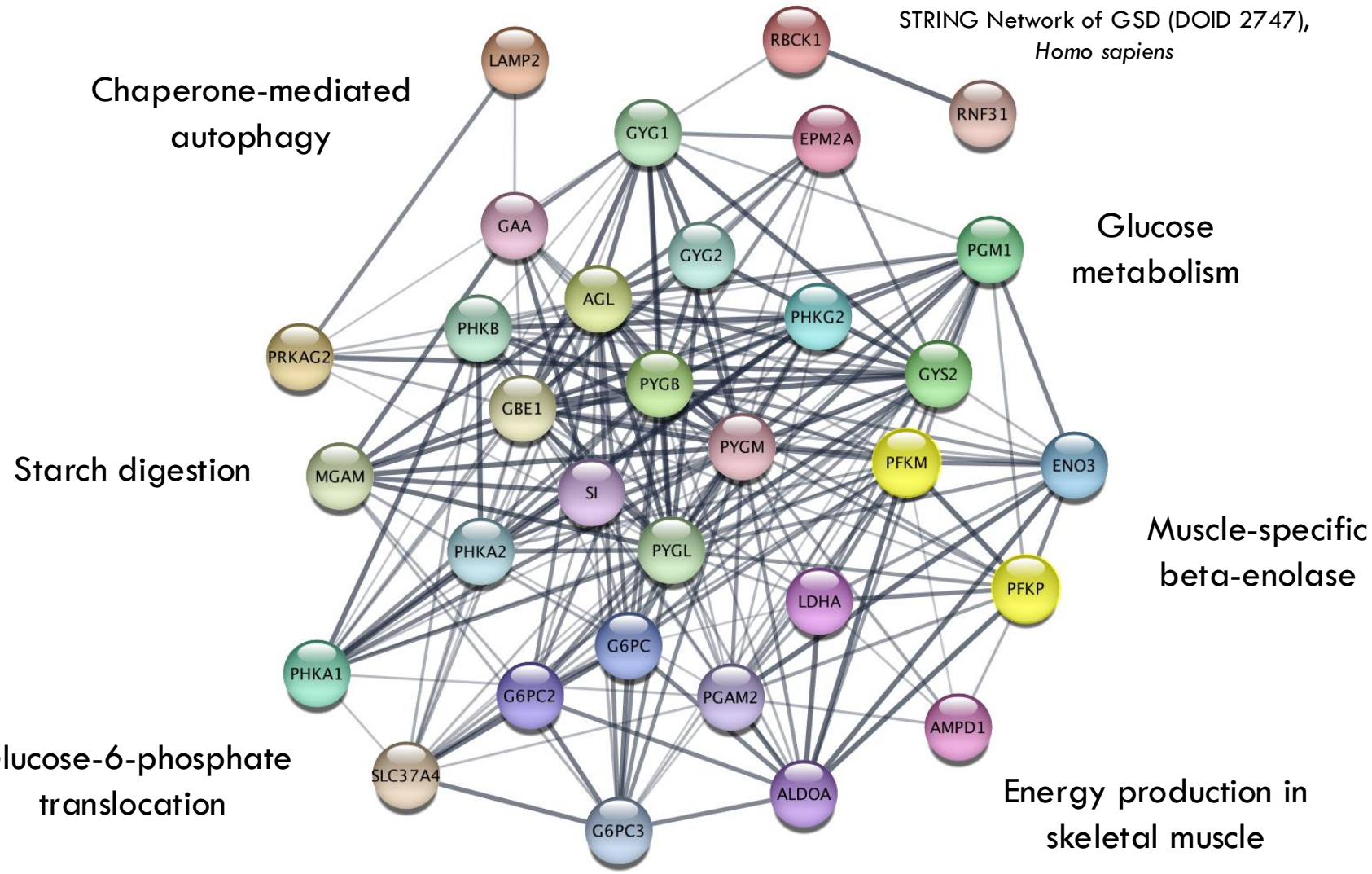
Validation of a ΔPFK2 *Saccharomyces cerevisiae* Model of Glycogen Storage Disease 7

THE UNIVERSITY OF SYDNEY

BCMB3888 Group E

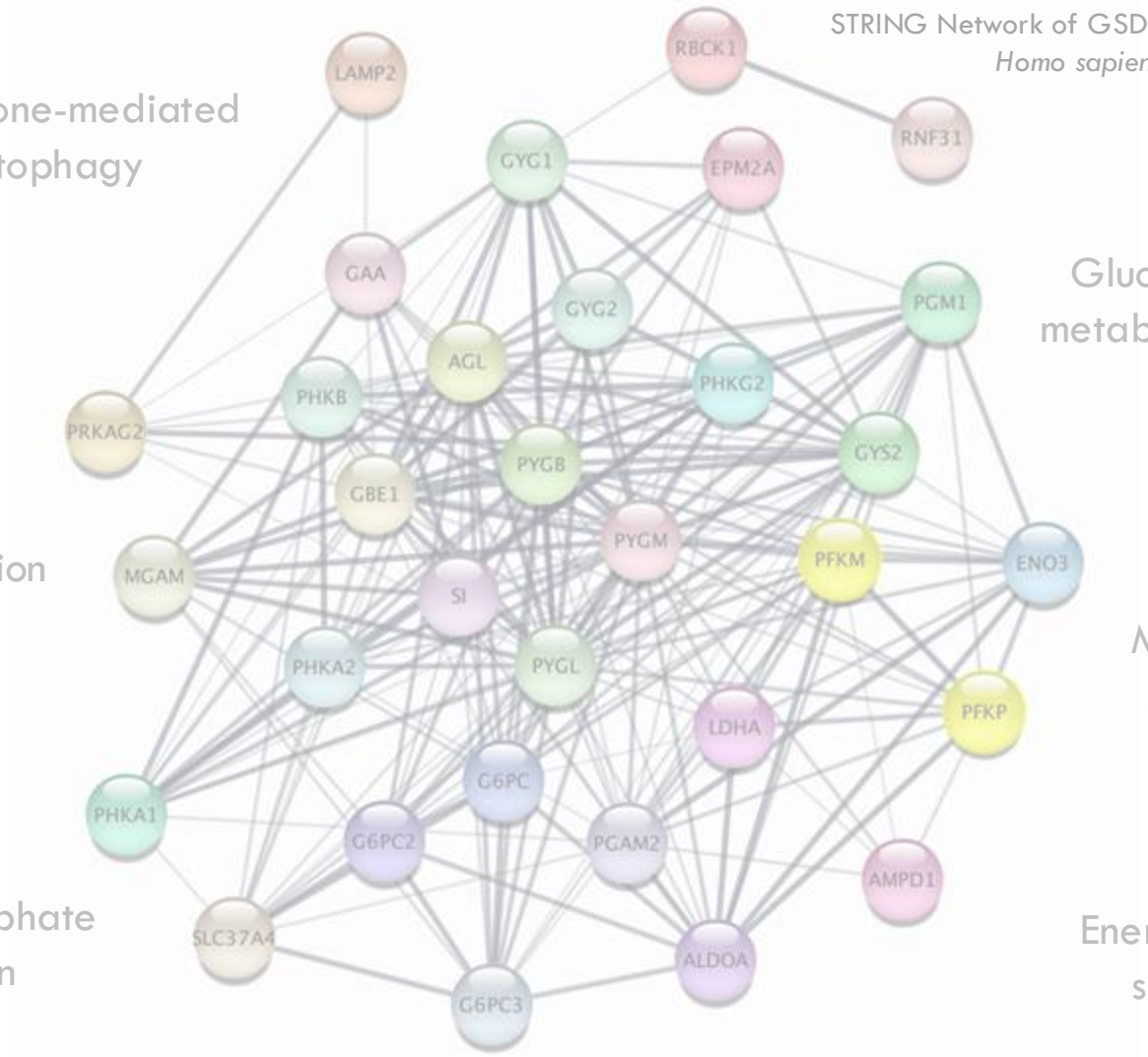
Glycogen Storage Disease 7



Glycogen Storage Disease 7

Metabolic flux through glycolysis

TKL1



Starvation-Induced Autophagy

+

Glycogen Synthase Inhibitor

PHO85

Project Aims

- Understand the role of the glycolytic pathway in regulating the oxidative stress response
- Determine whether transcriptional regulation of PHO85 and TKL1 expression is relevant to GSD
- Conduct pathway analysis of YMR205C by testing growth conditions and metabolite profiles
- Identify new proteins which undergo functional or transcriptional changes in ΔPFK2 *S. cerevisiae* by network analysis for future work
- Develop alternative hypotheses which may explain the GSD phenotype

Experiment Workflow

1. YMR205C
   Deletion Confirmation
2. Yeast Viability Assay
   Test: Oxidative Stress
3. qPCR
   Test: PHO85 and TKL1
4. Glycogen Staining and
   Growth Curve Assay
   Test: Nutrient conditions
5. Metabolomics

