



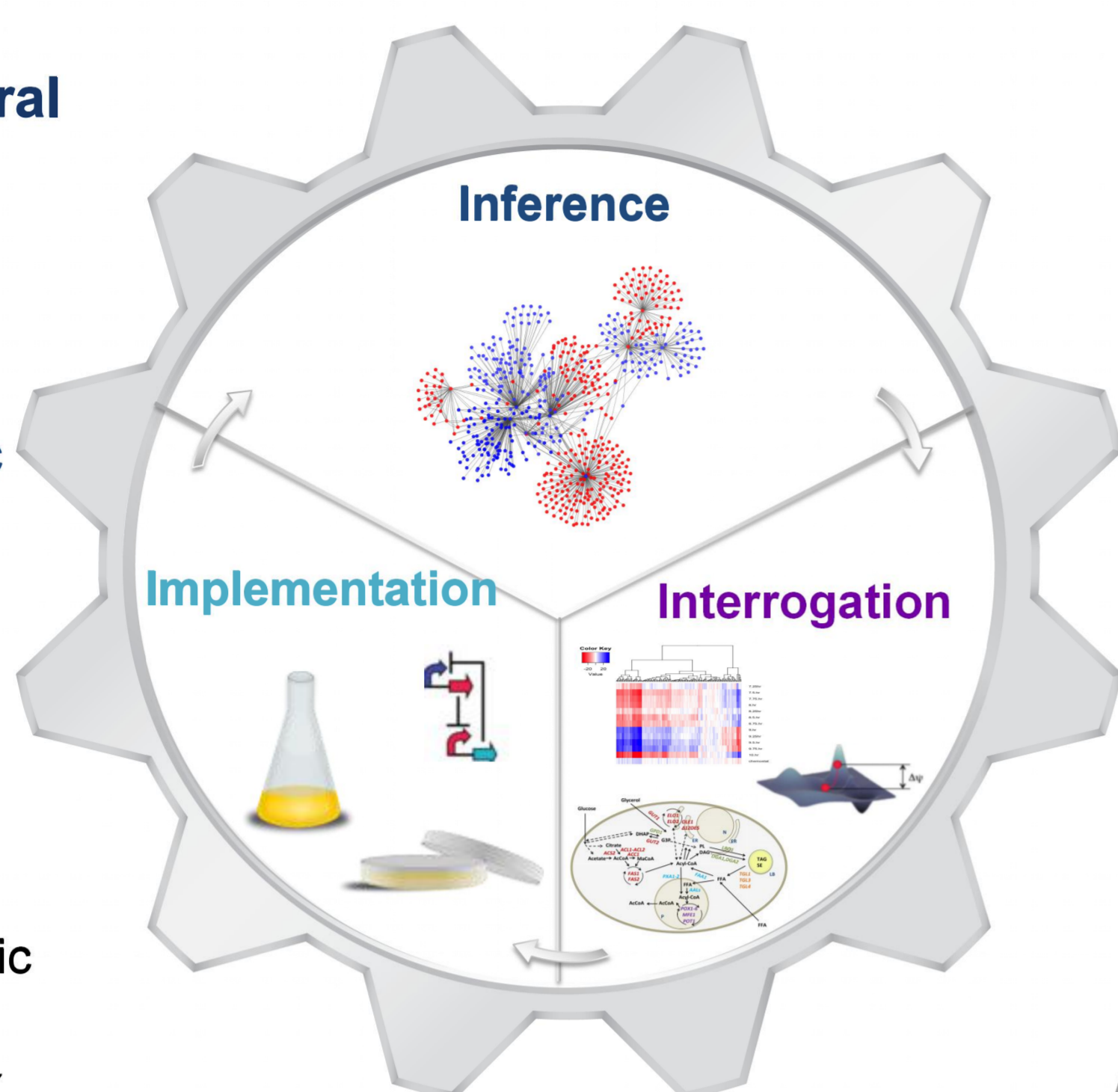
In this study, we aimed to identify **context-specific regulators and mechanisms**, to guide explorations of the regulation of lipid accumulation in *Y. lipolytica* during nitrogen limitation. Using **gene regulatory network (GRN) inference**, and considering the expression of **6539 genes over 26 time points** from GSE35447 for **biolipid production** and a list of **151 transcription factors (TF)**, we reconstructed a GRN comprising **111 transcription factors, 4451 target genes and 17048 regulatory interactions (YL-GRN-1)** supported by evidence of protein-protein interactions.

