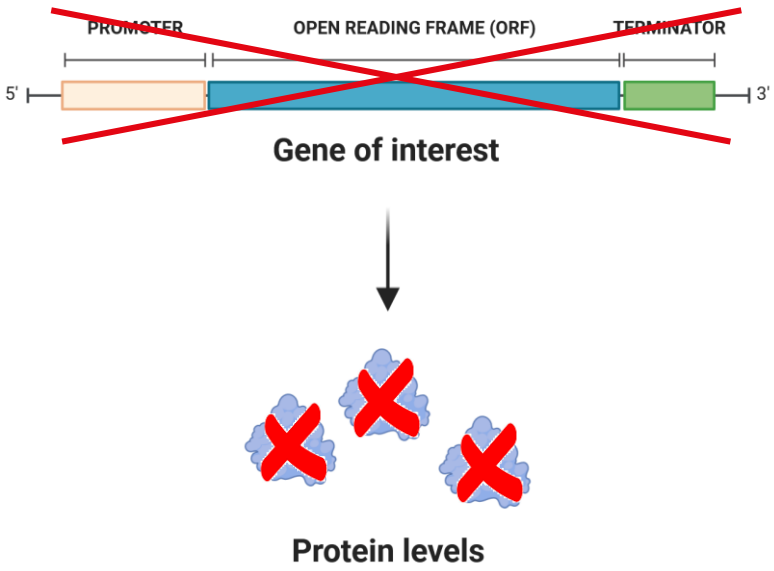
2 The decrease in **Ddc2** abundance could attenuate Mec1's initiating activity.

