

A targeted proteomic assay quantifies the
periodic expression of cell-cycle regulators in
yeast S. cerevisiae

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Duke University Proteomics and Metabolomics Shared Resource

Acknowledgements

DPMSR



Duke Biology



Steve Haase

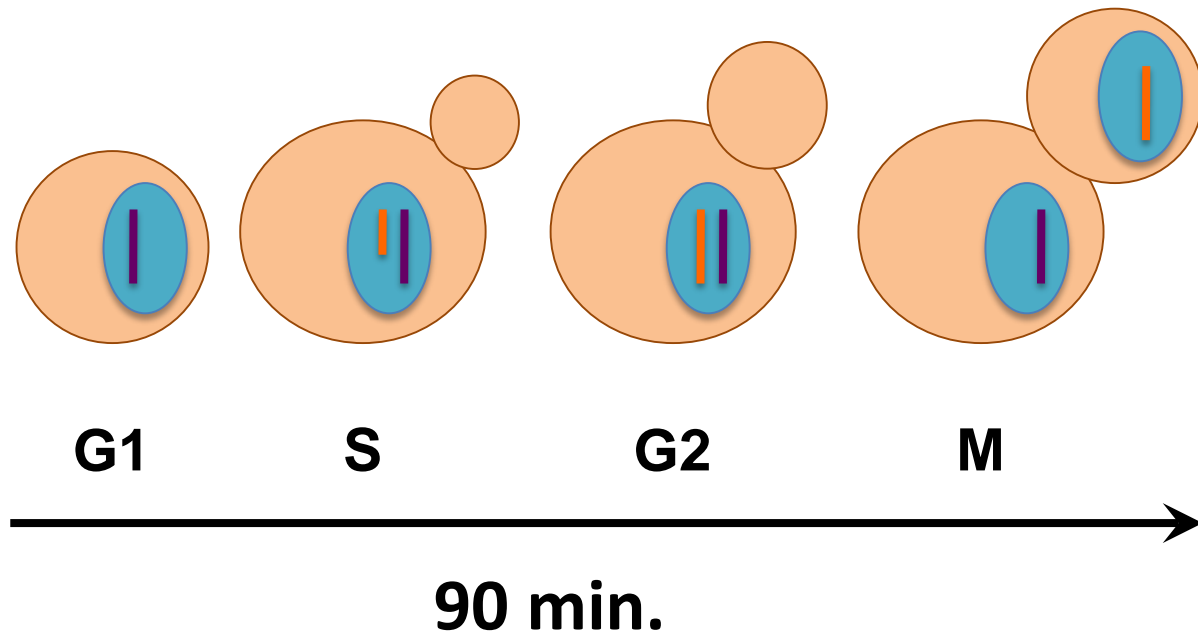
\$\$\$ - DARPA



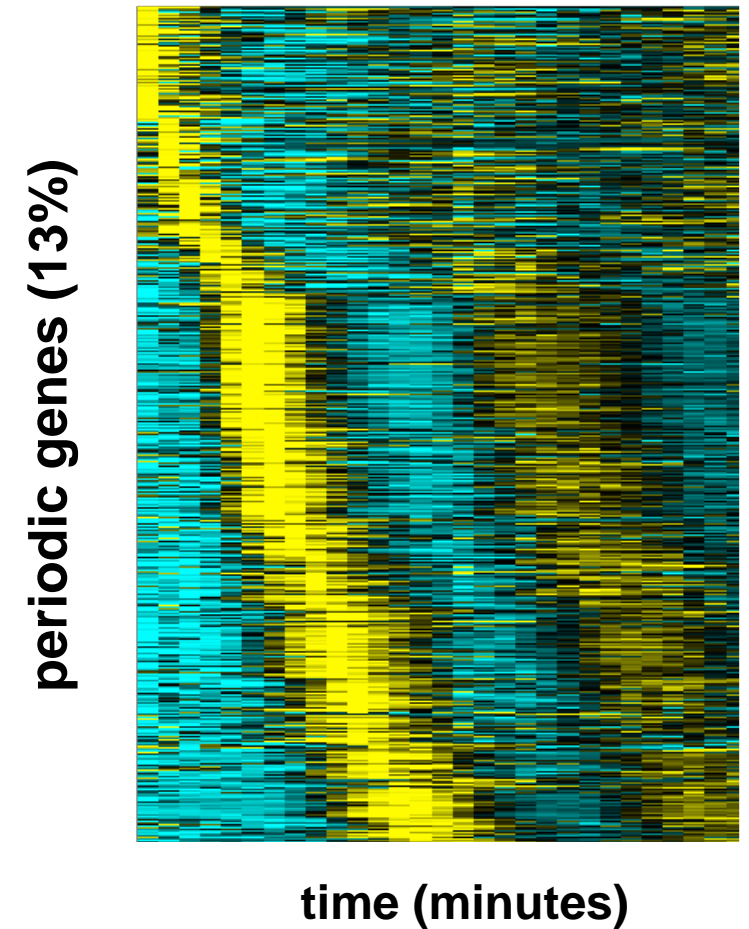
Tina Kelliher

Central Question & Model System

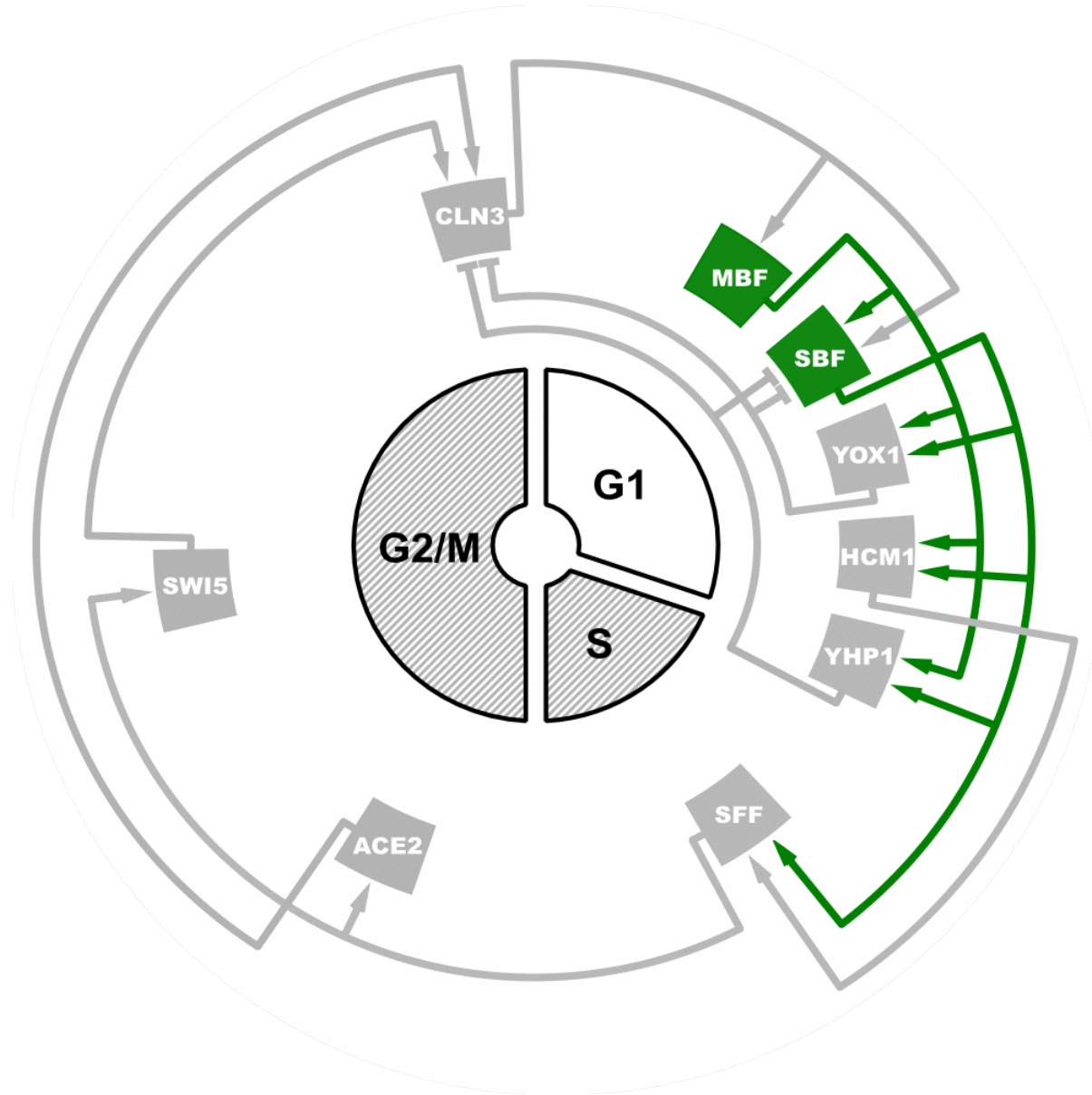
Q: What controls oscillating genes across the cell cycle?



