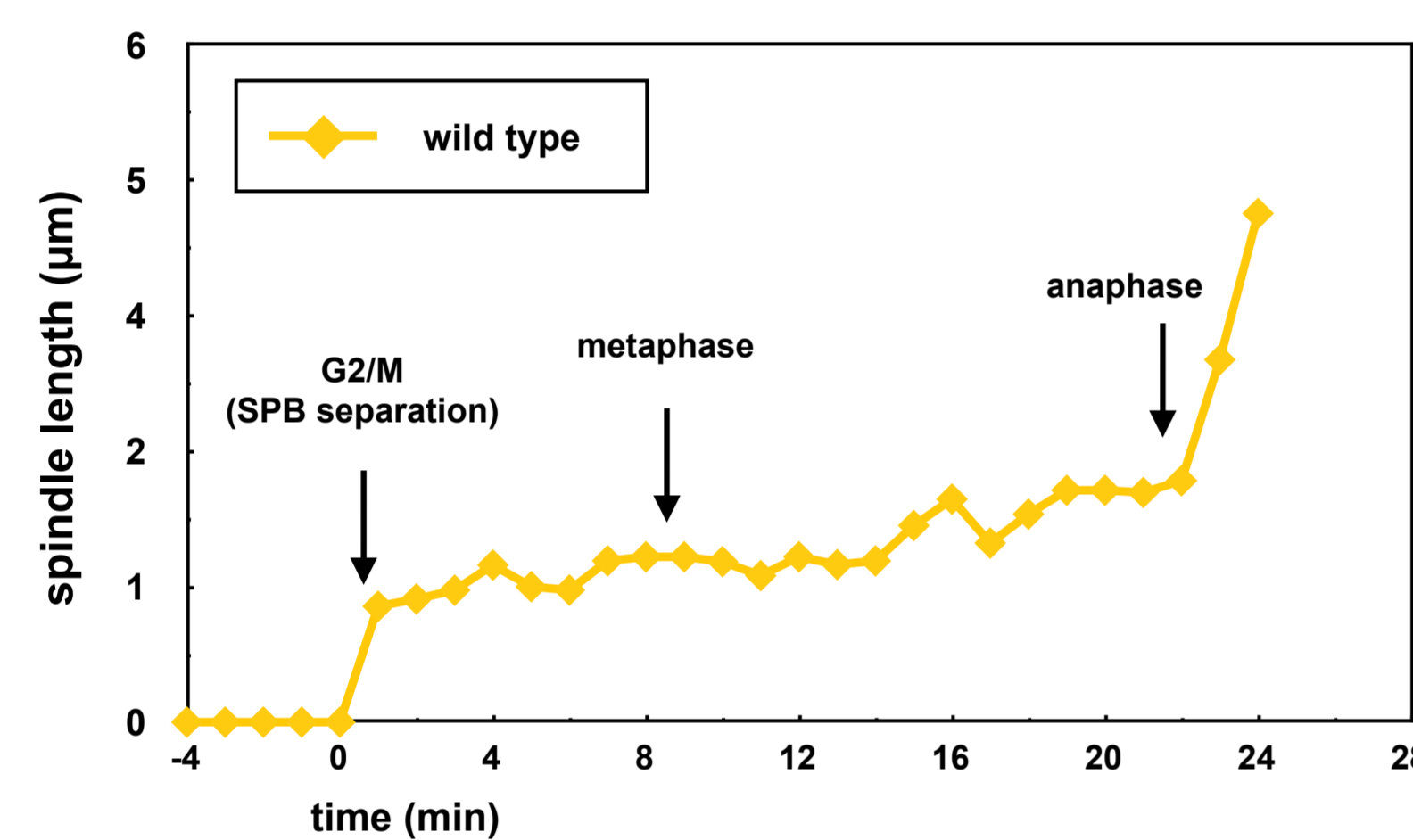
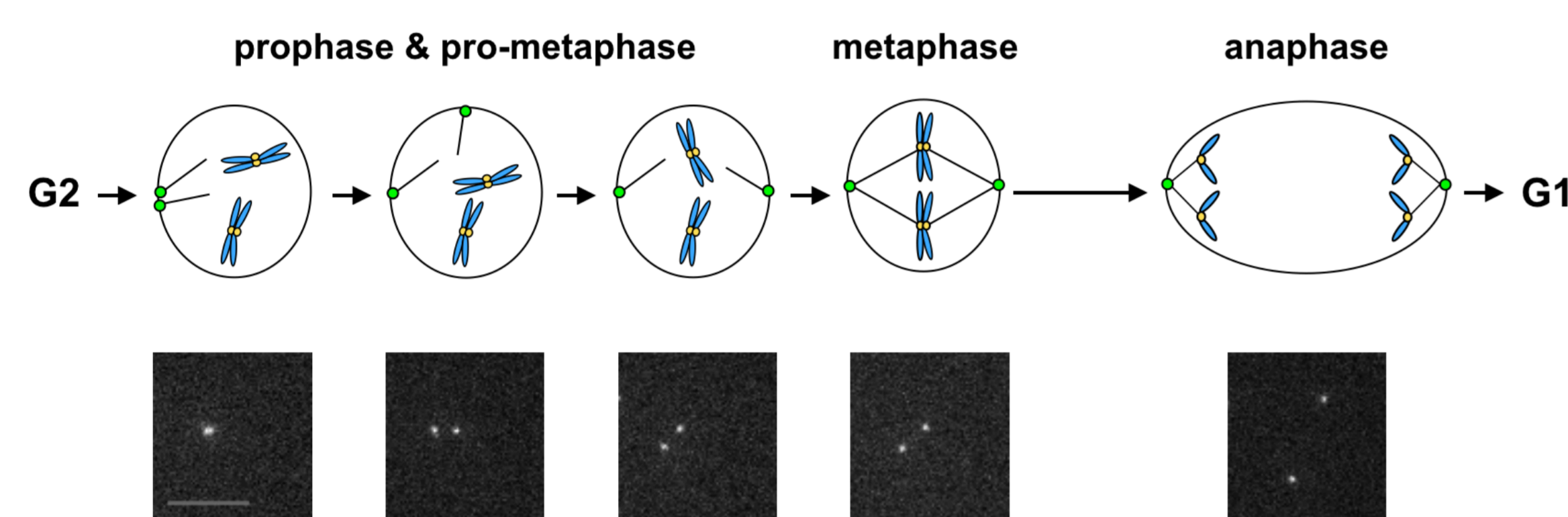


