



# Redundant Regulation of Cdk1 Tyrosine Dephosphorylation in *Saccharomyces cerevisiae*

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## Introduction

In all eukaryotes, the Wee1 protein kinase acts as a negative regulator of the entry into mitosis by phosphorylating and inhibiting Cyclin-dependent kinase (Cdk1). Cdc25 phosphatase works antagonistically as positive regulator by dephosphorylation of Cdk1, allowing its activation and entry into mitosis. Wee1/Cdc25 regulation of mitosis is a target of both the DNA damage and a G2 cell size checkpoints

Cdc25 is an essential mitotic inducer in the fission yeast, *Schizosaccharomyces pombe*, where it was first identified. In the budding yeast, *Saccharomyces cerevisiae*, however, the deletion of *MIH1* (the *cdc25* homologue) is viable and has little phenotype. Our lab has recently shown that two other phosphatases, Ptp1 and PP2A-Rts1/B56, function redundantly with Mih1, and mutation of all three phosphatases is lethal.

This recent work, however, was unable to determine if this mutant combination arrests the cell cycle. This current project is focused on developing an assay to answer this question. A yeast strain in which Swe1 (the budding yeast Wee1) was created and tagged with an auxin inducible degron (AID) which allows the rapid degradation of Swe1-AID when grown in the presence of auxin. This strain is then used to test if a Swe1-dependent cell cycle arrest is maintained after degradation of Swe1 when the three redundant phosphatases are also mutated.

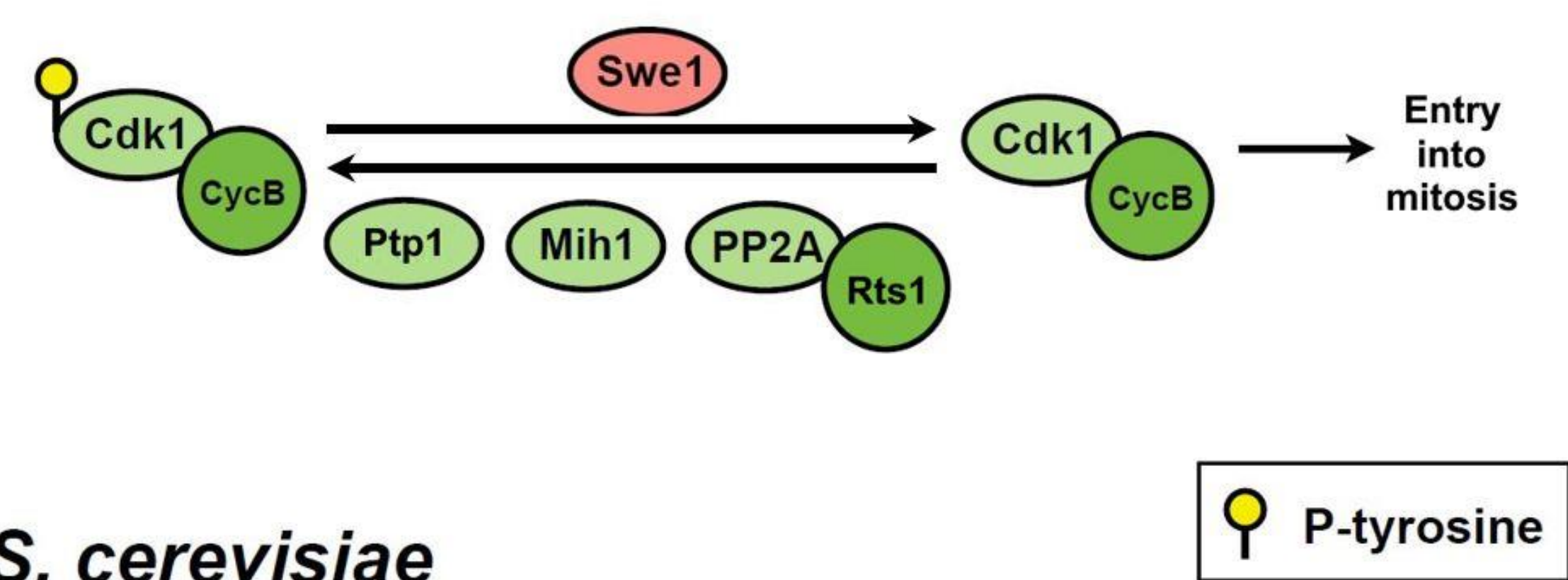


Figure 1. Redundant dephosphorylation of Cdk1 in budding yeast. In budding yeast, Swe1 phosphorylates Cdk1 while Mih1, Ptp1 and PP2A<sup>Rts1</sup> dephosphorylates Cdk1. Figure modified from Lianga, 2014.