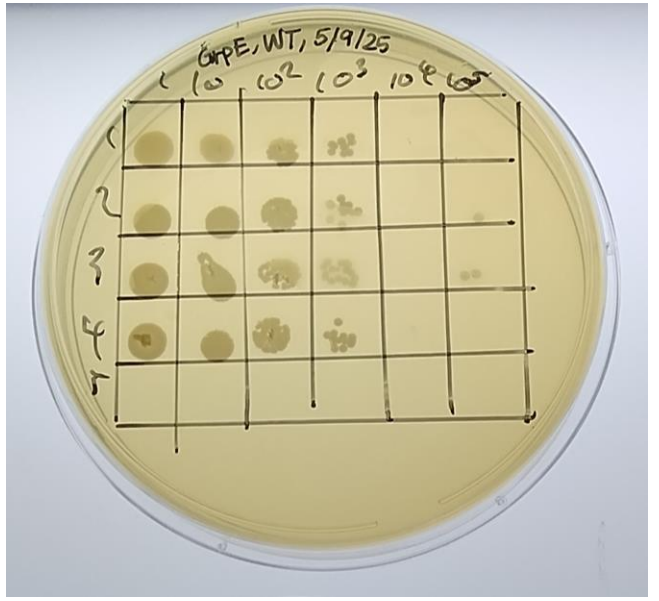
Network Theory
with MATH3888

Discussion of Preliminary Results

Viability Assay

- There was little difference between the WT and KO and between the varying H_2O_2 concentration.
- This result is limited as we did not have a dilution with a meaningful, discernible colony count.
- There may be some genome-level reasons for these observations, but these cannot be validated without a replicate experiment.

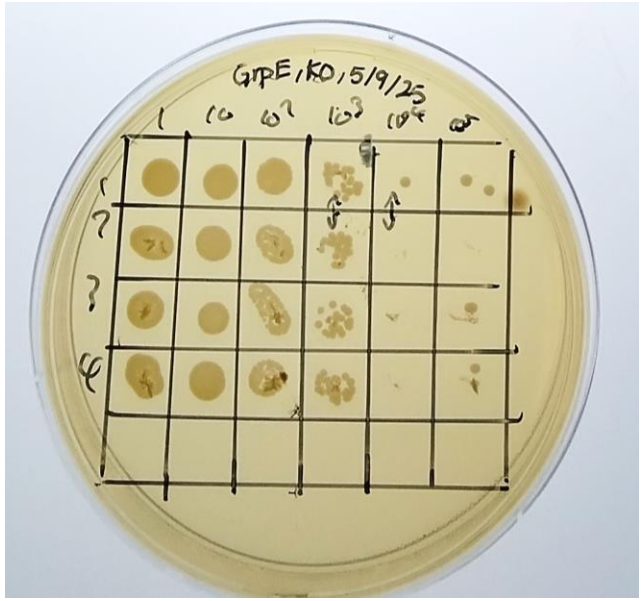
Increasing $[H_2O_2]$



WT: BY4741

Dilutions

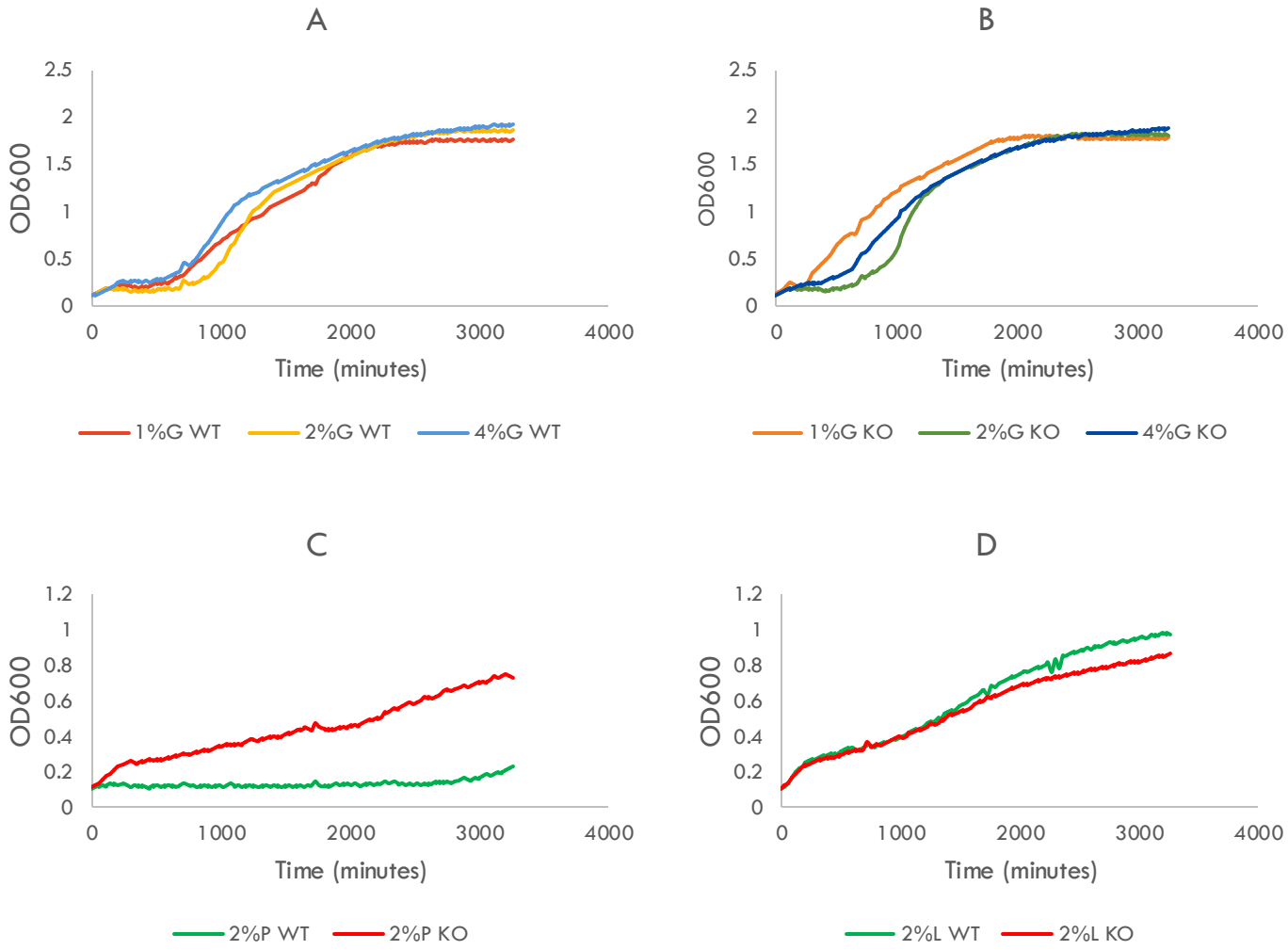
Increasing $[H_2O_2]$



KO: YMR205C

Growth Curve Assay

- WT, KO grown in 1, 2, 4% Glucose, 2% Lactate/Pyruvate
- Key Results
  - Cell growth of glucose fed cells was mostly limited by cell density, not feed
  - 1% Glucose KO samples grew surprisingly well, despite consistently having low OD values in our other experiments
  - Both yeast strains were able to metabolise the 3 Carbon feeds but with greater difficulty exhibited in their growth rate compared to the glucose fed yeast
  - The WT strain struggled to grow in pyruvate but eventually started growing. This is likely because it took some time to localise the relevant transporter to the membrane
  - Lactate was a better carbon source than pyruvate