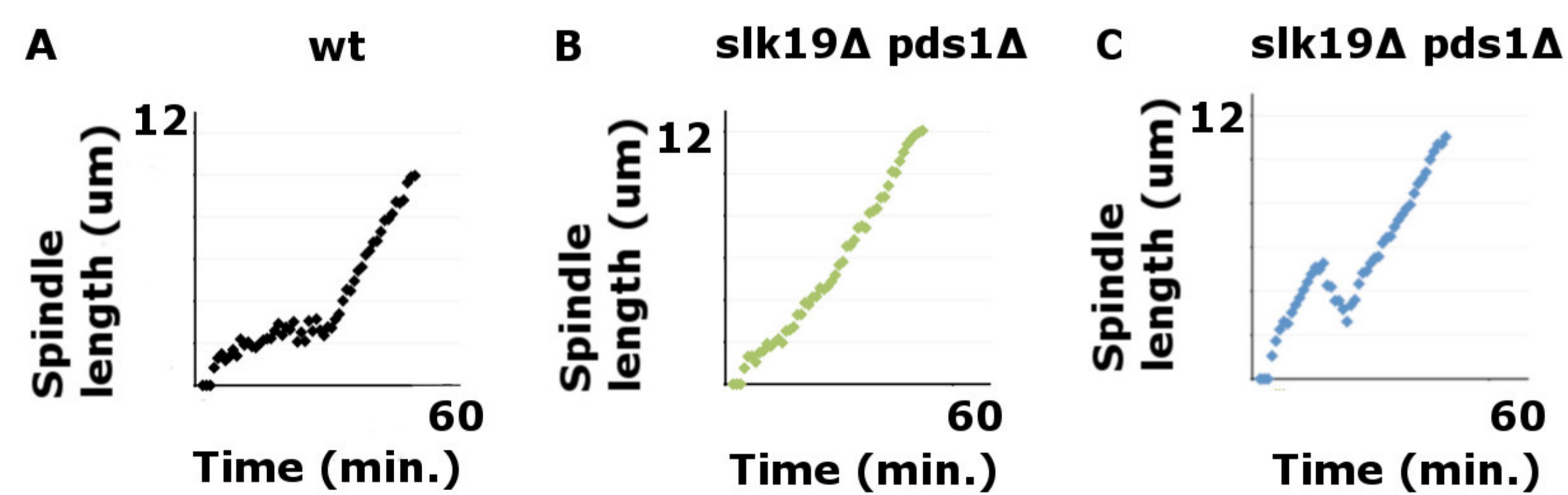
## Background

- Anaphase onset is triggered by the activation of Esp1/Separase, a protease that cleaves Mcd1/Scc1 subunit of the Cohesion ring complex that physically holds sister chromatids together. Separase is regulated by the anaphase inhibitor Pds1/Securin.