Saccharomyces cerevisiae
cell cycle



Cell-cycle regulated transcription factors (TFs)



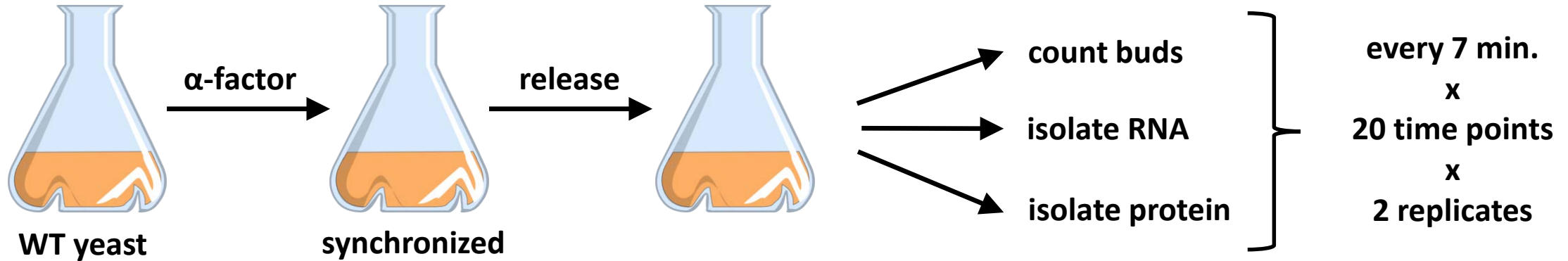
Model built on mRNA expression and ChIP.
(Orlando et al. Nature 2008)

Model fails if protein expression is not similarly regulated.

Target selection & assay development

- Selected 48 TFs that: 1) oscillate (mRNA); and 2) are regulated by **and** regulate other periodic TFs (ChIP).
- Targeted ~3 peptides per protein, selected from data in PeptideAtlas & Aebersold (Cell 2009; PNAS 2012) → 138 JPT SpikeTides TQL standards.
- Assay development w/ asynchronous cells. Most endogenous peptides were undetectable by MRM; 26 peptides detected by PRM.
- Skyline used to generate spectral library, refine assay to 2 peptides per protein, select best charge states, schedule retention times, etc.