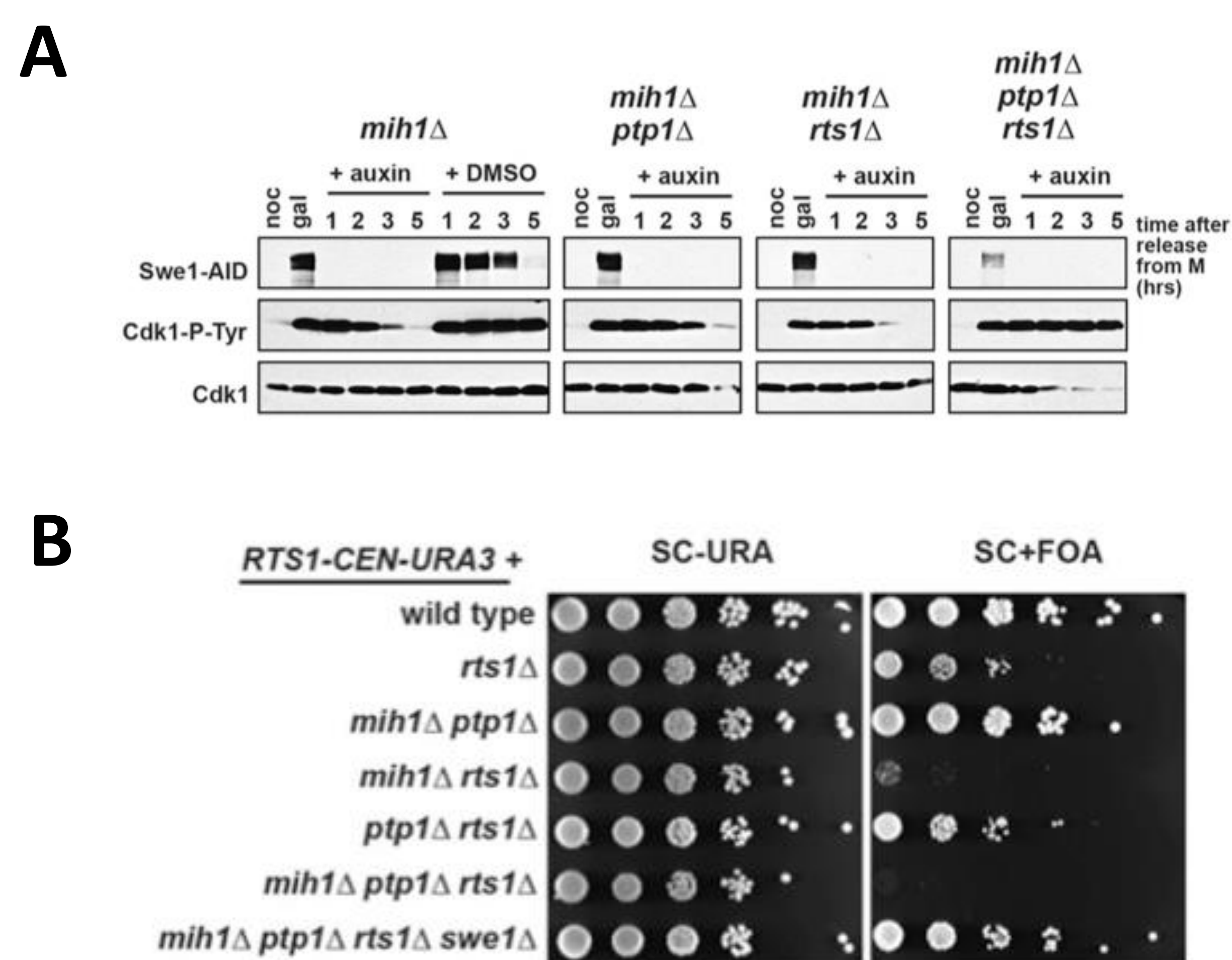
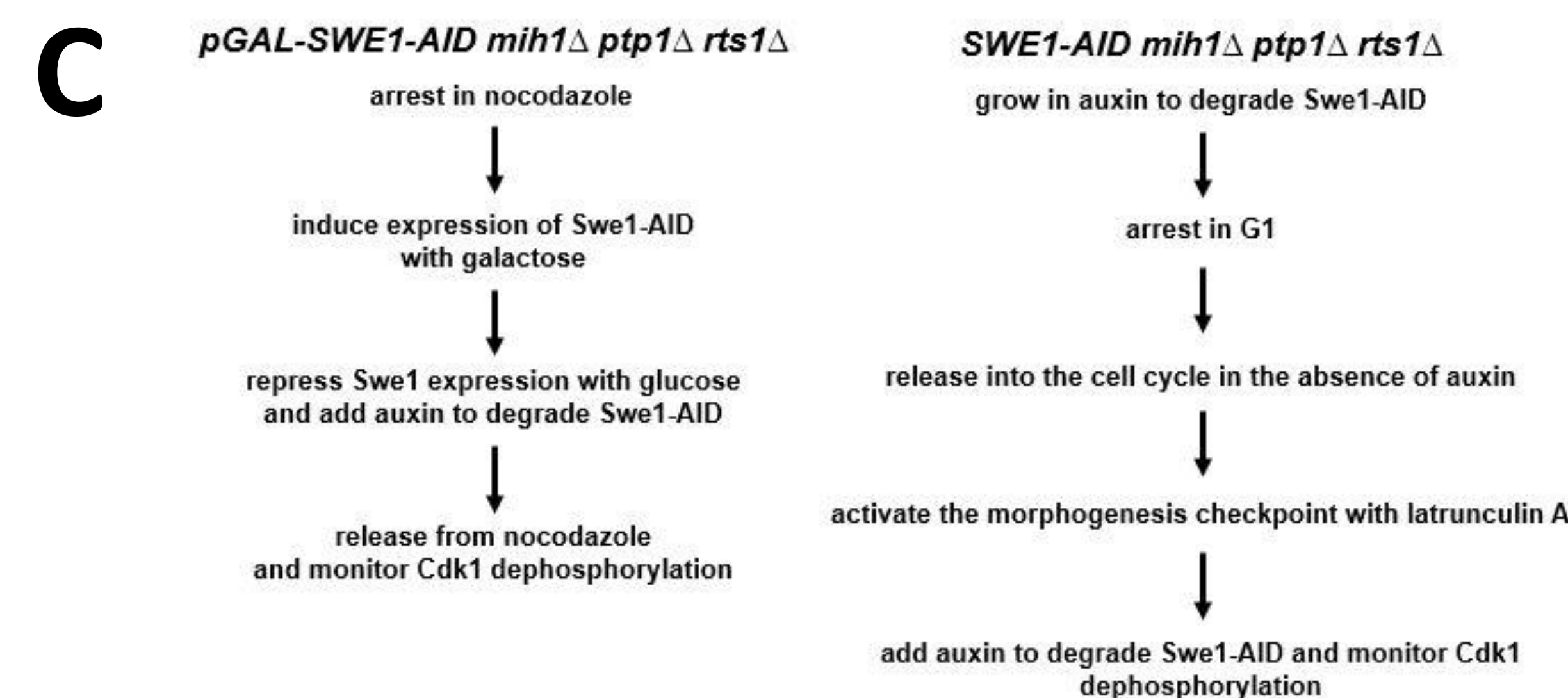