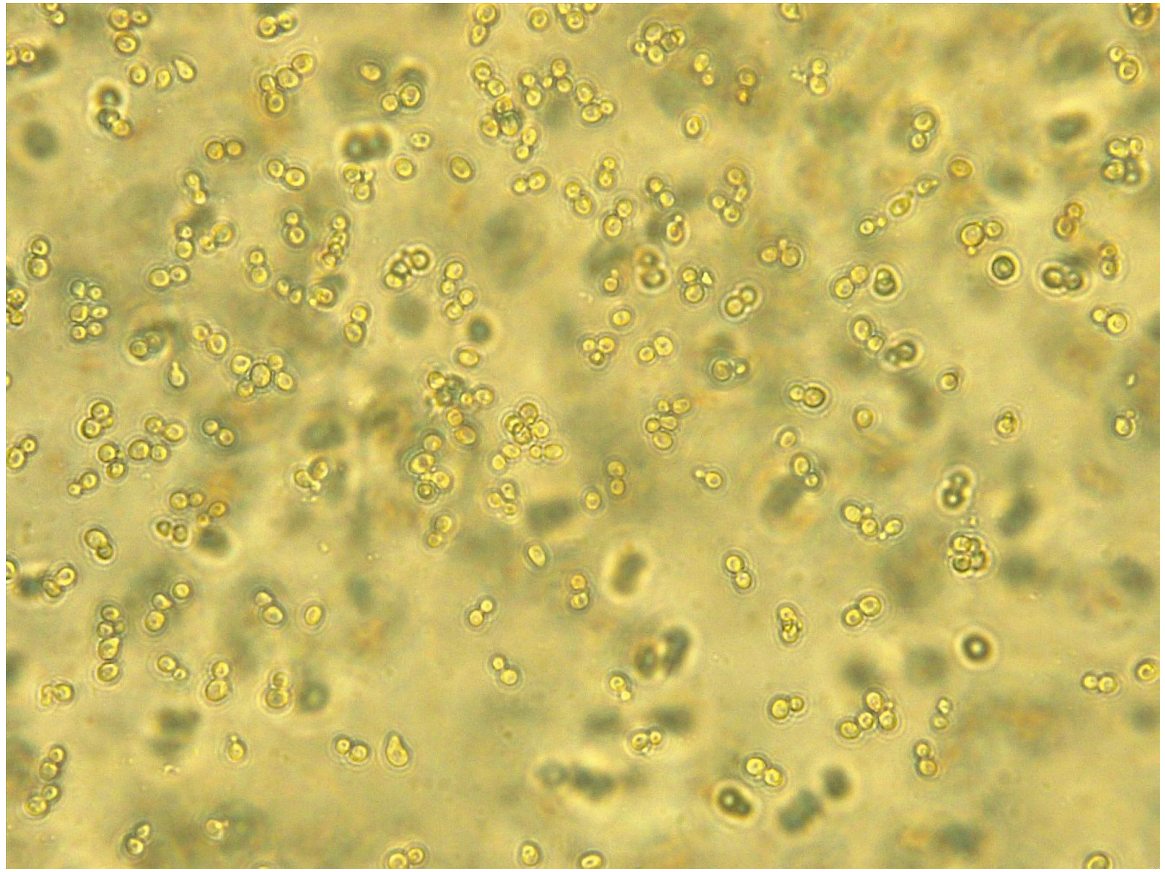


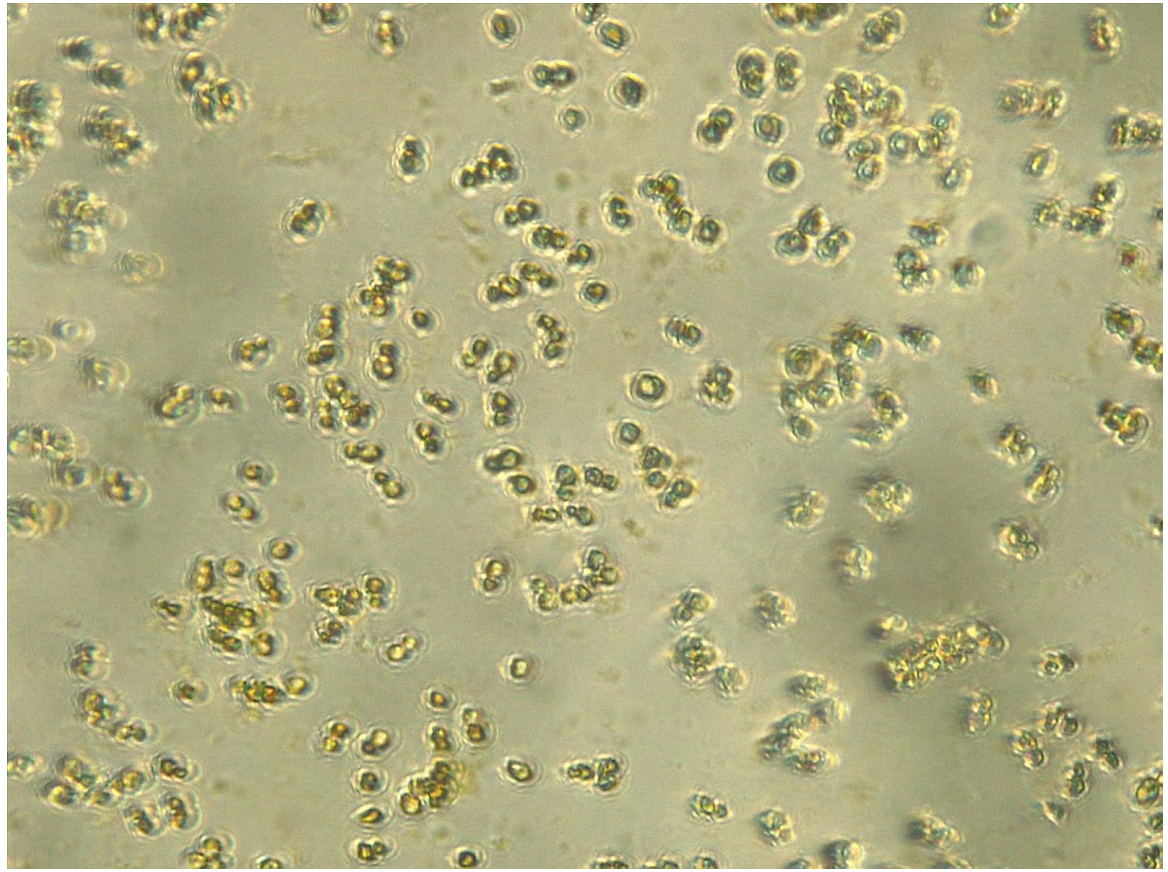
Fujita *et al. J. Biol. Chem.* **2018,** *293* (28).