T. Schleker et al, Cell Cycle (2010)
G. Vidanes et al, PLoS Biology (2010)
J. Harrison et al, Annu. Rev. Genet. (2006)

F. Zhou et al, bioRxiv (2023)
G. Memisoglu et al, Cell Rep. (2019)
A. Pellicioli et al, Mol. Cell (2001)

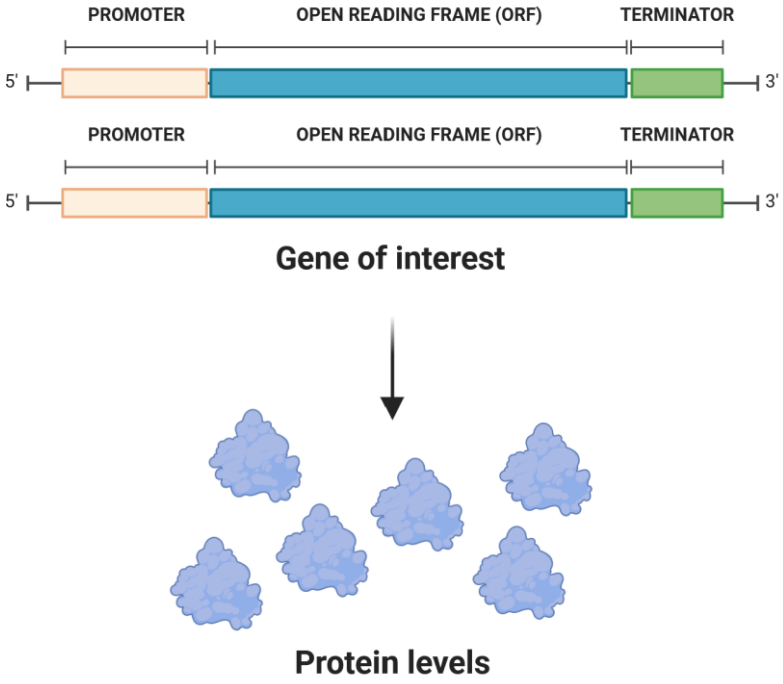
# RESEARCH PLAN

## Wild type scenario

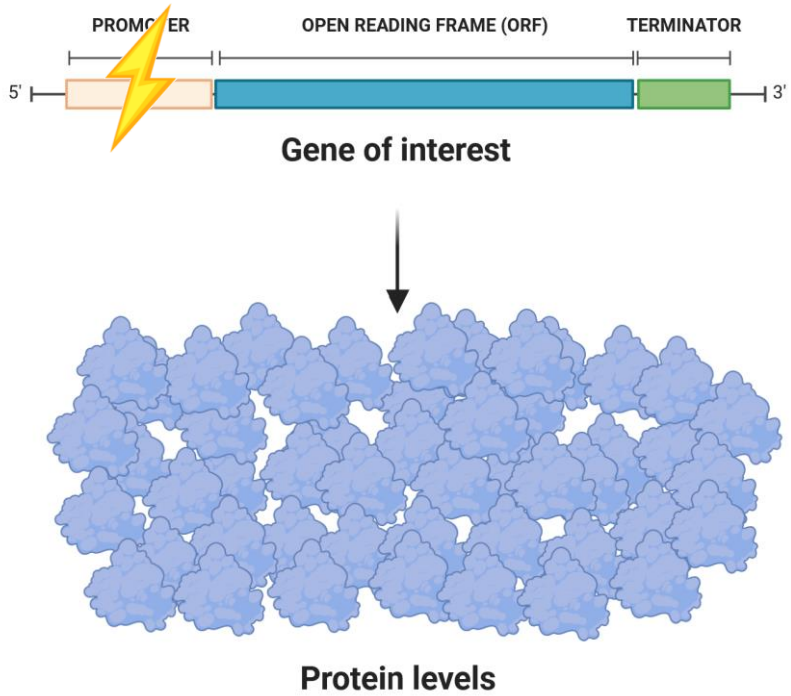