- Anaphase onset can be monitored by the lengthening of the spindle fibers as the spindle pole bodies move apart from each other after ~20 minutes in metaphase:

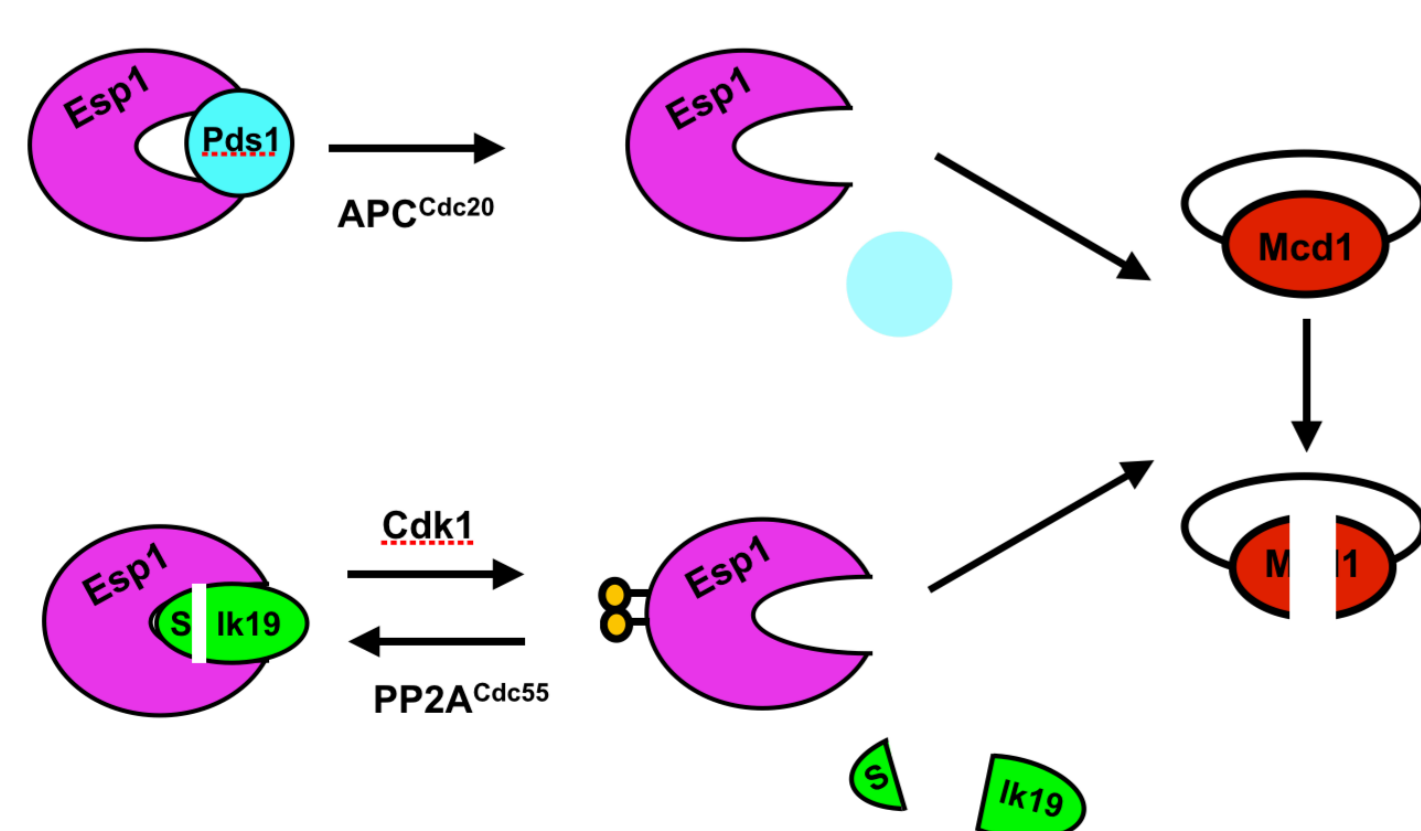
Using Spc42-GFP to measure progression through mitosis