Glycogen Staining

= Glycogen

= No Glycogen





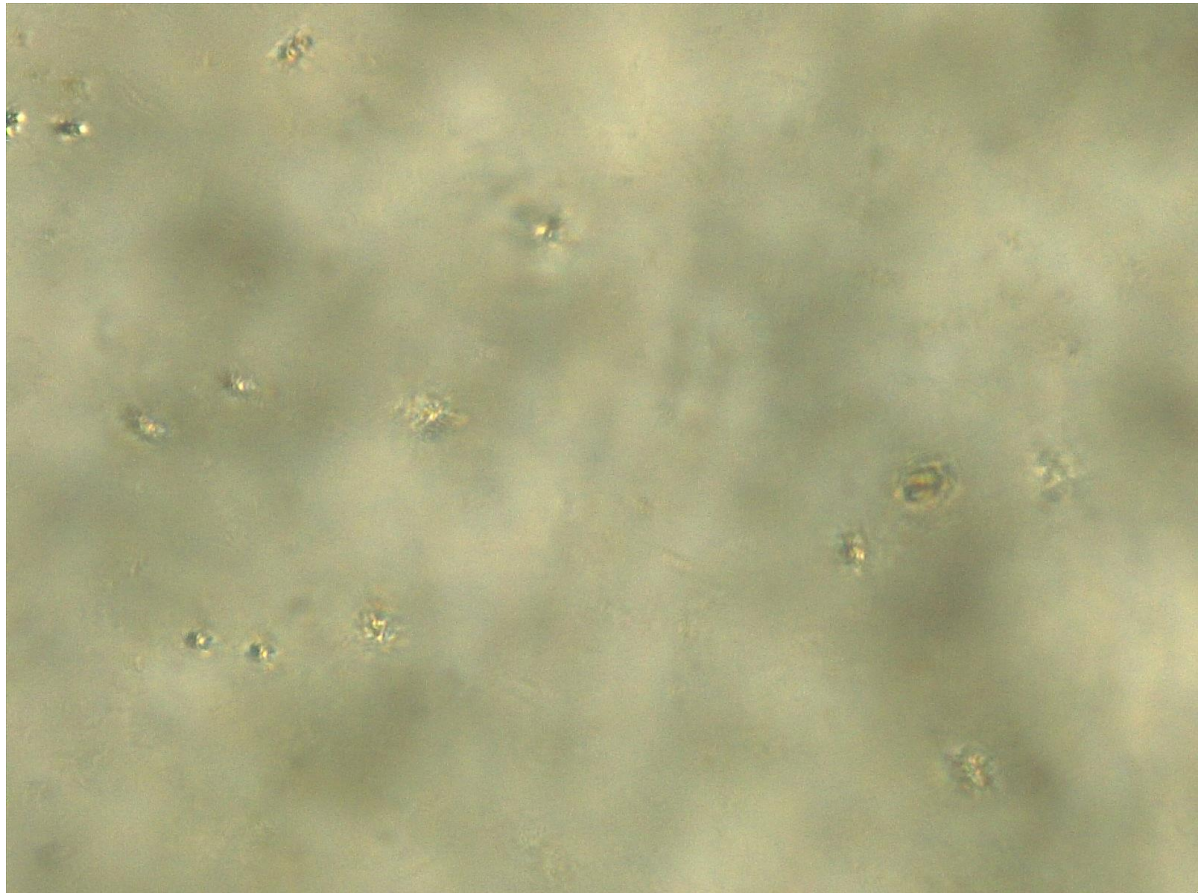
0 minutes → 15 minutes

4% Glucose Knockout treated with Lugol's Solution

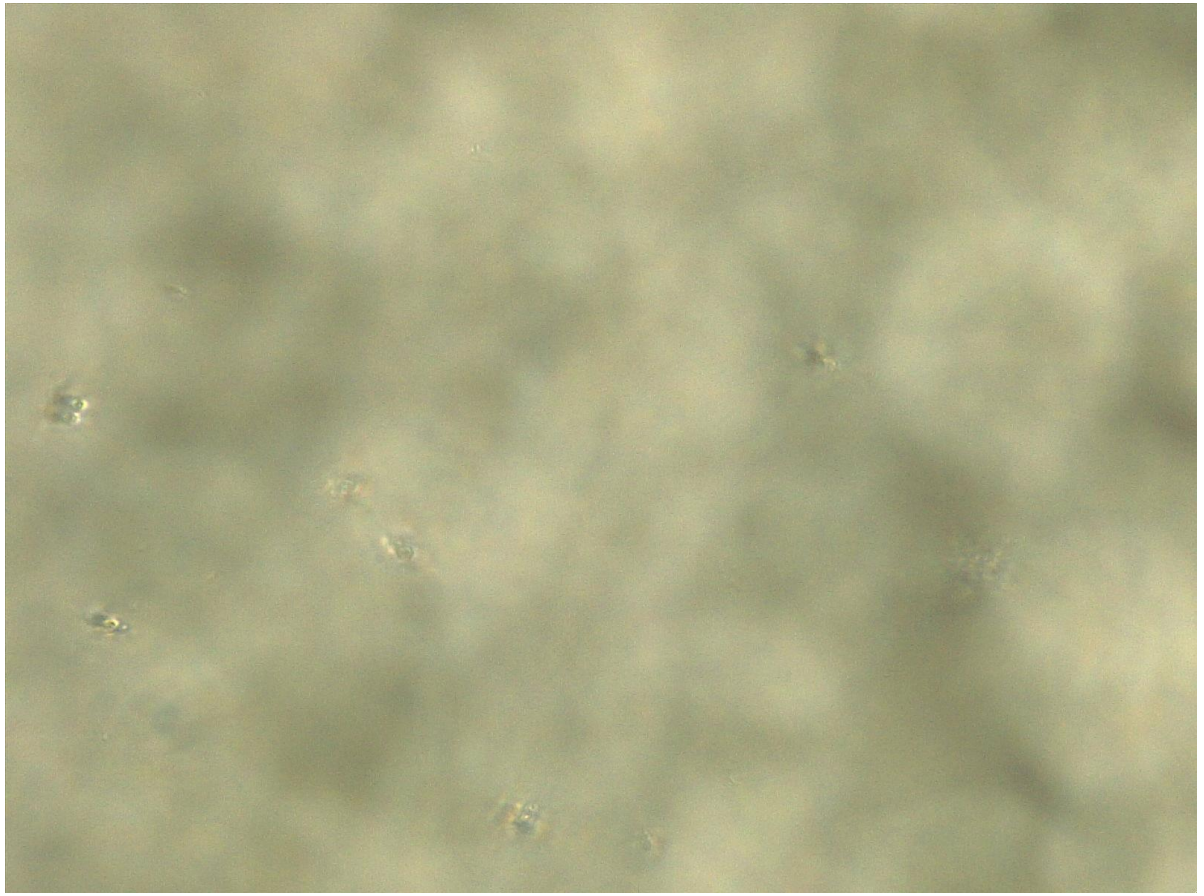
Glycogen Staining

= Glycogen

= No Glycogen



2% Lactate Knockout

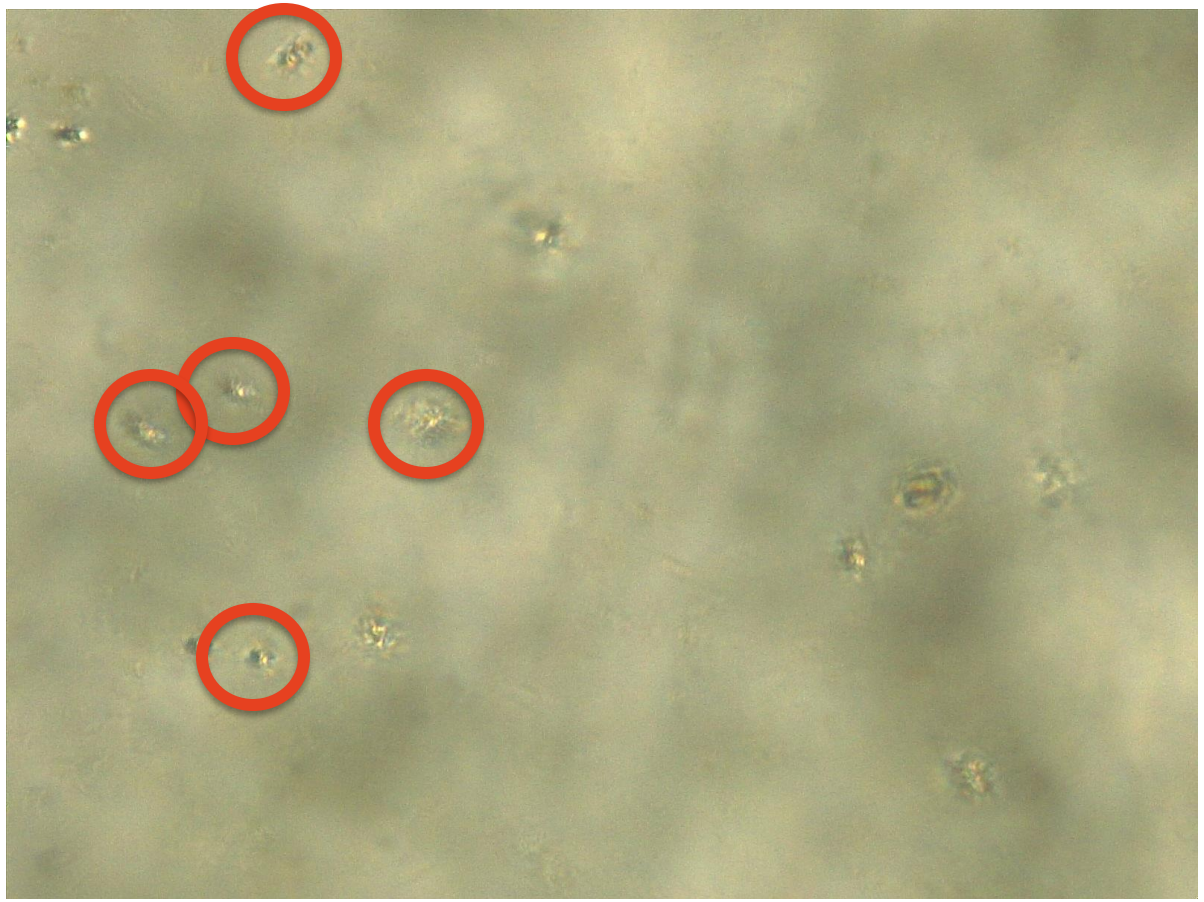


2% Pyruvate Knockout

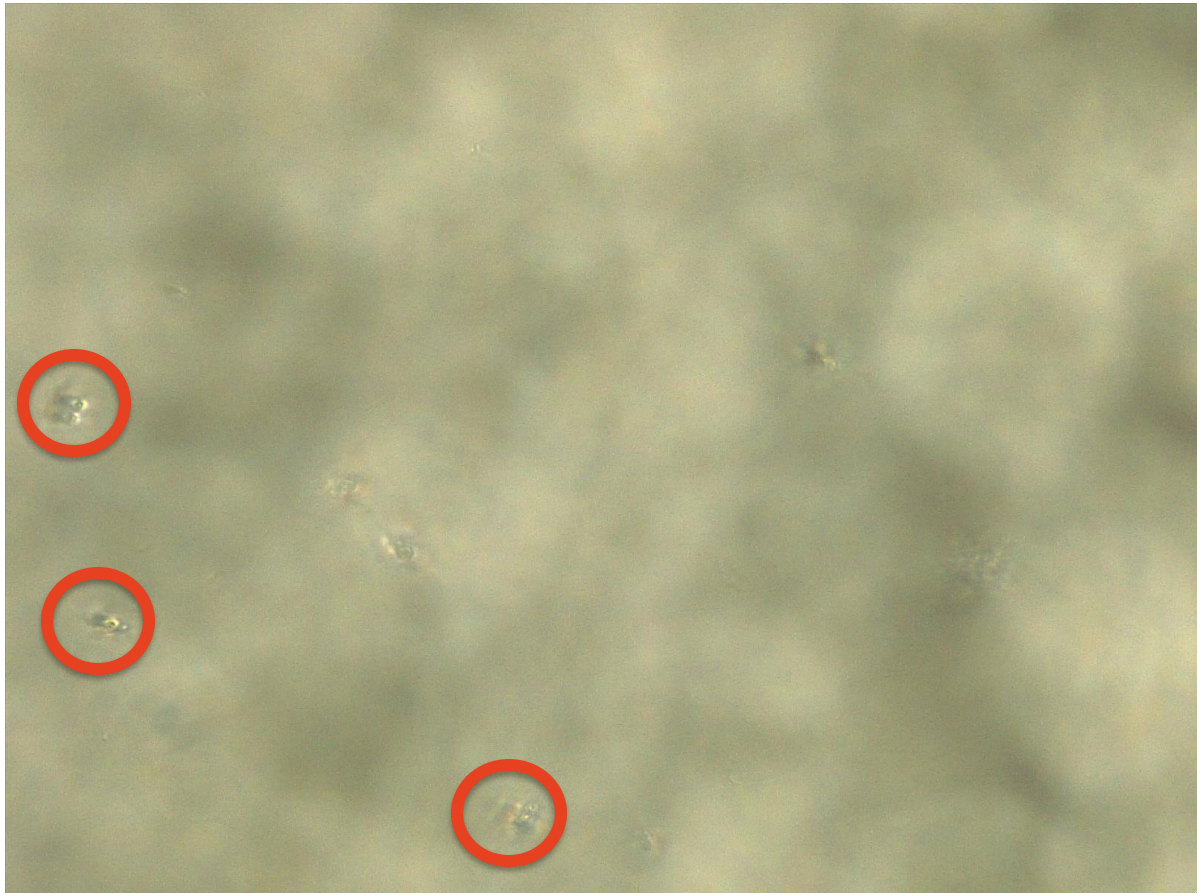
Without the presence of glucose, absence of glycogen formation was expected. Knockout strains are not producing glycogen through alternative biosynthetic pathways.