## Gene knockout

## What about adding a 2nd copy of the gene?



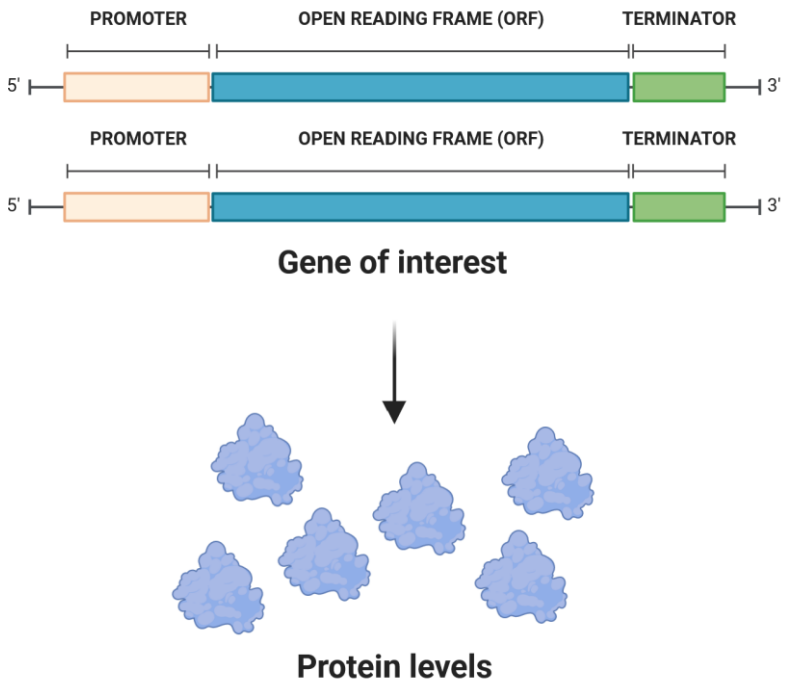

## Strong Over-expression

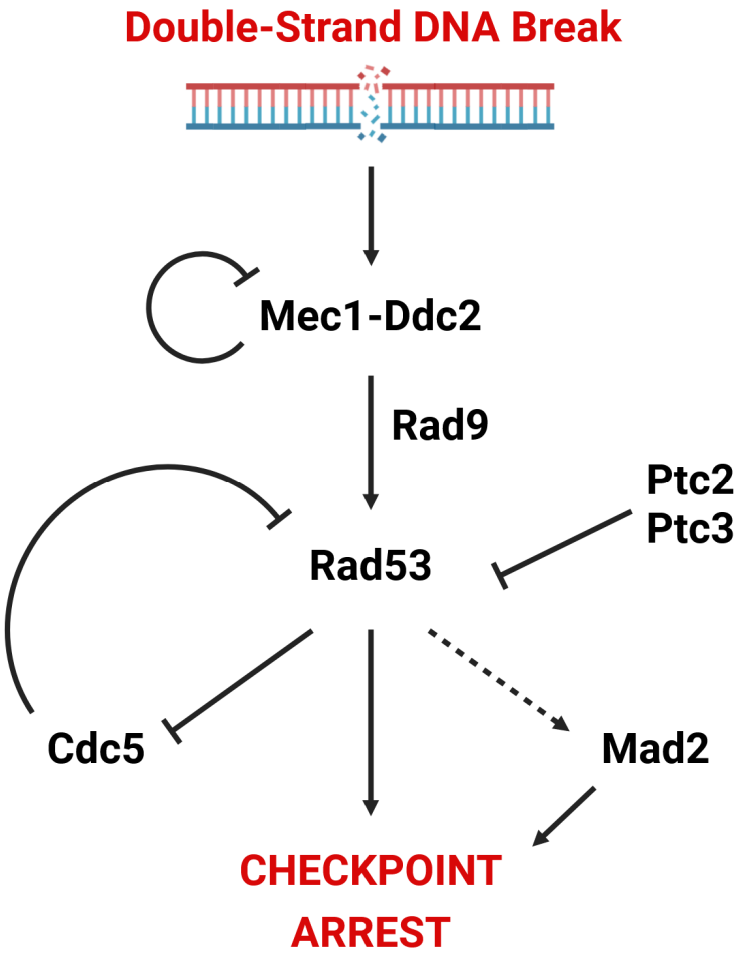


# RESEARCH PLAN

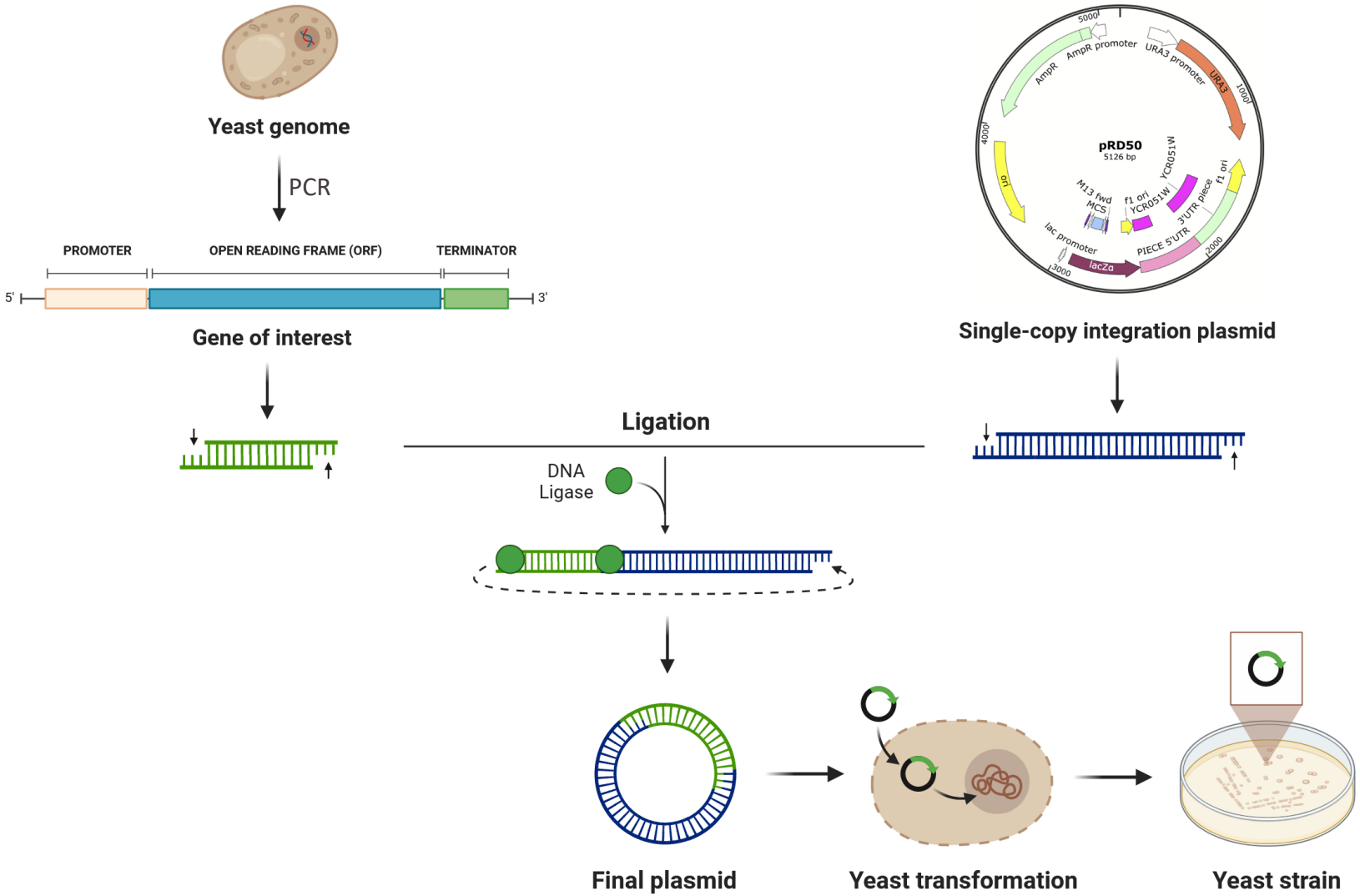
Override time should be extremely sensitive to the presence of a 2nd copy of the key regulator