- Wild-type cells: rapid anaphase lengthening of the spindle occurs after a pause in metaphase (95% of cells).
- slk19Δ* and *pds1Δ* cells display similar spindle elongation pattern as wild-type cells (graph A).
- Many *slk19Δ pds1Δ* cells display immediate spindle lengthening (40%) (graph B) or immediate spindle lengthening with short periods of shortening (40%) (graph C). No cells behave like wild-type.

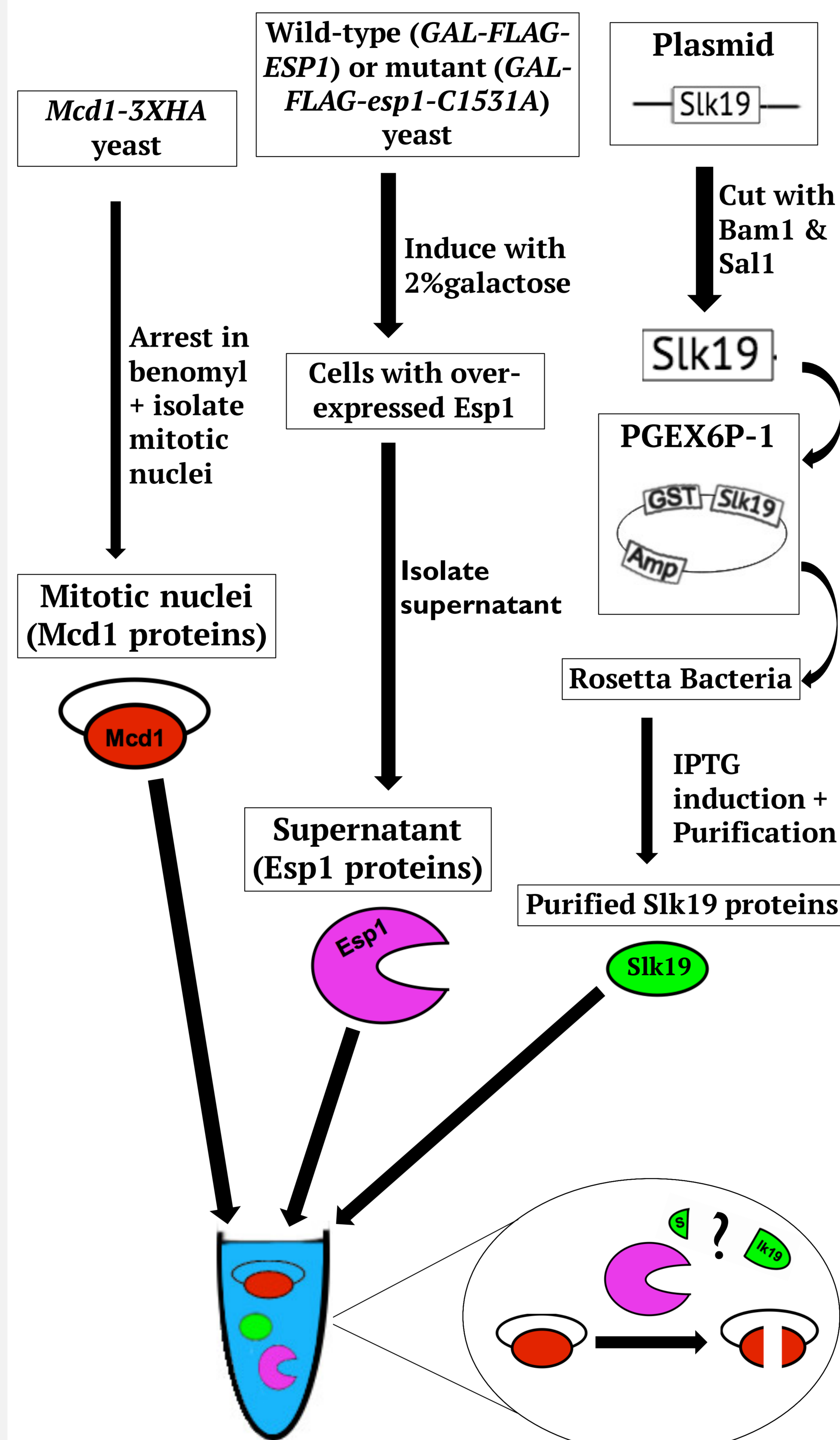


- MODEL—Slk19 acts redundantly with Securin to inhibit Separase:



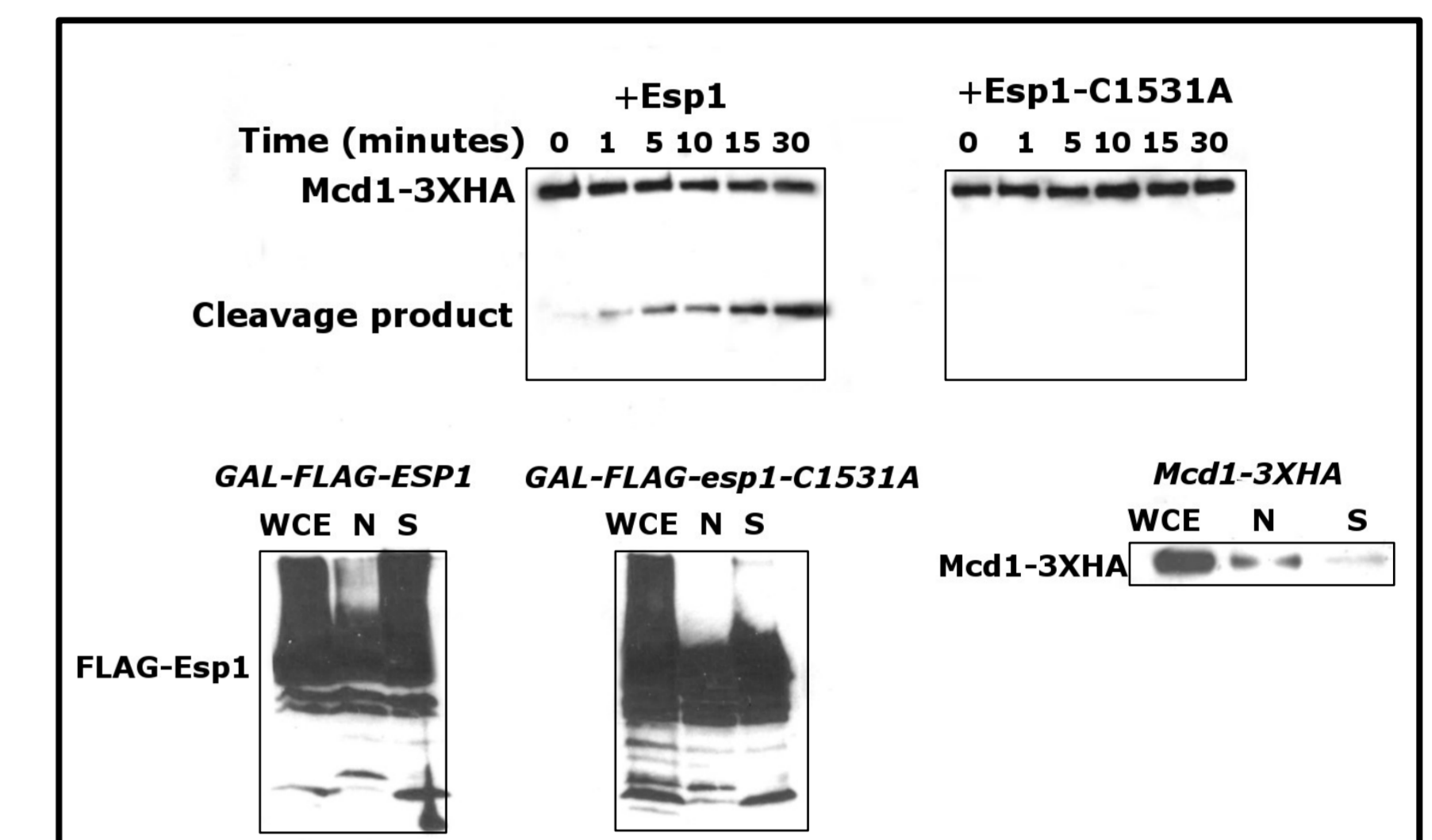
- GOAL: Test if Slk19 can inhibit Esp1 activity in an *in vitro* cleavage assay.