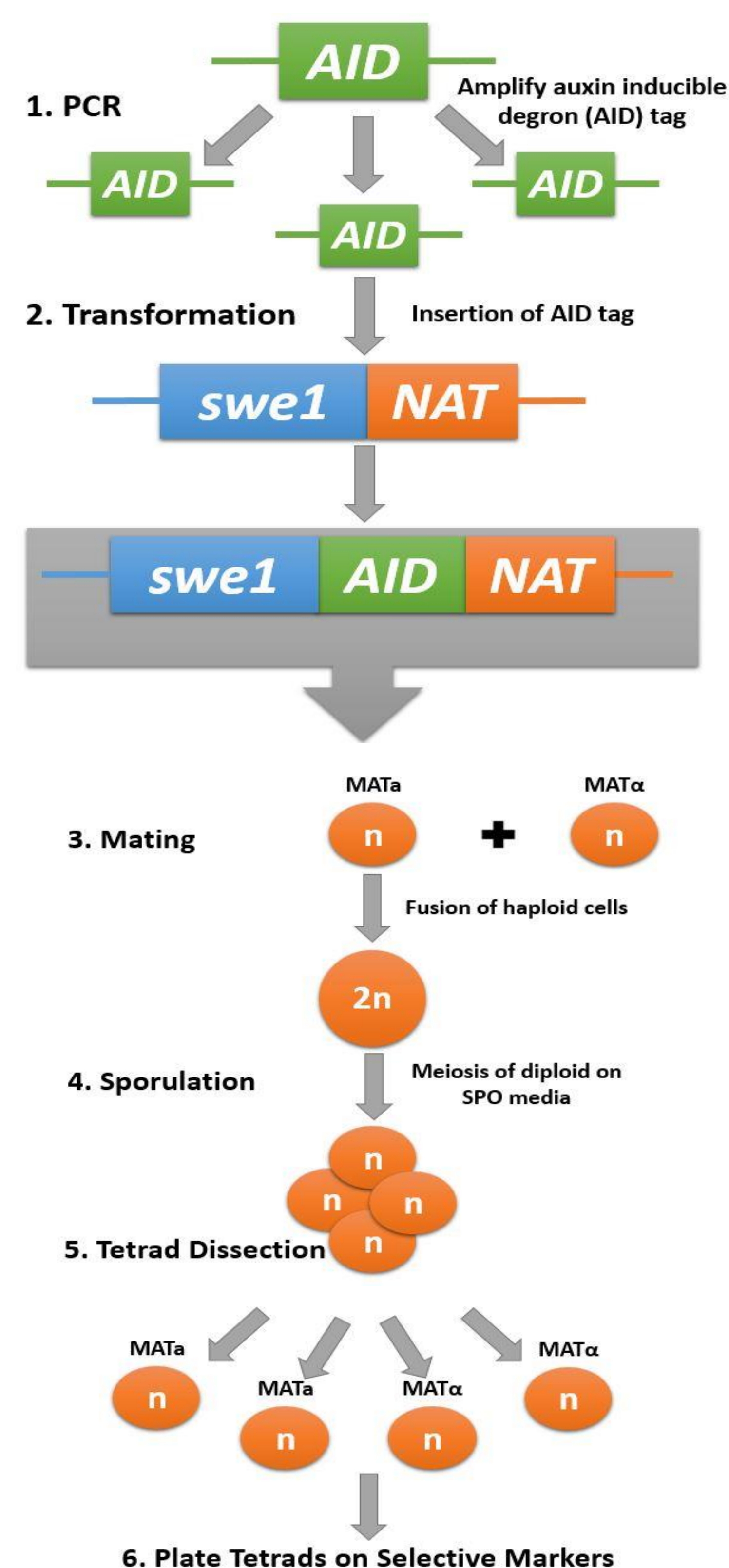