Analysis of TF expression



Sample Prep

TCA pellet resusp.
in ALS-1

reduction/alkylation
& trypsin digestion

spike with
5 fmol/ μg SILS

LC-MS/MS

1 μg peptide,
5 fmol SILS

75 μm x 25 cm HSS-T3
90 min gradient

PRM: \sim 100 pairs,
2 min. rt win.,
240 ms IT

nanoACQUITY
& Q-Exactive Plus

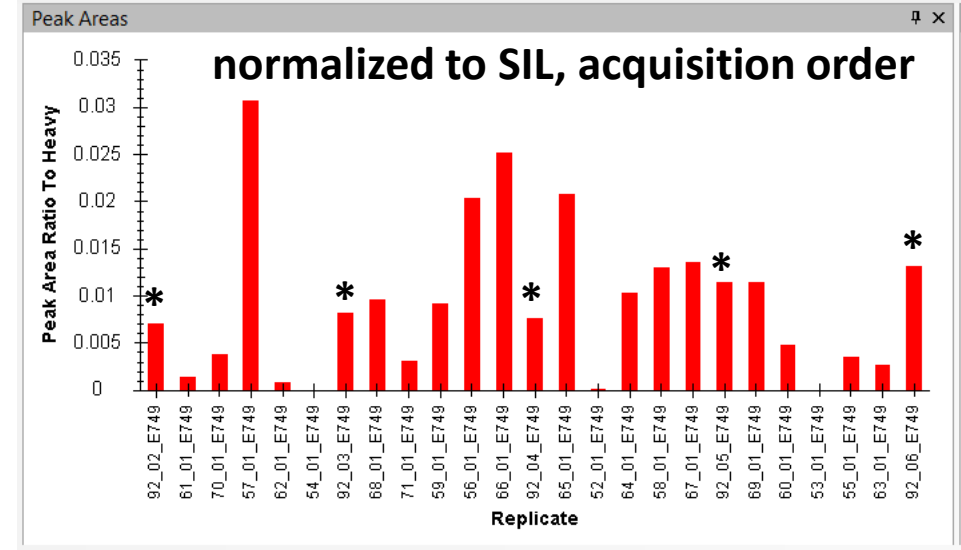
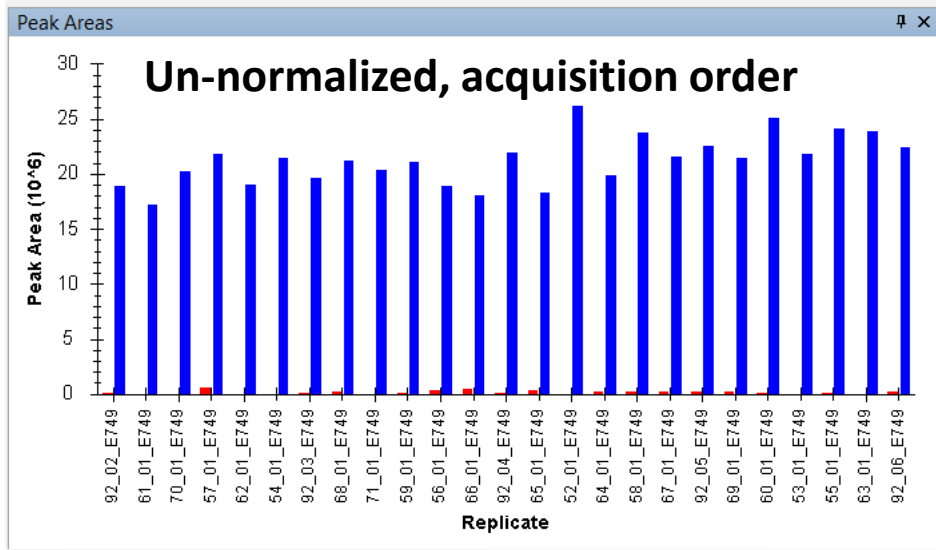