## Methodology

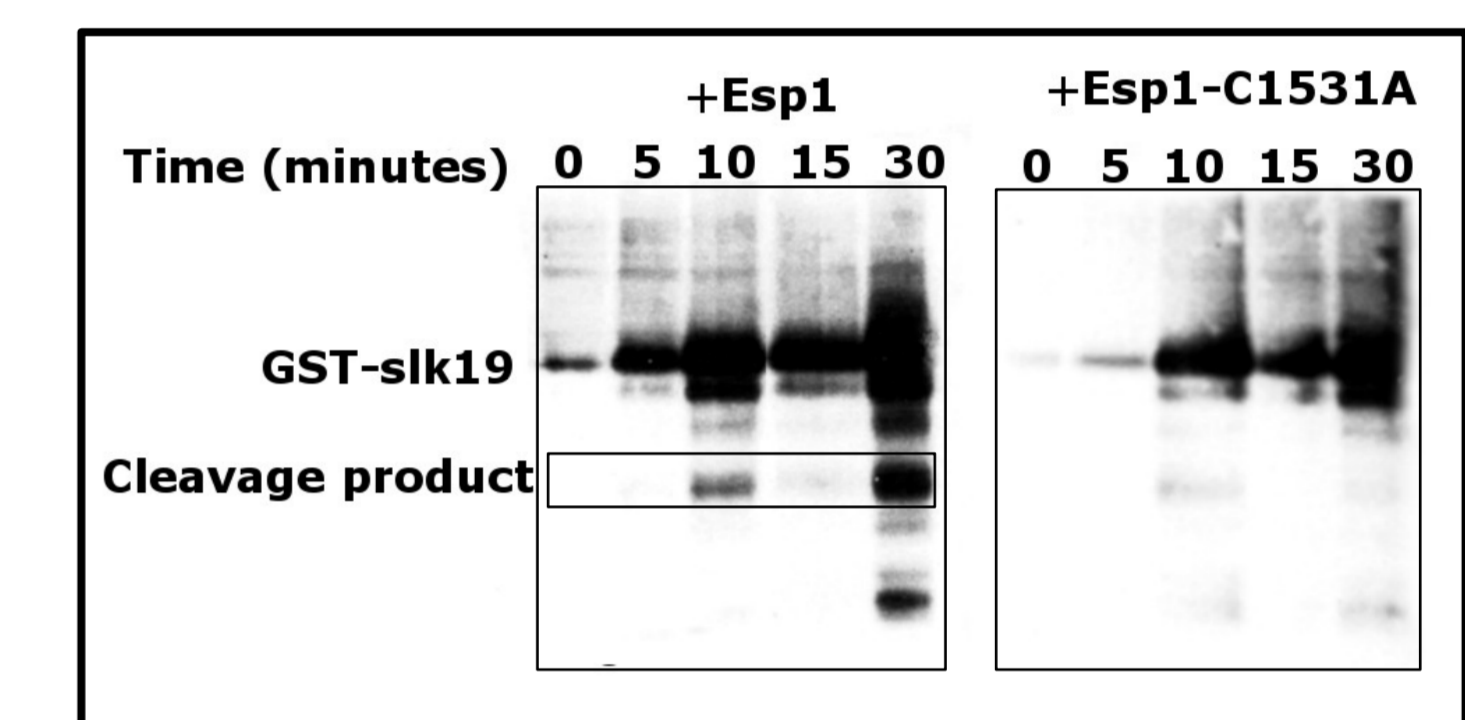


## Results