Figure 2: *MIH1*, *PTP1*, and *RTS1* redundantly dephosphorylate Cdk1-Y19. (A) *mih1Δ ptp1Δ rts1Δ* cells block tyrosine dephosphorylation *in vivo*. (B) *mih1Δ rts1Δ* and *mih1Δ ptp1Δ rts1Δ* cells are inviable, and this inviability is rescued by *swe1Δ*. Figures are obtained from Kennedy, 2016. (C) Flowchart of experiments with *pGAL-SWE1-AID mih1Δ ptp1Δ rts1Δ* and *SWE1-AID mih1Δ ptp1Δ rts1Δ*.



## Methodology



## Results

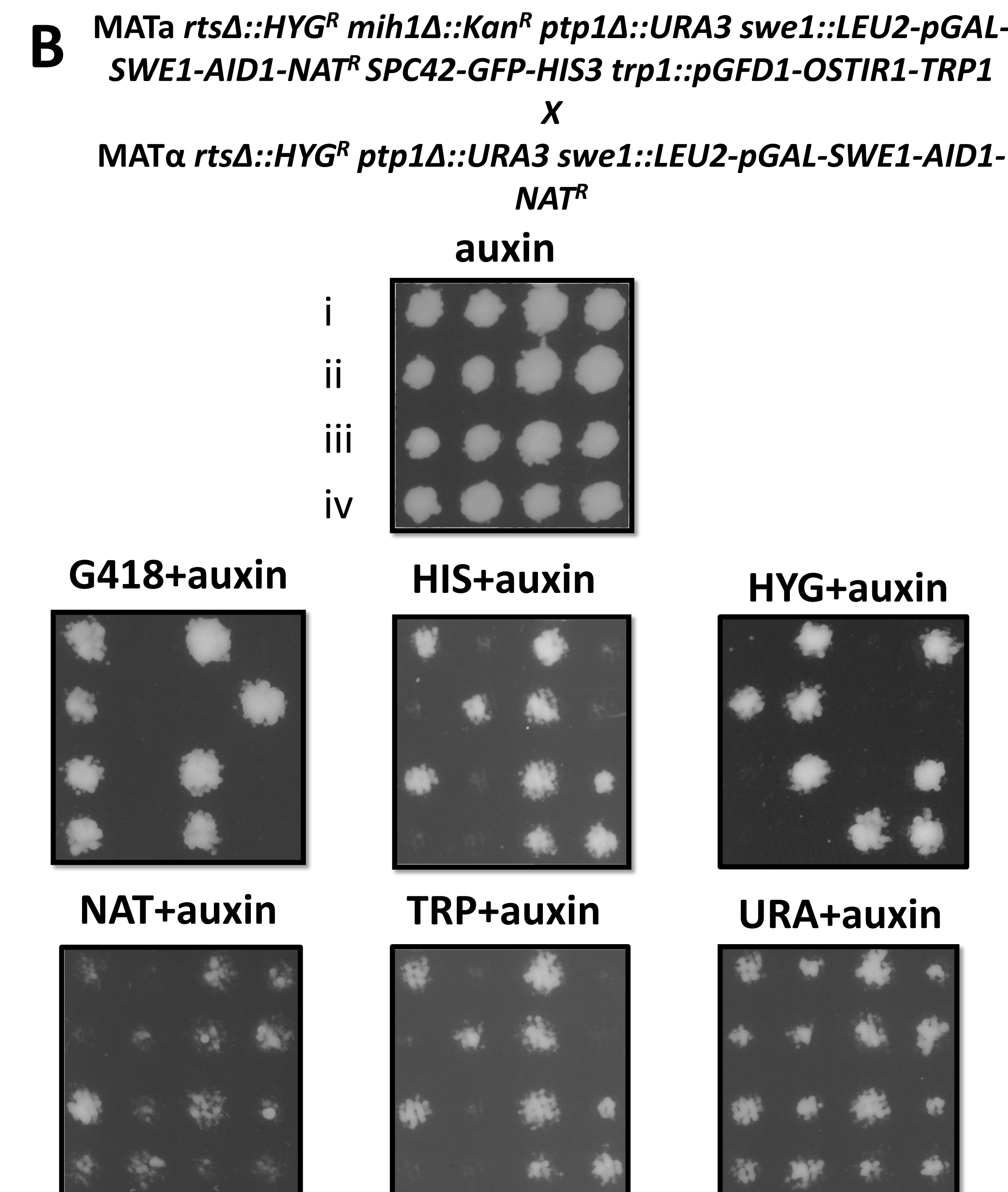
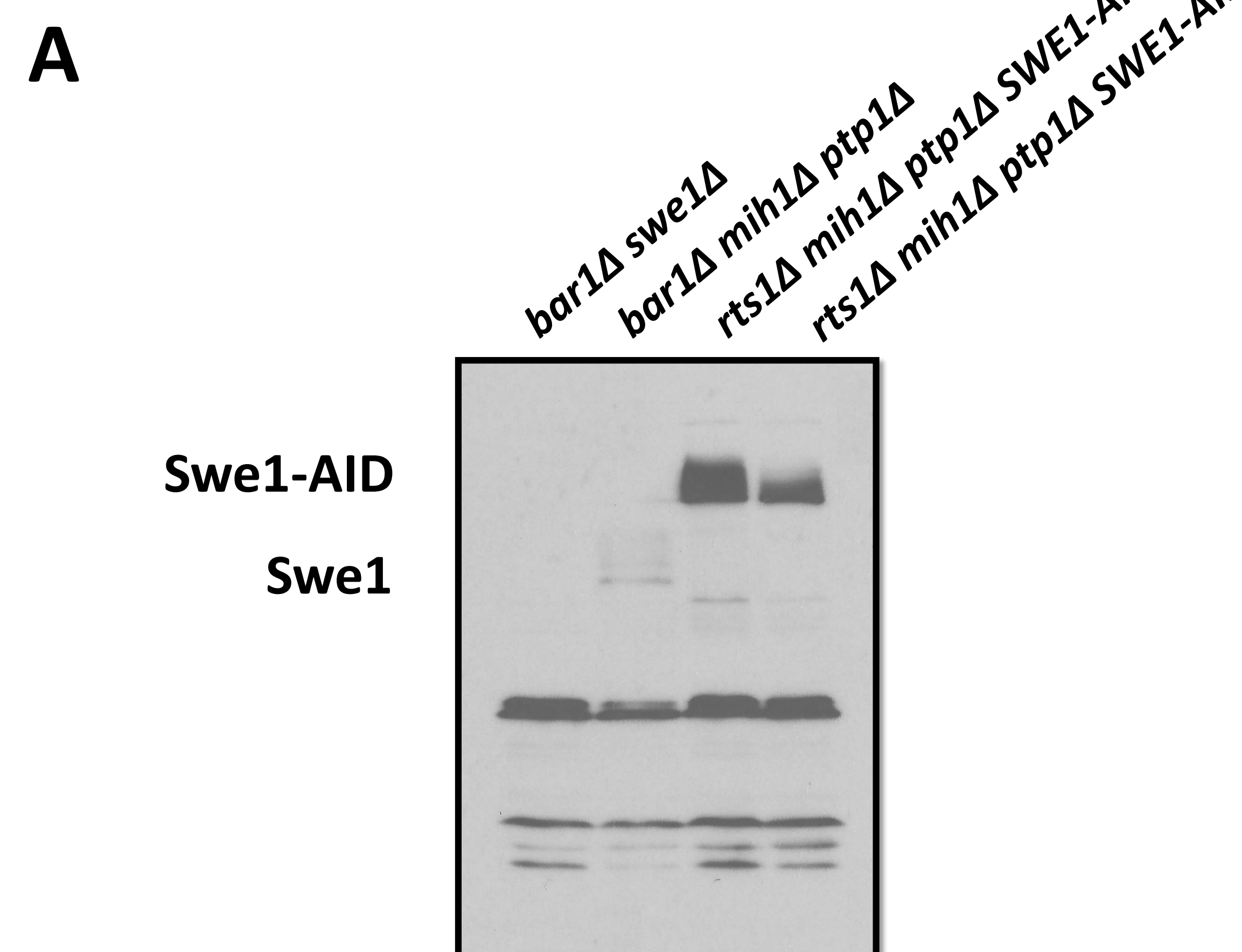