Analysis



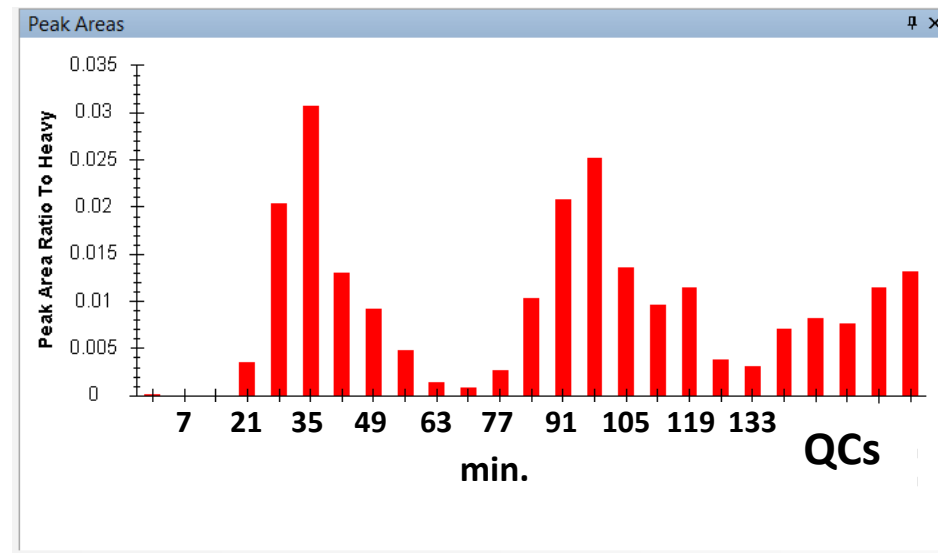
CLOCCS

Raw data – TOS4



*QCs

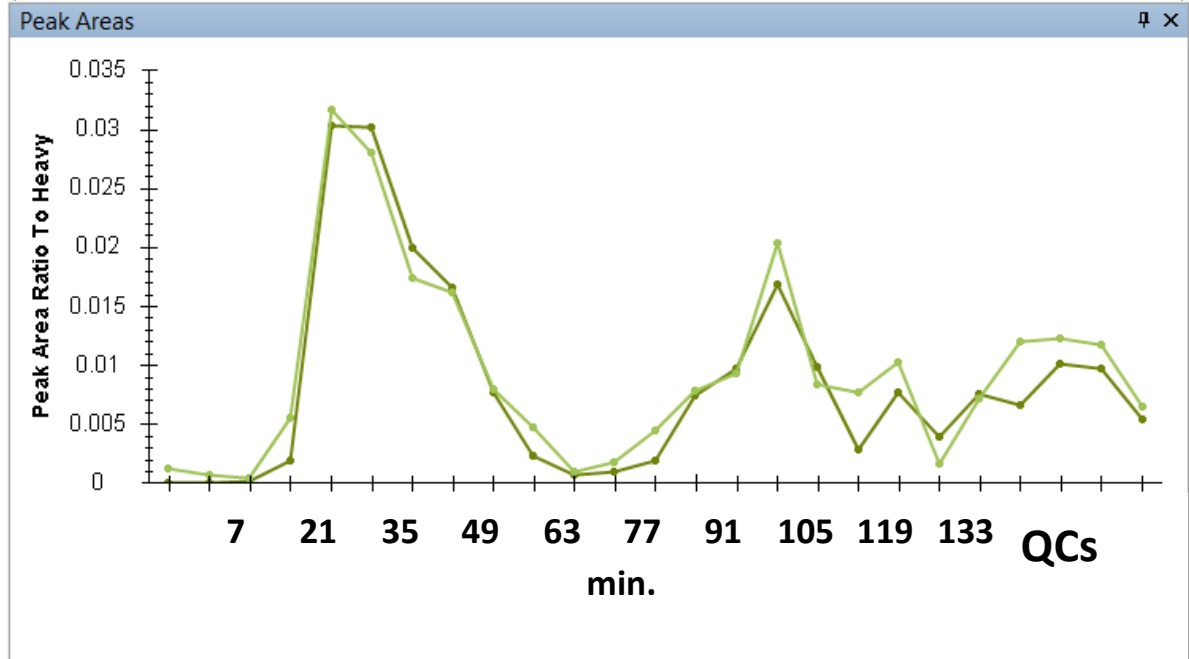