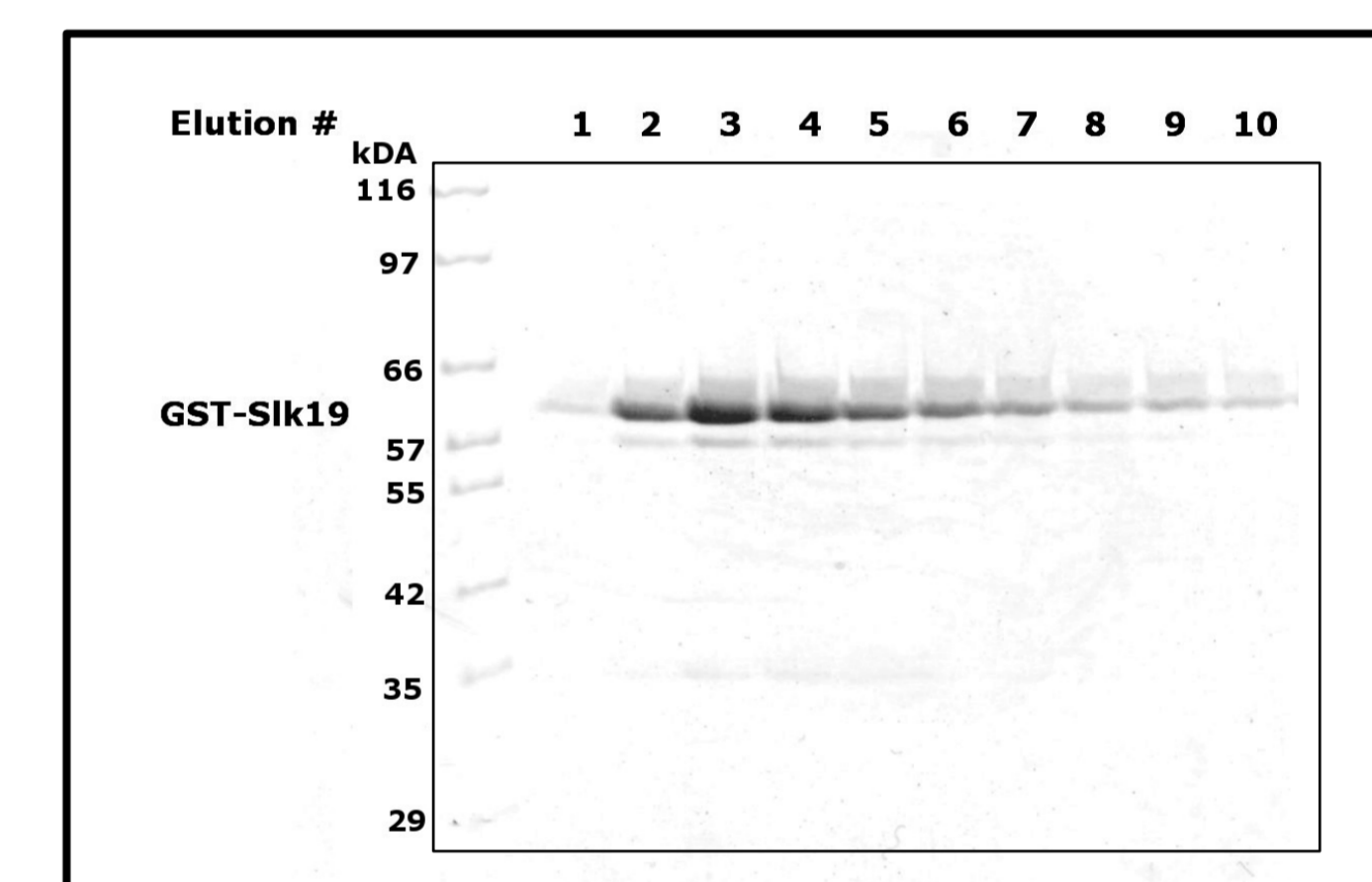
### In vitro cleavage of partially purified nuclear Mcd1