Figure 3. Immunoblotting of transformant strains and tetrad dissection of transformants after sporulation. (A) Two transformant isolates of *rts1Δ mih1Δ ptp1Δ SWE1-AID*, were immunoblotted with anti-Swe1 antibodies. (B) One transformant was crossed to a *rts1Δ ptp1Δ GAL-SWE1-AID* strain, sporulated and tetrads dissected. Haploid segregants were replica plated to selective media.

## Future Directions

Further study of the mutant combinations on Cdk1 regulation in *Saccharomyces cerevisiae* will be used to examine redundant dephosphorylation during the entry into mitosis. This will be accomplished by repeating the tetrad dissection of the crossed strains to obtain the triple mutant and with other mutant combination which will be confirmed by replica plating onto selective markers. Afterwards cell cycle progression in the various mutant can then be studied using time course microscopy in auxin media.

## References

Kennedy, E. K., M. Dysart, N. Lianga, E. C. Williams, S. Pilon *et al.*, 2016 Redundant Regulation of Cdk1 Tyrosine Dephosphorylation in *Saccharomyces cerevisiae*. GENETICS. 202(3): 903-910  
Lianga, N., 2014. Cdk1 regulates anaphase onset (Doctorate thesis, University of Ottawa, 2015) (pp. 2-3). Ottawa: © Noel Lianga.  
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