= Glycogen

= No Glycogen



2% Lactate Knockout

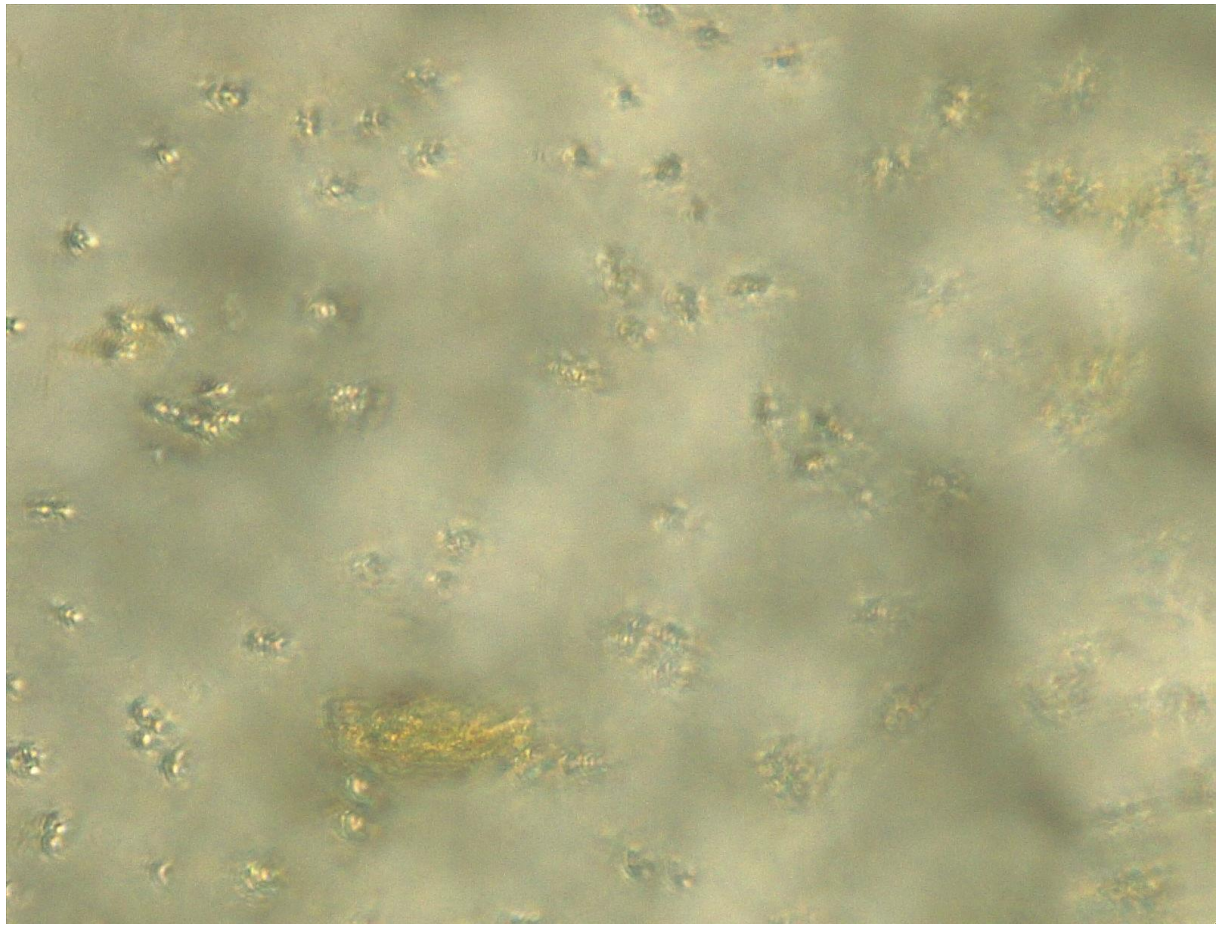


2% Pyruvate Knockout

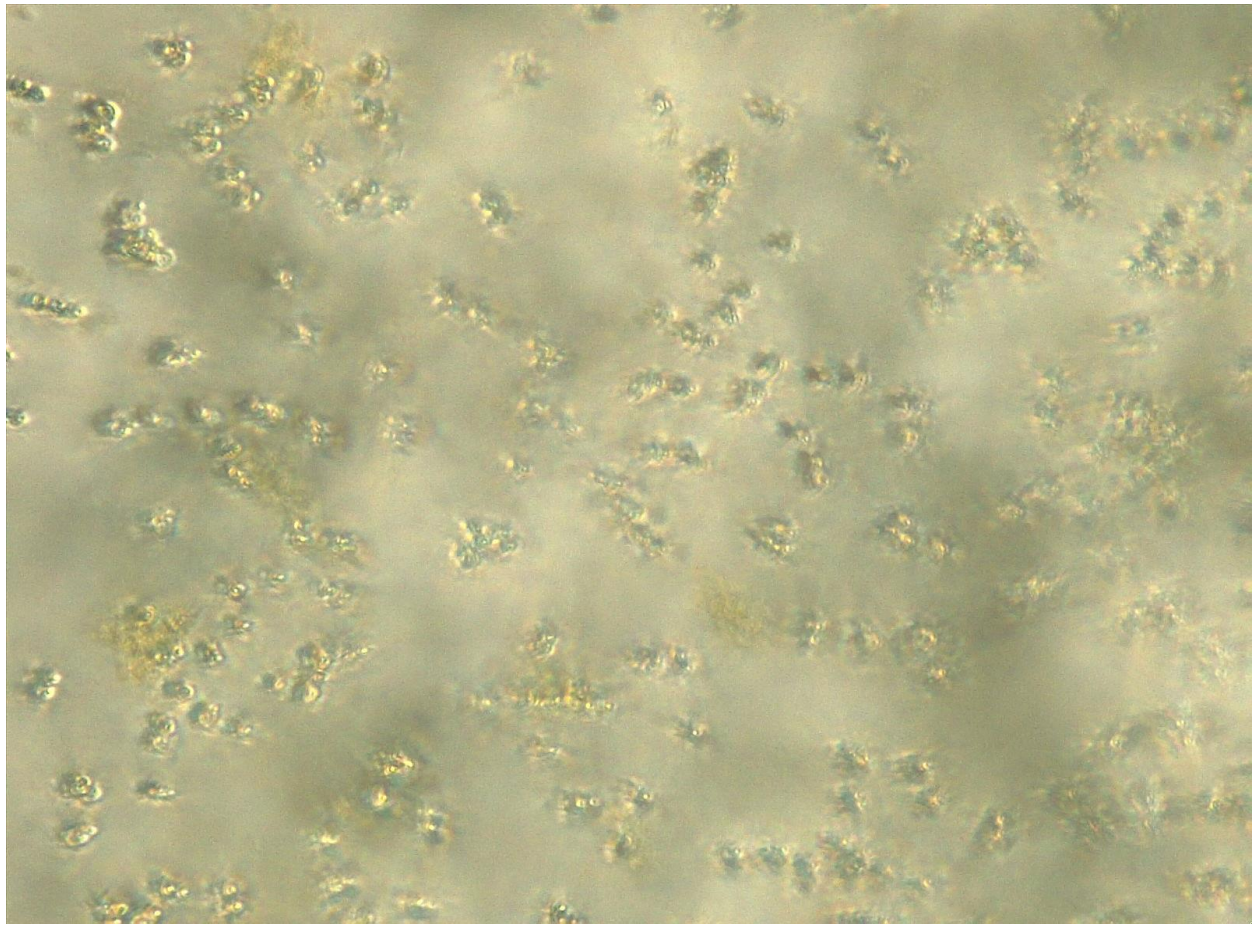
Glycogen Staining

= Glycogen

= No Glycogen



1% Glucose Knockout