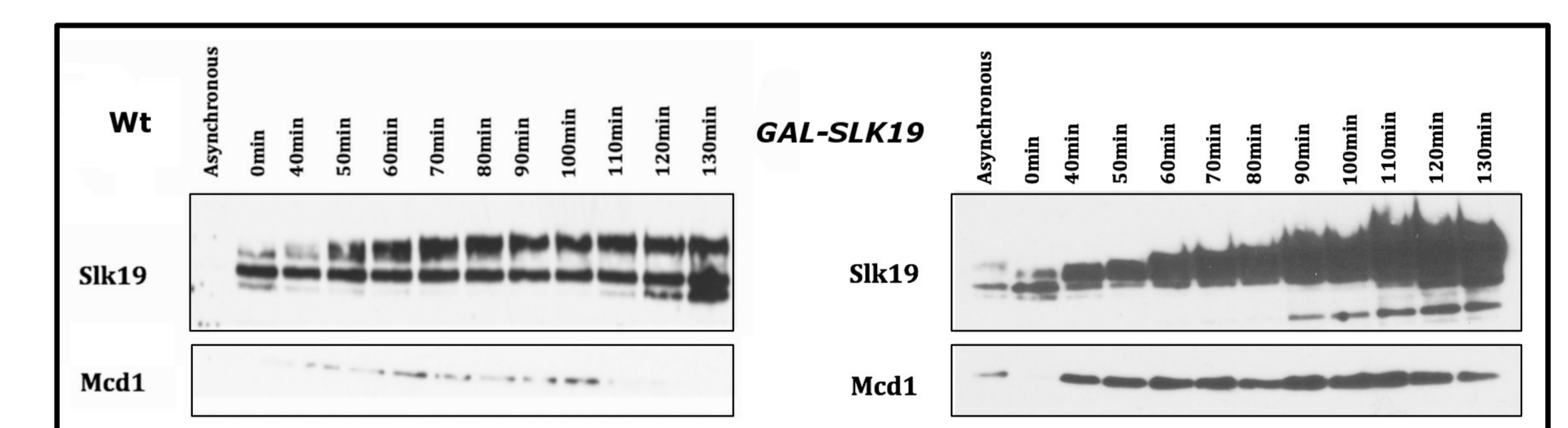
### In vitro cleavage of bacterially purified Slk19



### Elution of purified GST-Slk19



### Overexpression of Slk19 blocks Mcd1 cleavage *in vivo*



## Conclusions

- The results have shown that Esp1/Separase cleaves Mcd1/Scc1 and Slk19 separately as expected.
- Future steps:
  - Slk19 inhibition of Esp1 *in vitro*.
  - Inhibition with Slk19 mutants to compare phosphorylated vs unphosphorylated Slk19, and cleavage site mutants that suppress *pds1Δ* cells.

## Acknowledgements

I thank my supervisor Adam Rudner, lab members Camille Fortinez and Sean Stephenson, and Ryan Sanderage for their teaching, support and encouragement. I also thank University of Ottawa for giving me a chance to participate in the Undergraduate Research Program.

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