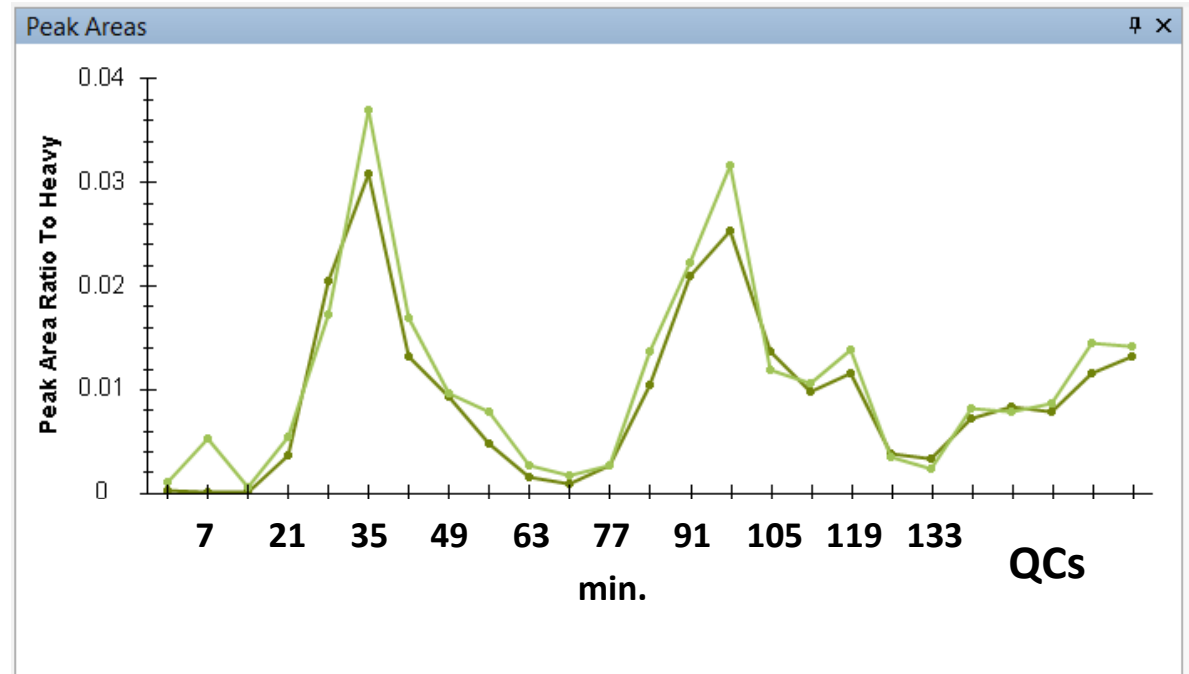
Normalized to heavy,
ordered to collection
time:



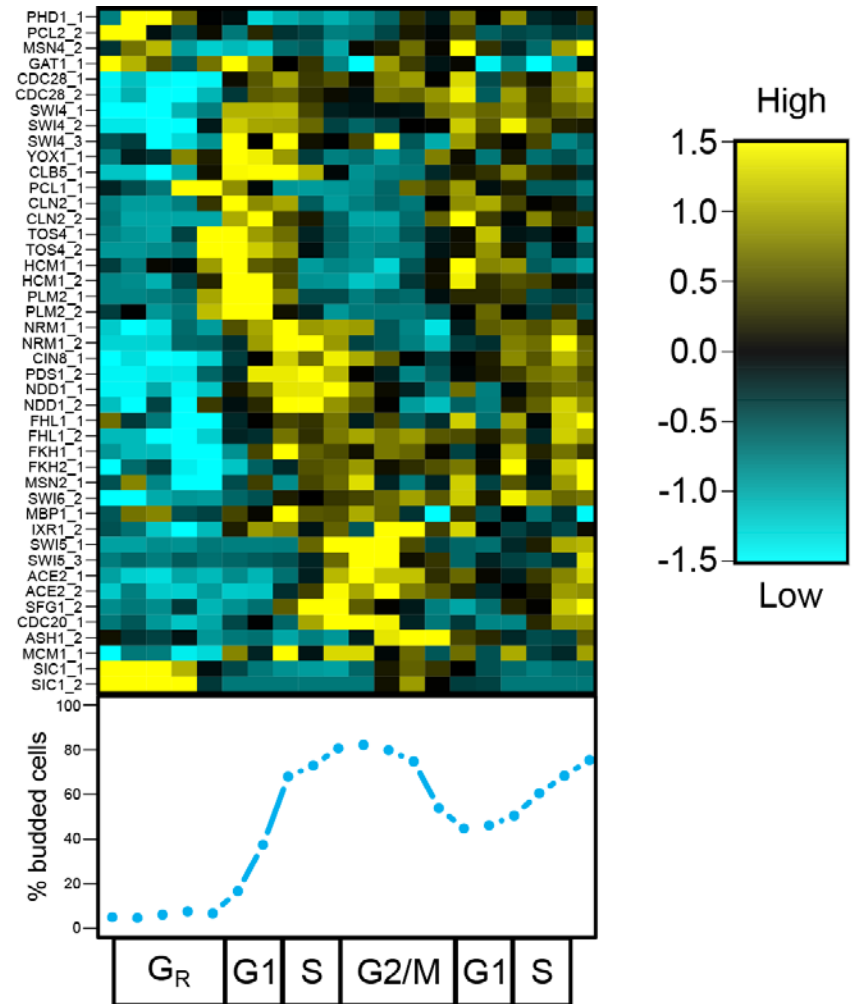
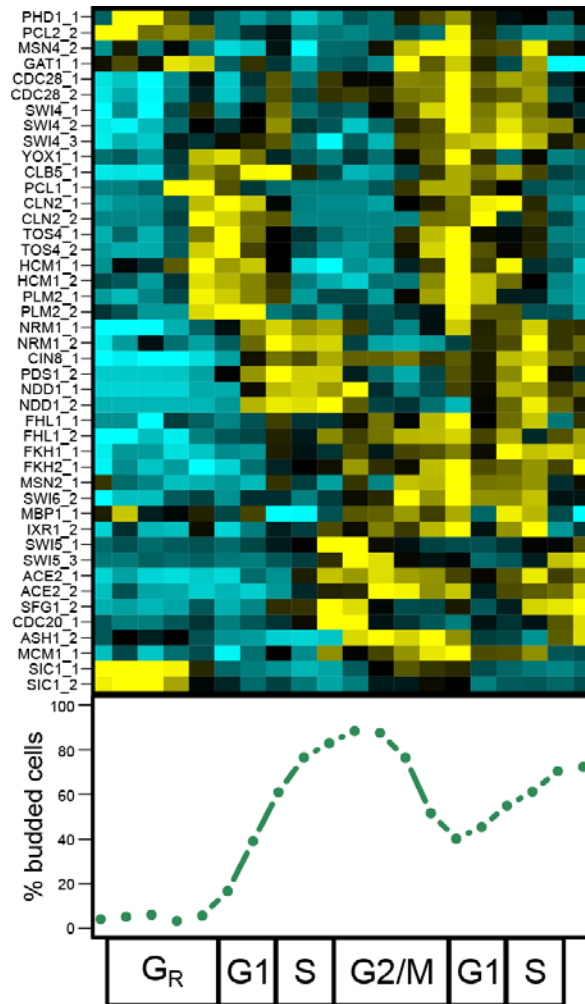
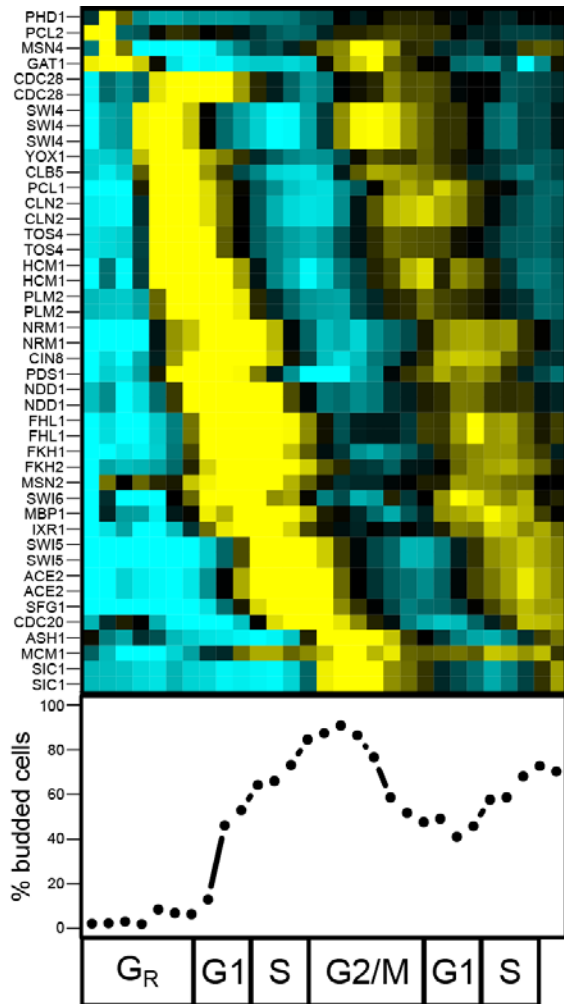
Raw data – TOS4