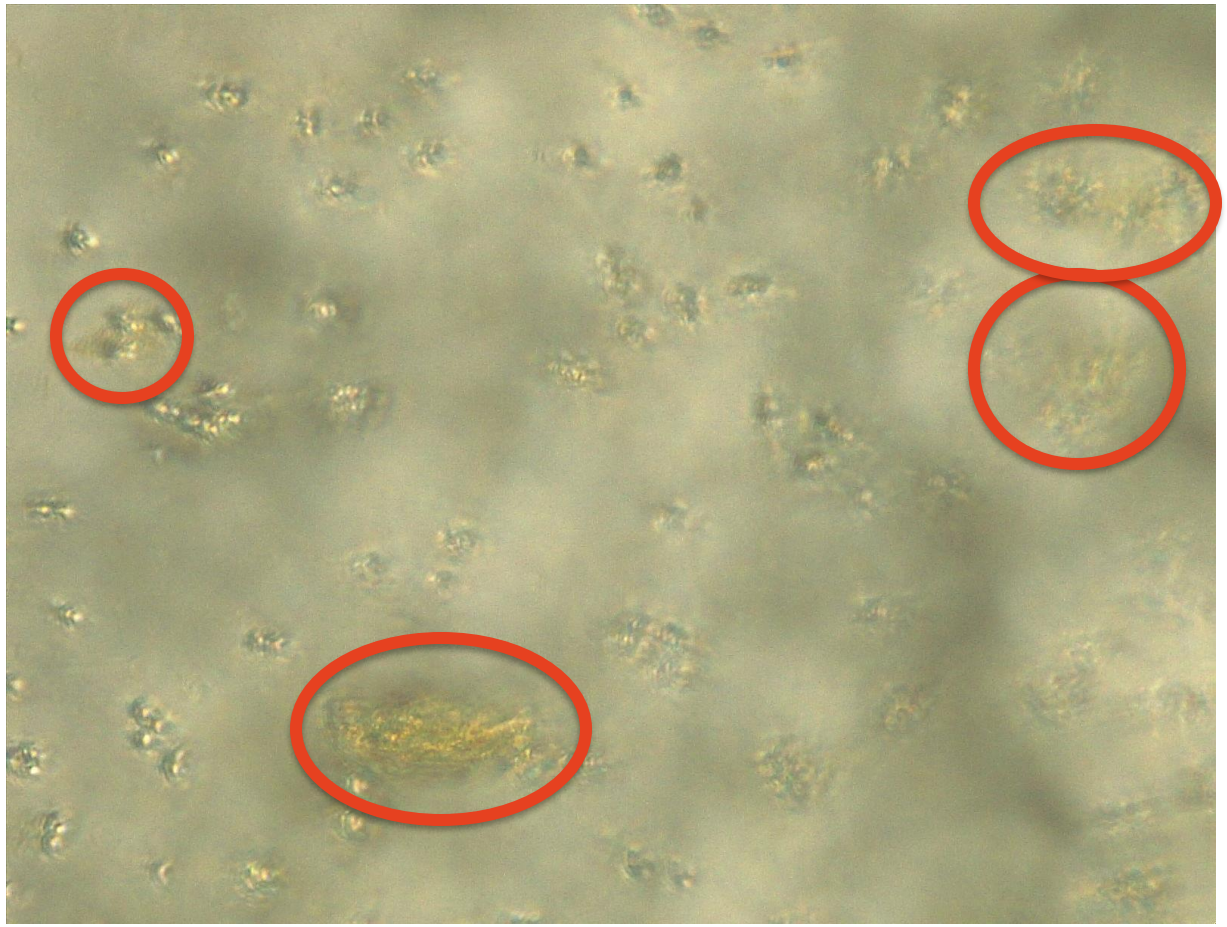


1% Glucose Wild-Type

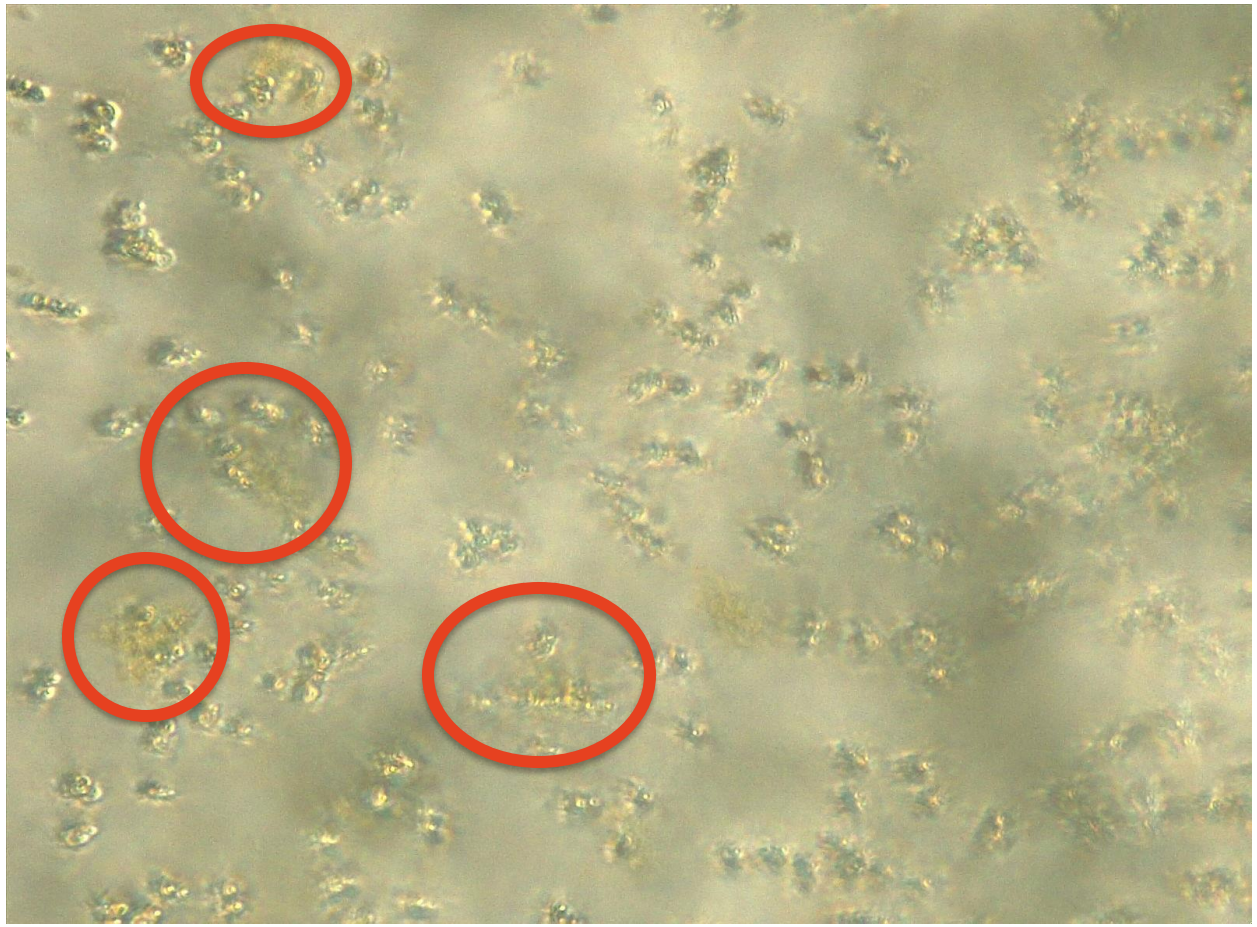
Glycogen formation present in both KO and WT strains.
Suggests that yeast does not accurately model GSD7.
Possible that glucose concentration is too high.