## What about adding a 2nd copy of the gene?



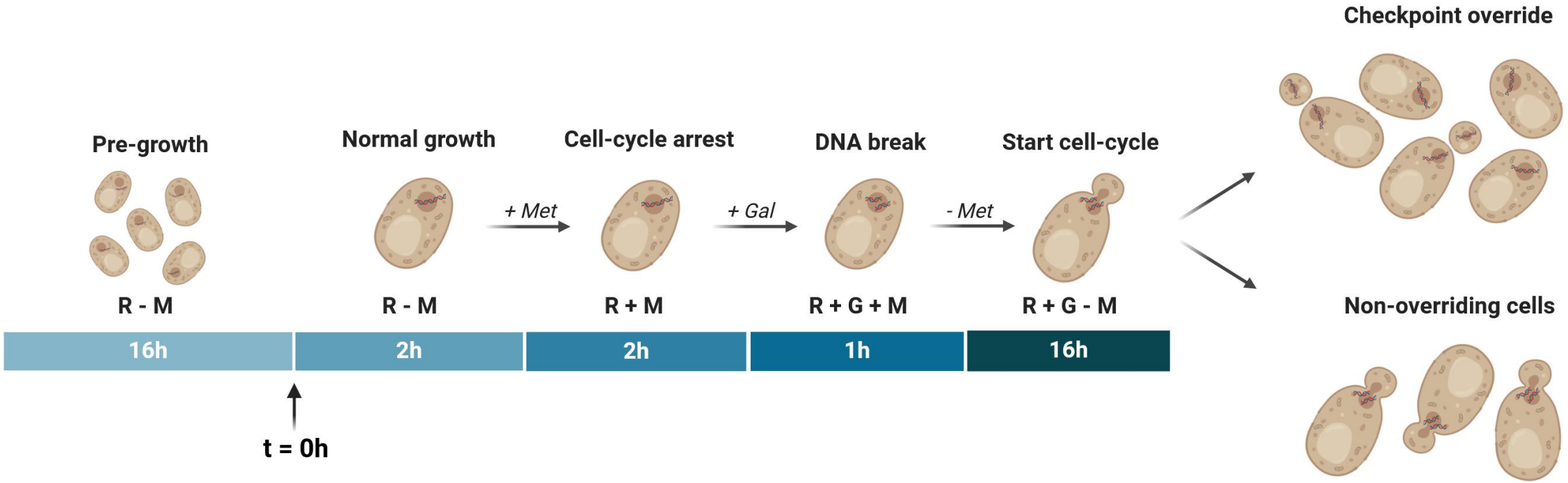
# CLONING STRATEGY



# INVESTIGATE OVERRIDE TIME USING MICROFLUIDIC CHIPS

**Genotype yeast strain**:

*cln1-3Δ MET3pr-CLN2 GAL1pr-HO URA3-HOcs HTB2-mCherry MATα-syn*



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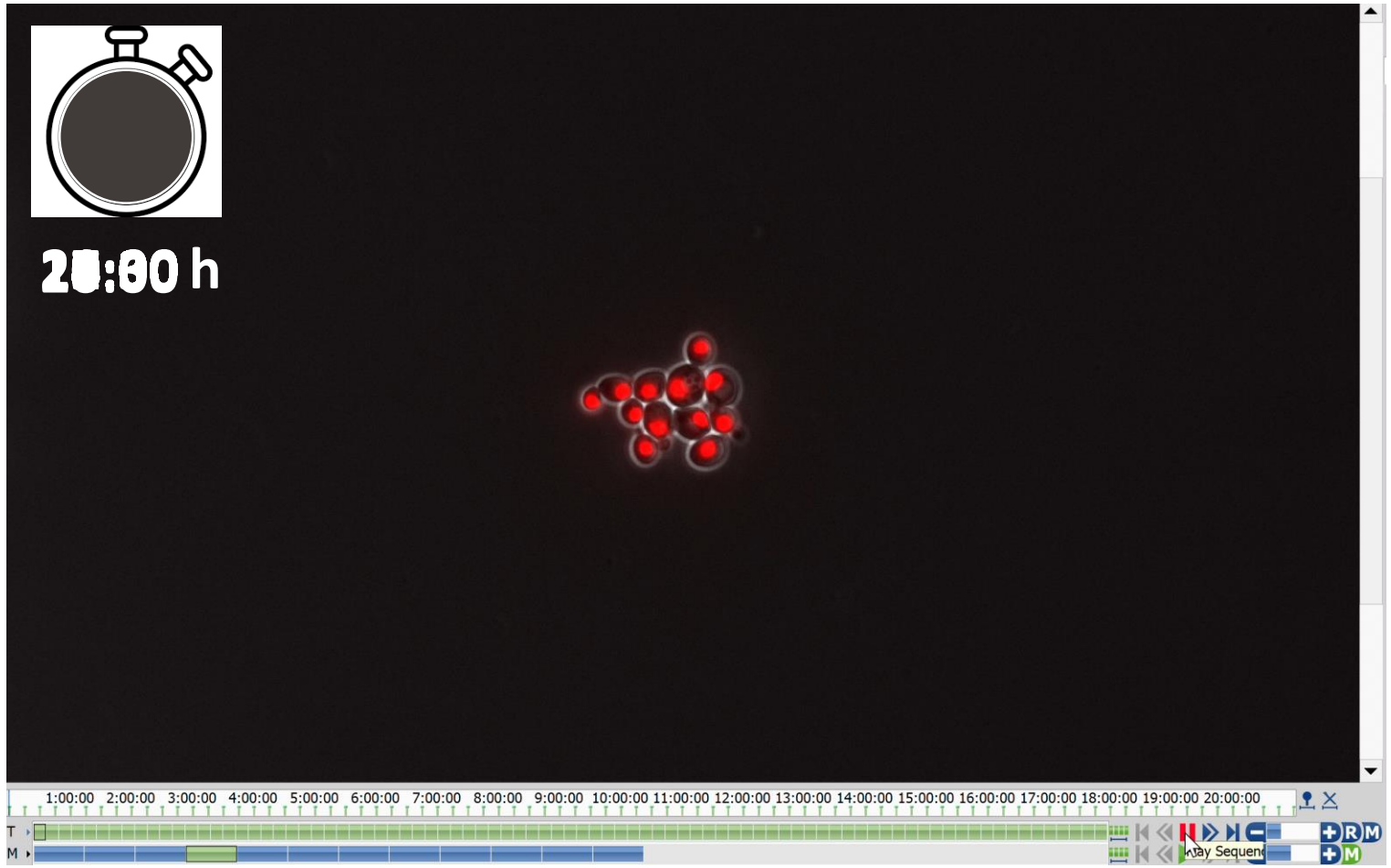
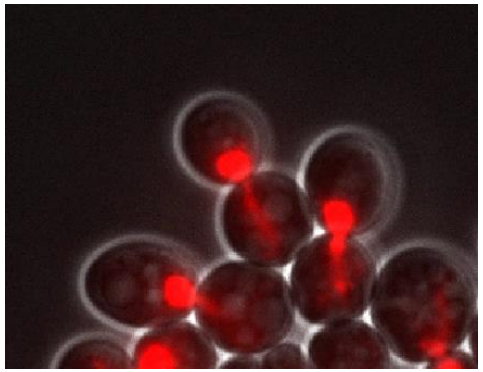
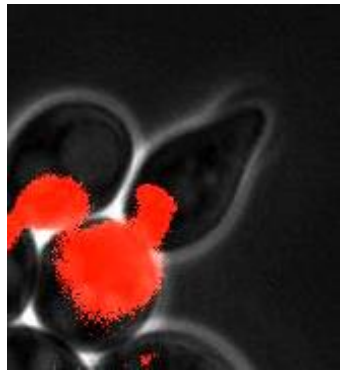