YLR183C TOS4

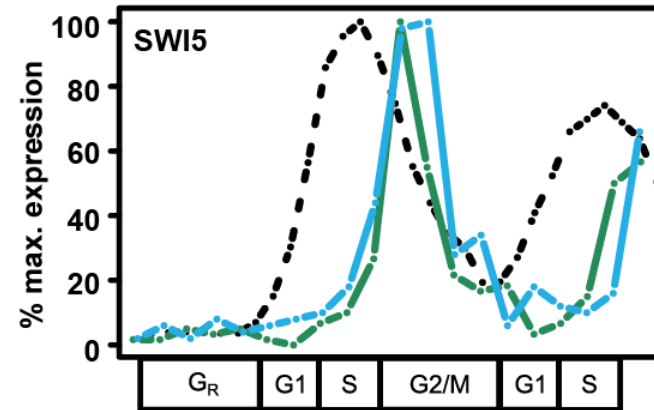
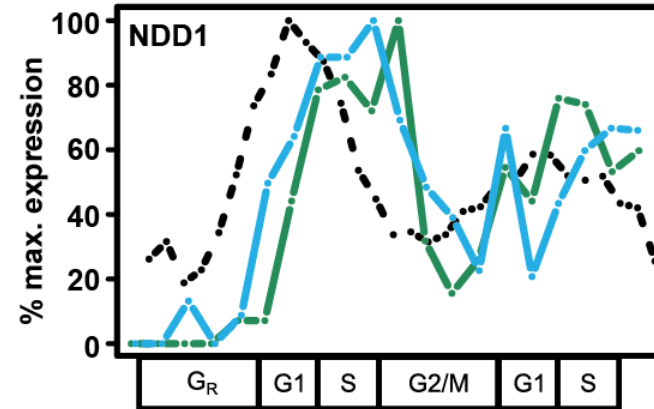
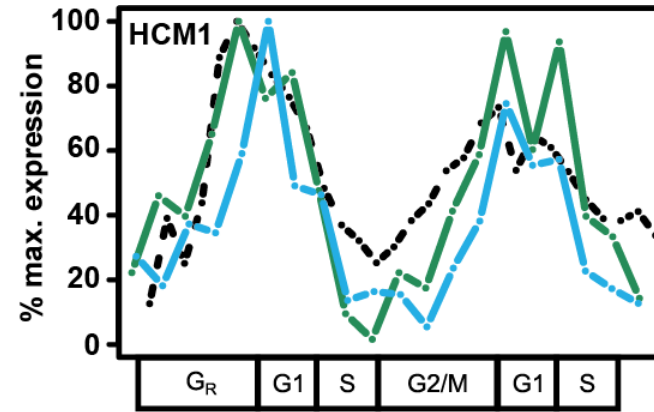
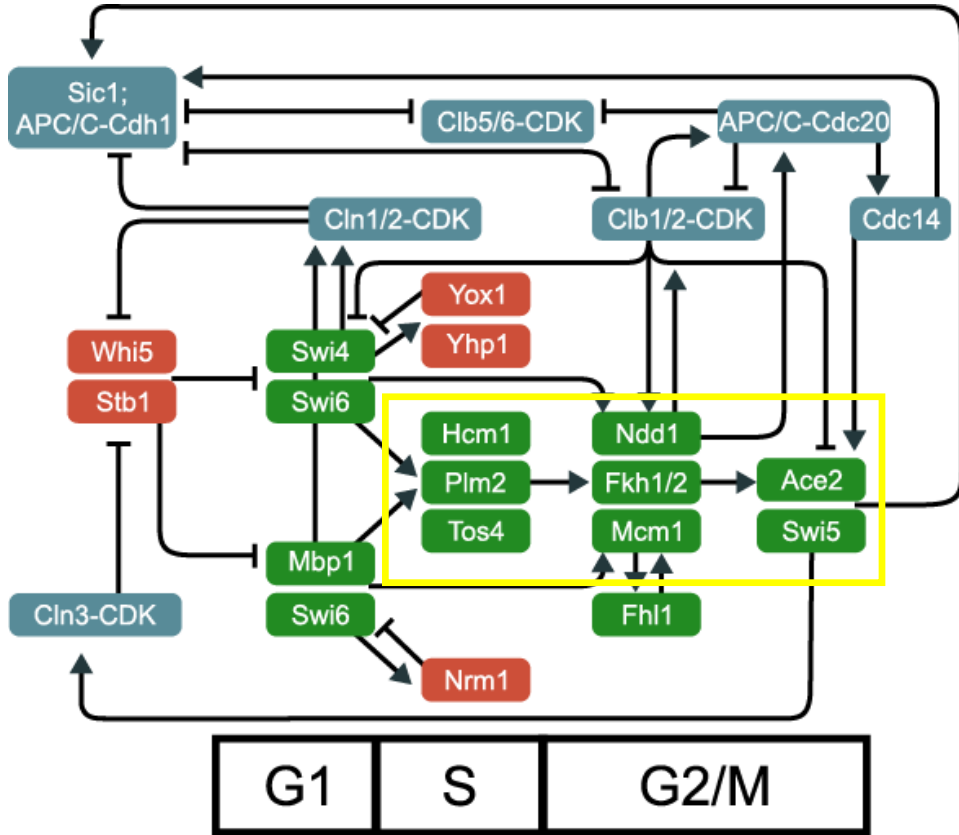
- K.TASESIGNFGK.G [37, 47]
- K.ISIGSDSGVAER.M [294, 305]



Results: comparison of mRNA vs. protein



Conclusions



mRNA
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Pep, rep. 1
- - - - -

Pep, rep. 2
- - - - -