= Glycogen

= No Glycogen



1% Glucose Knockout



1% Glucose Wild-Type

Quantitative PCR

- Pho85 downregulation is expected in KO
- qPCR validates mode of regulation of Pho85
  - Regulation at a functional level (cyclin)
- TKL1 upregulation is predicted and observed

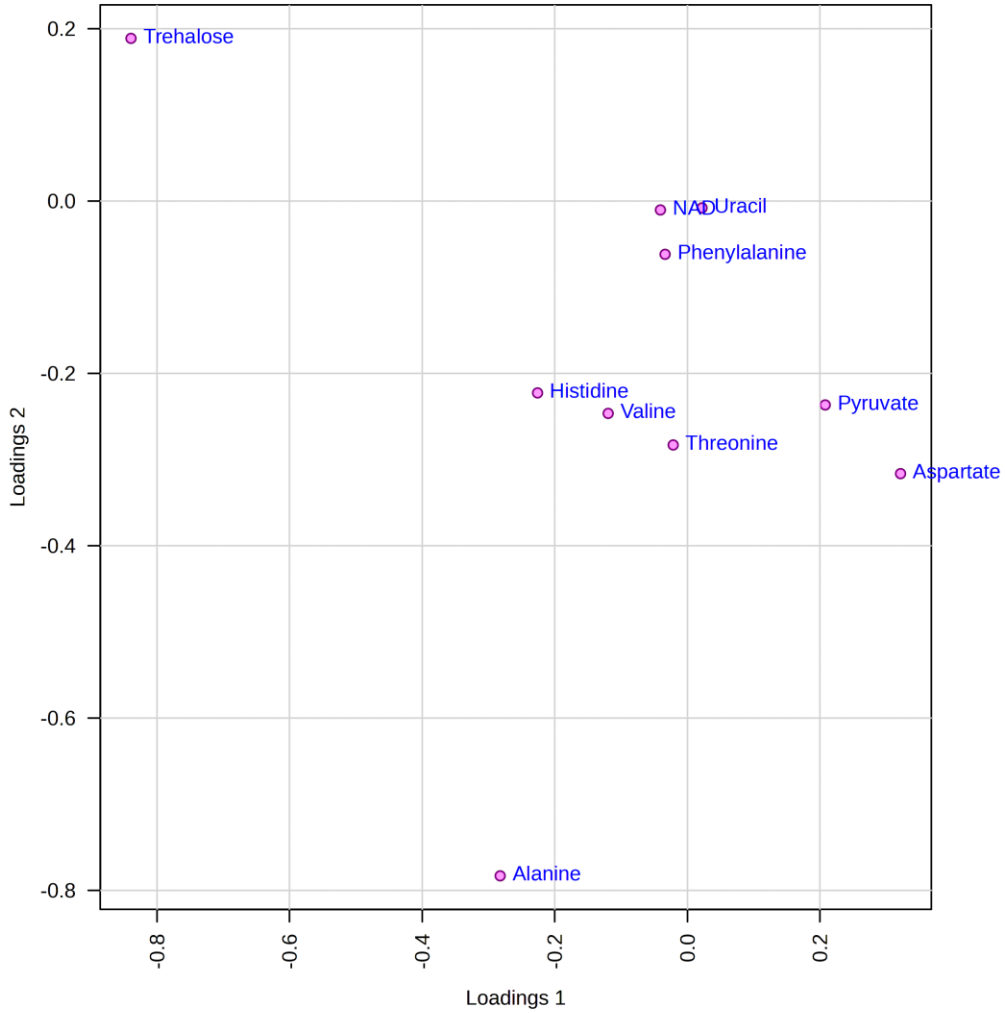
Gene	Predicted	Experimental
PHO85	Downregulated	Unchanged
TKL1	Upregulated	Upregulated

- **Under extreme glucose condition:**
  - Severely downregulated Pho85 transcript in KO low glucose (autophagy pathway)[1,2]
  - TKL1 inconclusive (too many data points removed)
- Lacks reliable result → additional research required

1. Ho *et al*. *Nature*. **2002**, *415* (6868), 180–183.
2. Yang *et al*. *Molecular Cell*. **2010**, *38* (2), 250–264.

Metabolomics

- Huge discrepancy in amino acid concentration
- Most amino acids exist in large quantity in WT low glucose yeast, likely due to stalled protein synthesis[1]
- Low amino acid concentration in KO yeast (except for aspartate!) unaccounted for



1. Dokládal *et al. Cell Reports.* **2021,** *37* (13), 110149–110149.

Metabolomics

- Huge discrepancy in amino acid concentration
- Most amino acids exist in large quantity in WT low glucose yeast, likely due to stalled protein synthesis[1]
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Low Glucose Condition	Wild Type	Knockout
Threonine	High	Low
Histidine	High	Low
Valine	High	Low
Alanine	High	Medium
Aspartate	Medium	High
Pyruvate	High	High

1. Dokládal *et al*. *Cell Reports*. **2021**, *37* (13), 110149–110149.

Conclusions

- Growth Plates: Most Glucose samples' growth was limited by cell density; alternate carbon sources could be utilised by the yeast but at ~50% effectiveness
- qPCR was informative but largely inconclusive
- Metabolomics identified differential amino acid biosynthesis pathways between the WT and KO
    - Aspartate and threonine biosynthesis/metabolism are of interest in future studies
- The glycogen staining protocol should be modified to include quantification (e.g. by spectrophotometry) or test the activity of enzymes involved in glycogen synthesis between the strains and treatment conditions (e.g. branching enzyme)
- qPCR was informative but largely inconclusive
- Metabolomics identified differential amino acid biosynthesis pathways between the WT and KO
    - Aspartate and threonine biosynthesis/metabolism are of interest in future studies

Investigation of PFK2 PPI Networks

Collaboration with MATH3888

THE UNIVERSITY OF SYDNEY

Collaboration Workflow

BCMB: How can we explain Glycogen Storage Disease at the genome and proteome level?

BCMB: How do we expect glycolysis to be circumvented in ΔPFK2 *S. cerevisiae*?

MATH and BCMB: Protein targets in ΔPFK2 *S. cerevisiae* to investigate: TKL1, PHO85, and BST1

MATH: Create algorithms based on importance of protein targets and distance and frequency of their connections (nodes and edges)

MATH and BCMB: First draft of novel proteins to review (Method 1)

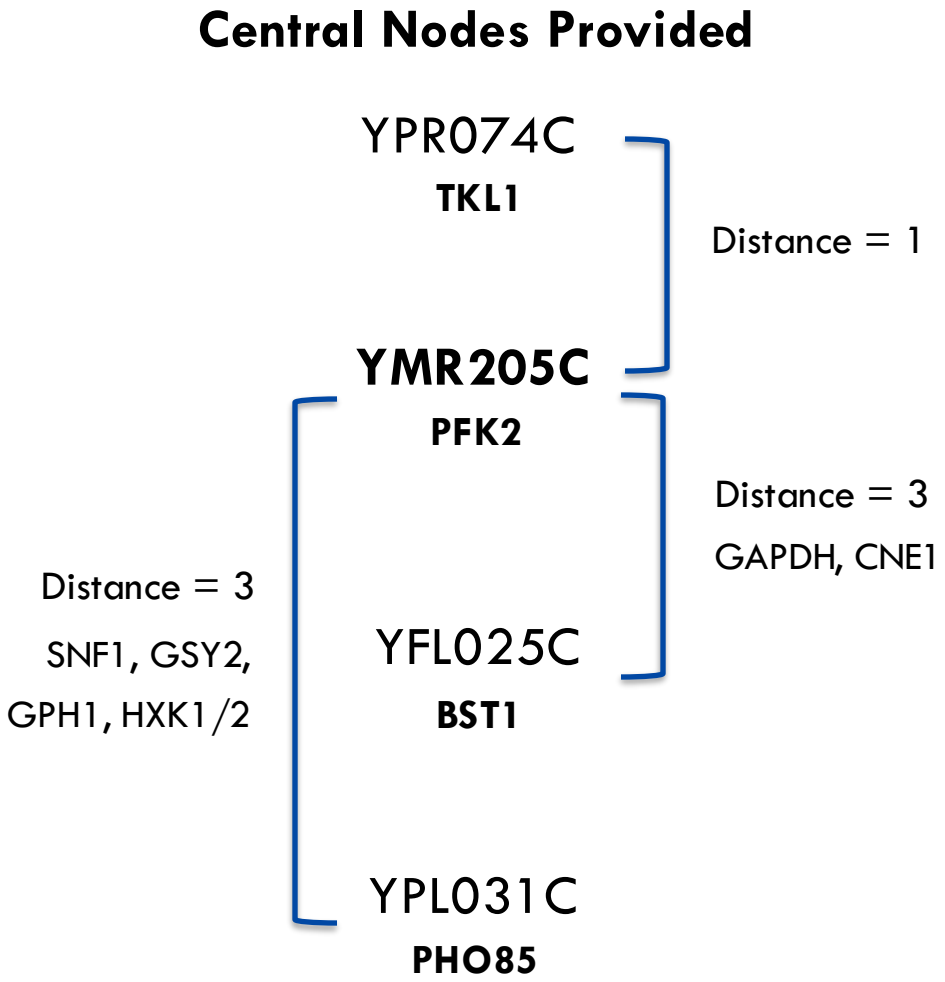
MATH and BCMB: Second draft of novel proteins to review (Method 2)