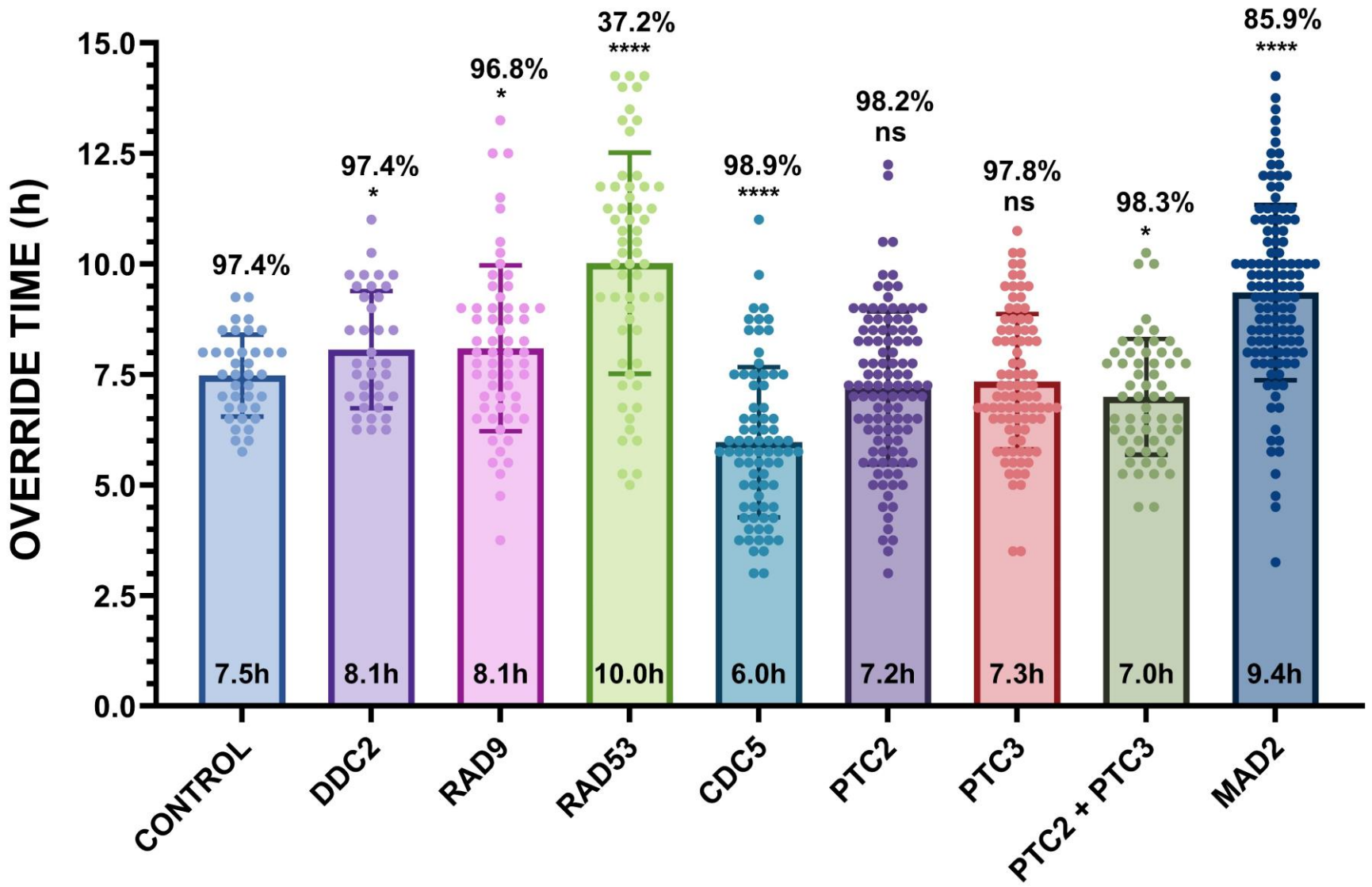





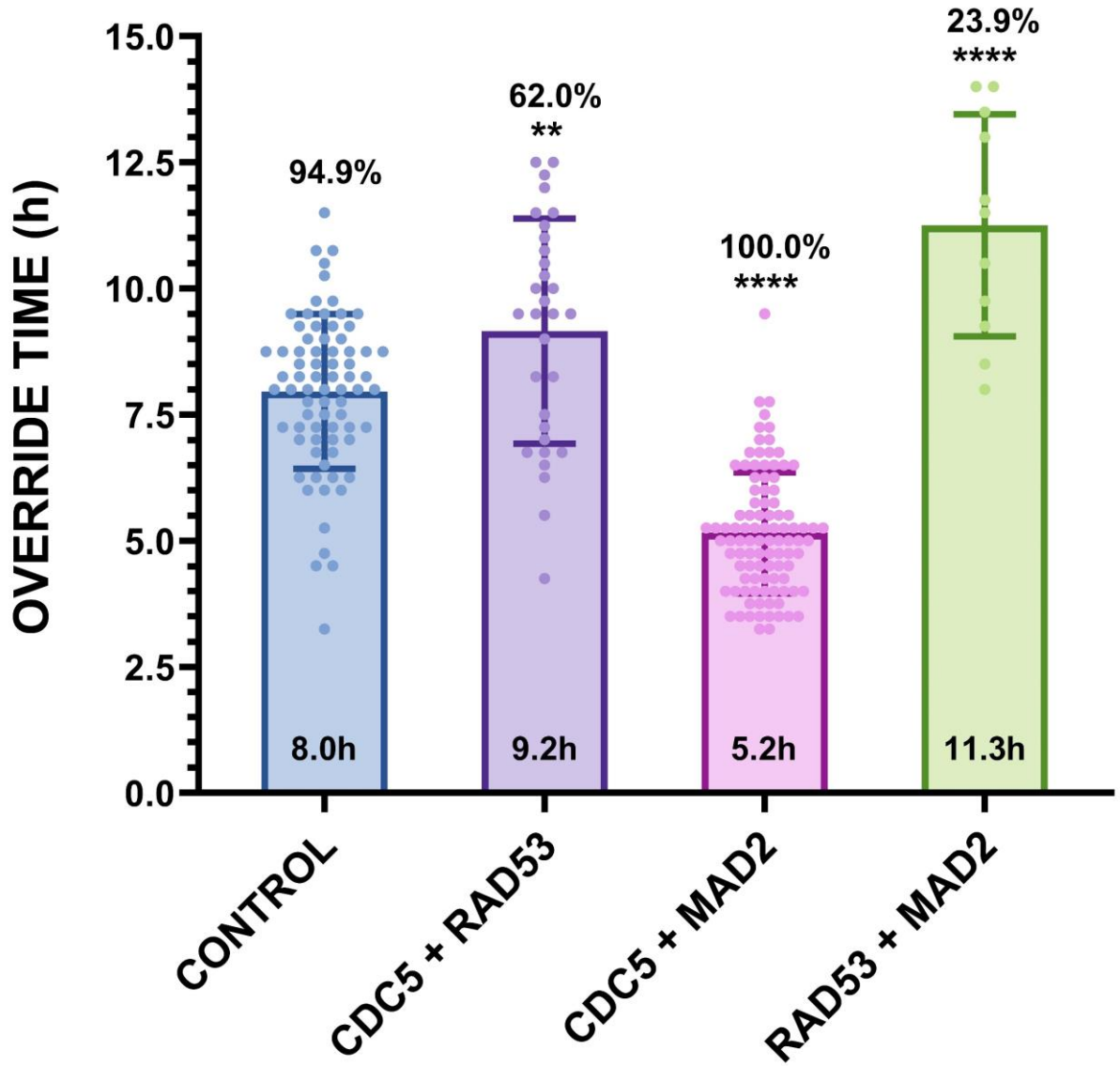
**Examples of DNA Damage Checkpoint override events**