MATH and BCMB: Final clarifications on projects. Discussion of relevance of new targets to human disease

Rationale for Target Confirmation

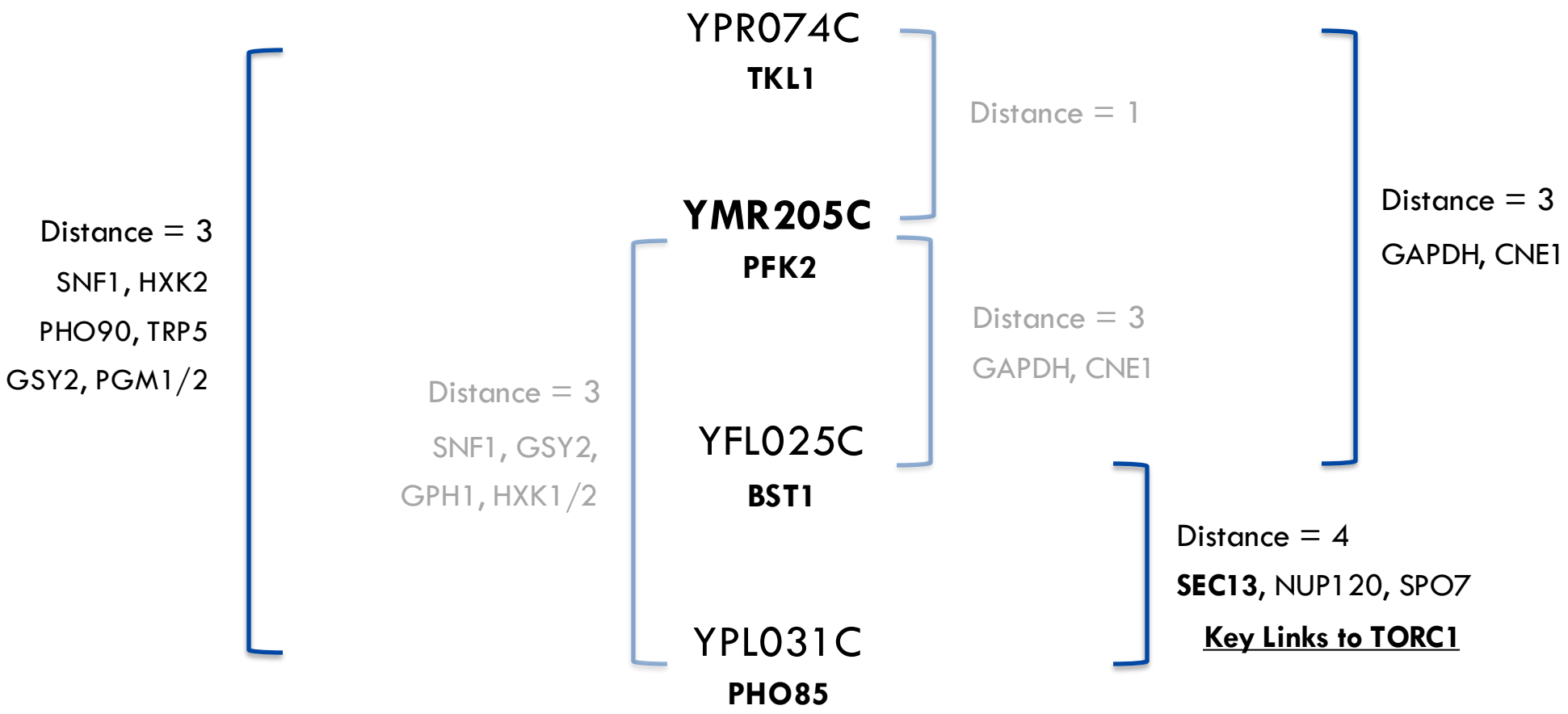
1. Do we expect the proteins to interact **directly** (i.e. physically) or **indirectly** based on literature evidence/pathway analysis?
2. Are the proteins localised in the same **subcellular** compartment?
3. Are there specific **conditions** under which the proteins are expressed, or are they expressed **constitutively**?
4. Can we correlate changes in the **transcription/expression** of these proteins to a **deletion** of PFK2?

Network Analysis



Method 1: Pairwise Shortest Paths

Network Analysis



Method 1: Pairwise Shortest Paths

Network Analysis

- Centrality of a node is dependent on the centrality of its neighbours
- HXK2, GLK1, and GAPDH reported within the top ten nodes of highest importance

(Relative to PFK2)

Gene	Importance	Centrality
ERV25	0.850	0.355
TAL1	0.816	0.224
TKL2	0.814	0.223
NQM1	0.807	0.218
EMI1	0.748	0.176

Method 2: Katz Centrality

Network Analysis

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Regulation of protein folding in ER

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Non-oxidative PPP transaldolase, induced in diauxic shift

Oxidative transaldolase

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Minor isoform of TKL1 (WGD)

Method 2: Katz Centrality

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Putative GLK
Expressed during low glucose growth
Essential for sporulation

Method 2: Katz Centrality

Experimental Validation

Targets: **SEC13** and **TORC1**

(Human Homologue **SEC13**/**mTORC1**)

Pathway of Interest

Activation of TORC1 via inhibition of SEACIT

Hypothesis

Since TORC1 activity is repressed in ΔPFK2 *S. cerevisiae*, SEC13 is downregulated (i.e. SEACIT activity upregulated)

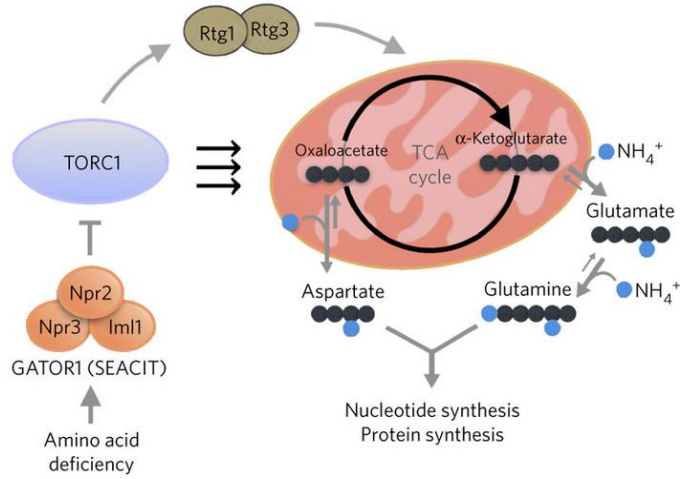
Strategy

1. Co-immunoprecipitation or pull-down assay. Tag targets:
SEC13–3xHA → SEC13 **bait** (IP protein)
SEA3–3xMyc → marker for SEACAT
2. Western blot on IP protein

Expected Result

WT: ↑ SEC13–SEACAT interaction (**TORC1 active**)

KO: ↓ SEC13–SEACAT interaction (**TORC1 repressed**)



Chen *et al.* 2017

Experimental Validation

Targets: **SEC13** and **TORC1**

(Human Homologue **SEC13**/**mTOR**)

Limitations:

- Difficult to isolate native SEC13 interactions within complexes rather than as a single protein.
- Scaffold-dependent binding can lead to non-specific or background interactions.
- SEACIT is activated when amino acids are depleted, and it is inhibited by ubiquitination when amino acids are abundant, which makes it challenging to incorporate into the test conditions used thus far.
- SEA complex fragility could affect trueness of readouts for SEC13-SEACAT interactions.
- Epitope tags (HA and Myc) may slightly alter protein conformation or interfere with complex assembly.

New Targets (Discussed Further in Portfolio)

Human	Yeast	Rationale
TKT	TKL1	Catalyses reversible sugar-phosphate conversions linking glycolysis to NADPH and aromatic amino acid biosynthesis
CDK5	PHO85	Regulates metabolism, glycogen synthesis, cell cycle progression in response to nutrient availability
NEP1-R1	SPO7	Controls phospholipid biosynthesis, and nuclear envelope morphology; adjusts lipid synthesis under low ATP or glycolytic stress
ULK1 and ULK2	ATG1	Ser/Thr kinase required for autophagy initiation and phagophore assembly in response to energy or nutrient stress
AMPK	SNF1	Activates energy stress responses and switches metabolism from glycolysis to alternative pathways during glucose limitation

Future Directions

- Integrate multi-omics studies
  - e.g. Large-scale proteomics, phosphoproteomics to enrich analysis of specific cascades, metabolomics of more specific glycolytic and PPP intermediates/products of interest
- Optimise our glycogen staining/quantification protocol
- Select alternate qPCR targets which may give better insight into transcriptional regulation in ΔPFK2 *S. cerevisiae*
- Decipher where and how transcriptional vs functional regulation occurs in ΔPFK2 *S. cerevisiae*, and whether this is relevant to GSD
- Address the limitations of using a yeast model to investigate biomarkers/phenotypes of a human disease