$$\textit{Override time} = \textit{Division time} - \textit{Budding time}$$

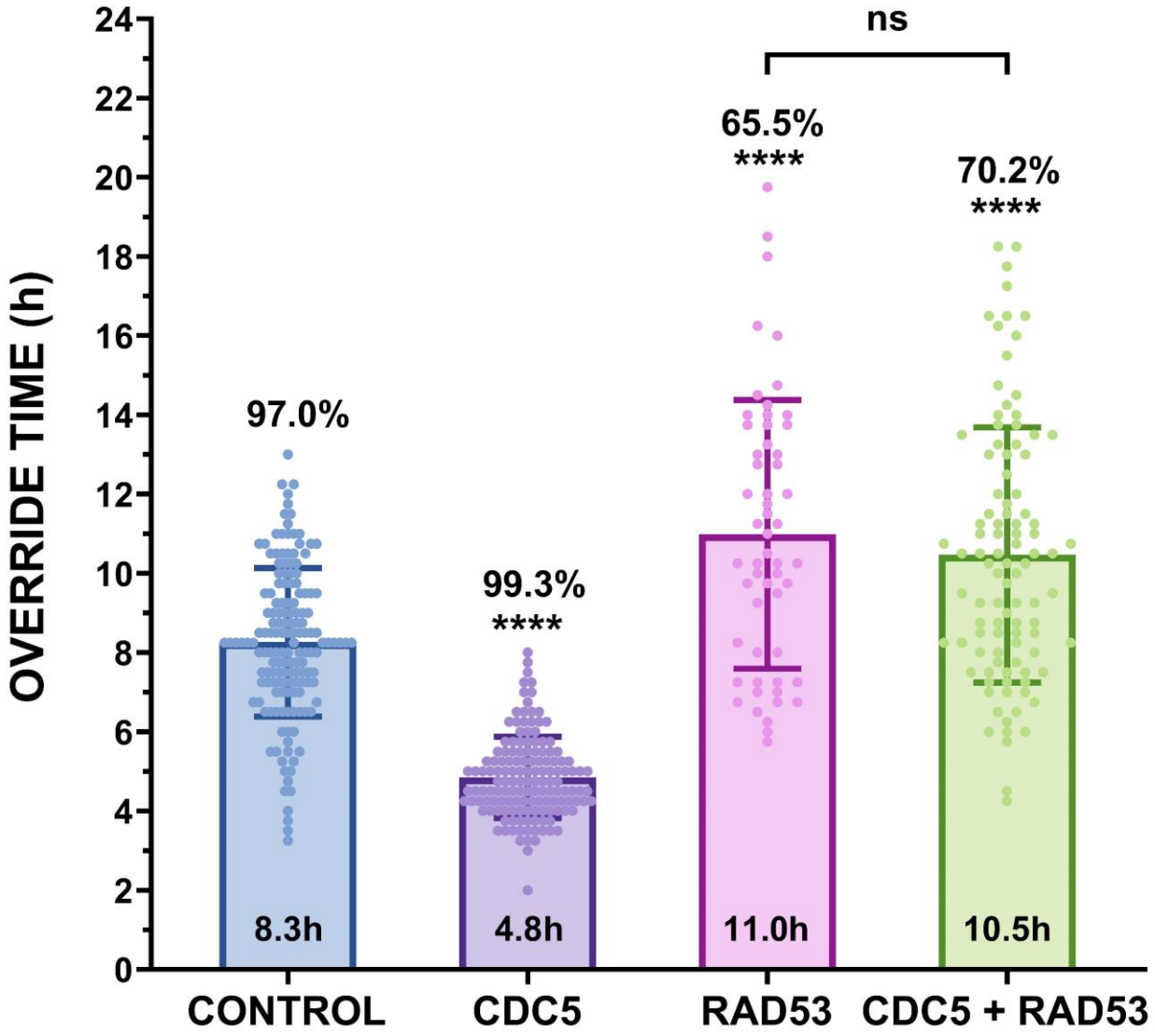
# OVERRIDE TIMES FOR 2nd COPIES OF DDC REGULATORS



# OVERRIDE TIMES FOR COMBINATION OF 2nd COPIES



# RAD53 CONTROLS OVERRIDE TIMING OVER CDC5

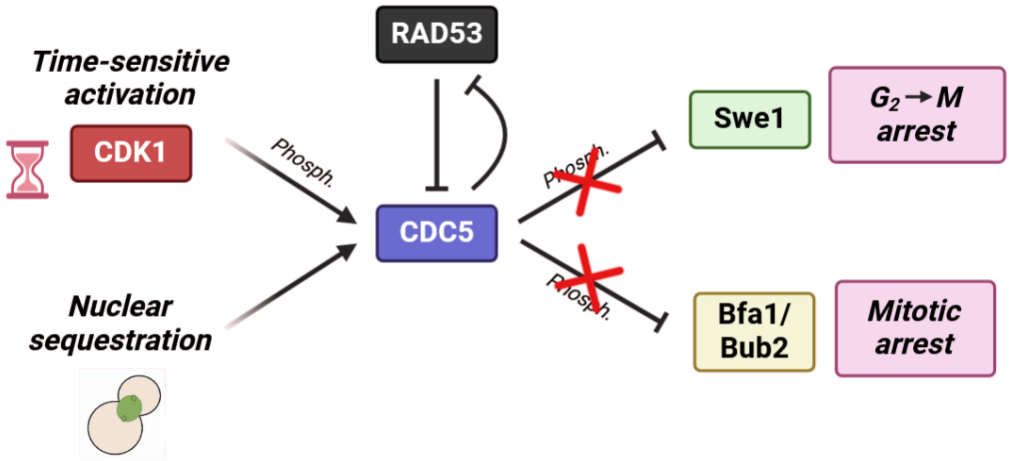



**Number of overriding cells**

- Control: 161/166
- CDC5: 144/145
- RAD53: 55/84
- CDC5+RAD53: 99/141

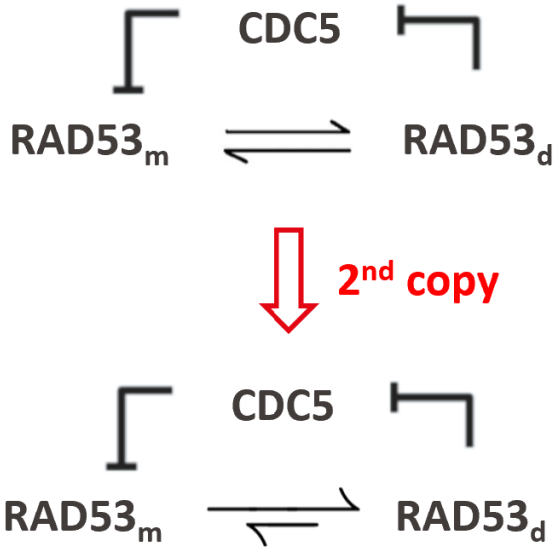
# MODELS AND HYPOTHESES

## RAD53 SEQUESTERS CDC5 IN THE NUCLEUS




Cdc5 targets its substrates in a timely manner by changing subcellular localization throughout the cell-cycle.

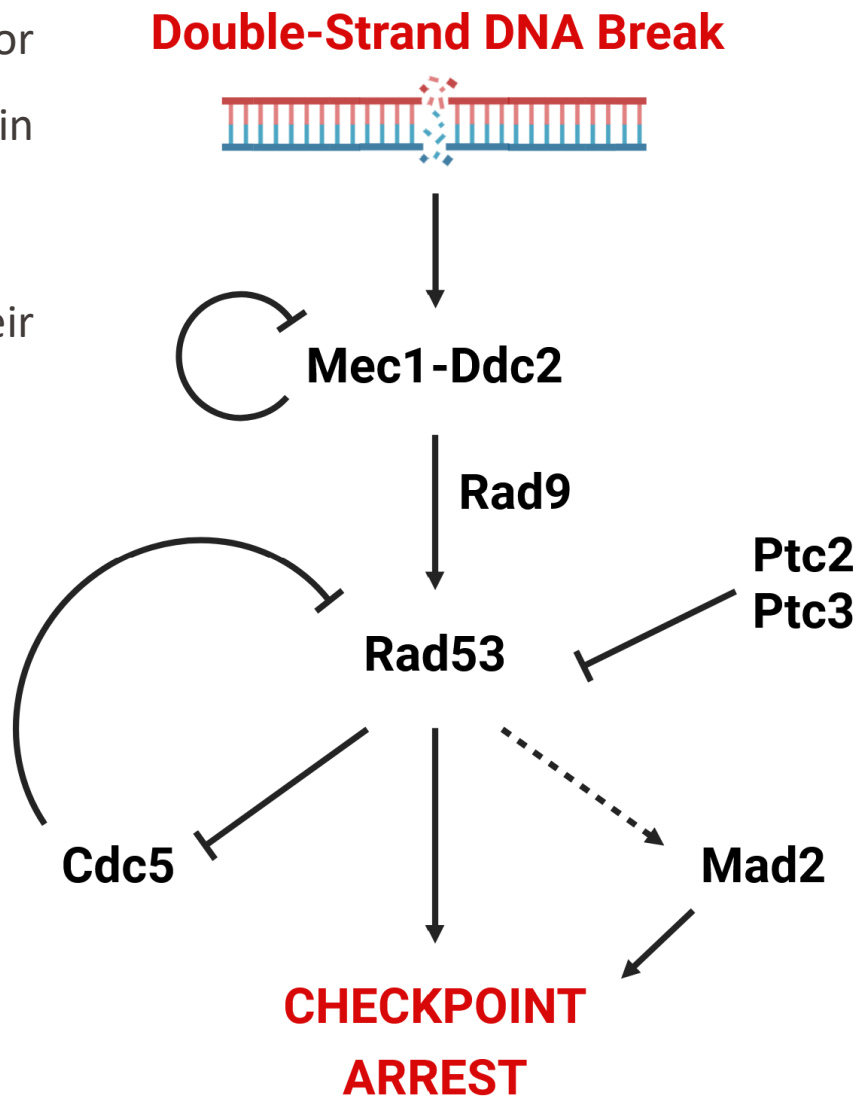
## CDC5 ACTS BY INHIBITING RAD53 DIMERIZATION




Cdc5 inhibits Rad53 by phosphorylating the dimer interface, preventing homo-dimerization. The 2nd copy of Rad53 increases the dimer:monomer ratio.

# CONCLUSION

- **DNA Damage Checkpoint override** has significant implications for cancer biology, yet its regulation and temporal dynamics remain poorly understood.
- We **expressed a 2nd copy of the key DDC regulators** to explore their impact on checkpoint override timing at the single-cell level.
- Our findings indicate two critical elements acting as key timers:
  - **RAD53 - CDC5 feedback loop**
  - **Handoff DDC → SAC**
- Future research directions:
  - **Testing CDC5 tagged with NLS and NES**
  - **Mutants of CDC5 and RAD53 with no reciprocal inhibition**
  - **Fluorescent labeling of CDC5 and RAD53**



# ACKNOWLEDGEMENTS

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Prof. Patrick Barth
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