This study, based on network interrogation and wet laboratory validation (a) highlights the relevance of **influence measurement for identifying phases corresponding to changes in physiological state without prior knowledge** (b) **suggests new potential regulators and drivers of lipid accumulation** and (c) **experimentally validates the role of 6 of the 9 regulators identified in lipid accumulation**, with variations in lipid content from +43.2% to -31.2% on glucose or glycerol.

1 Context and motivation

Multi-scale modeling of the central regulatory network of lipids metabolism

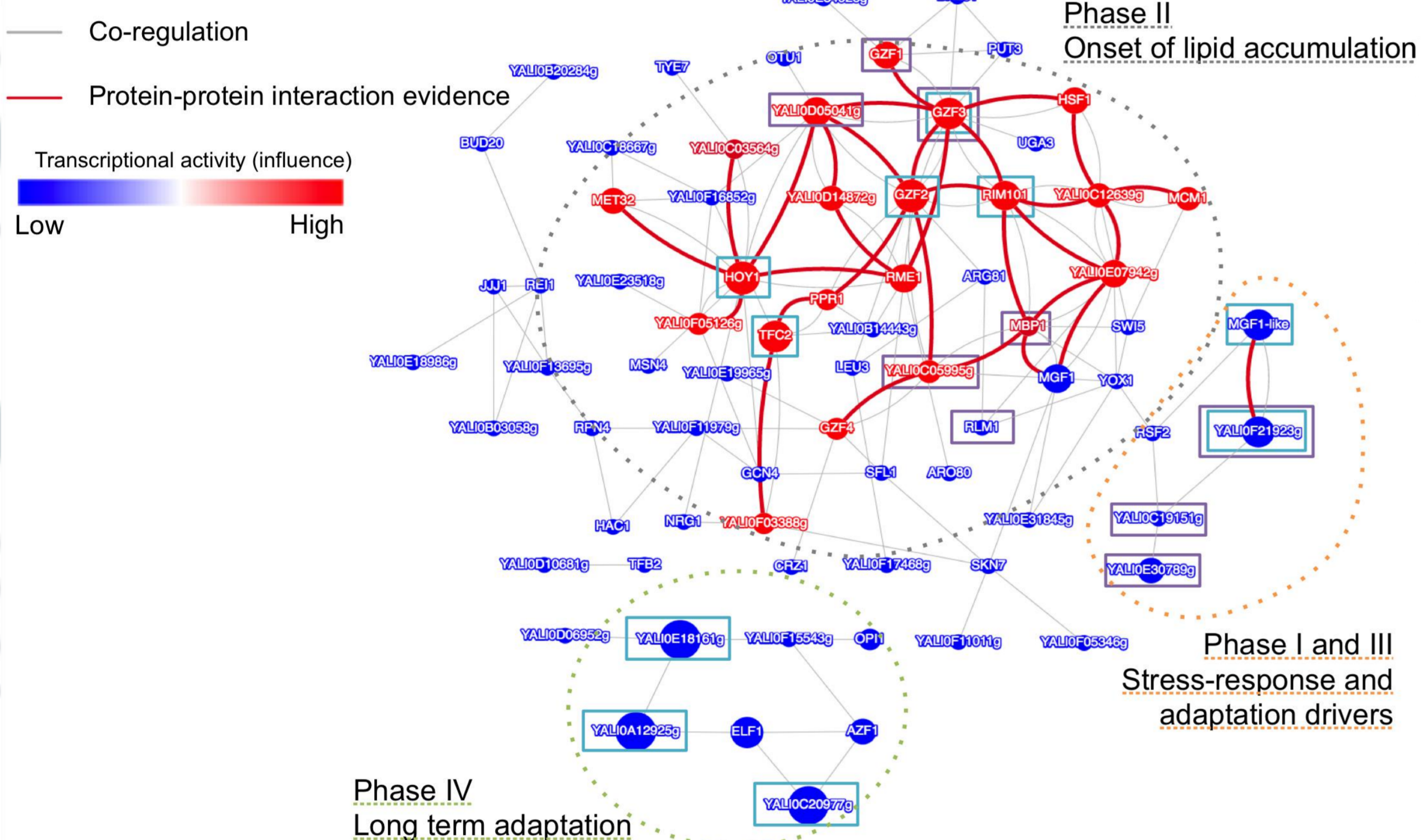
Experimentation and metabolic engineering



- Reduce time-consuming and costly laboratory effort
- Find non-trivial and context-specific regulators
- Leverage information from transcriptomic data
- Improve the understanding of complex phenotypes regulation

3 Use of a cooperativity network to identify evidence-supported coregulatory relationships and new candidate co-regulators

- Master regulators of lipids associated-genes
- Top 10 most influential TFs over the whole experiment

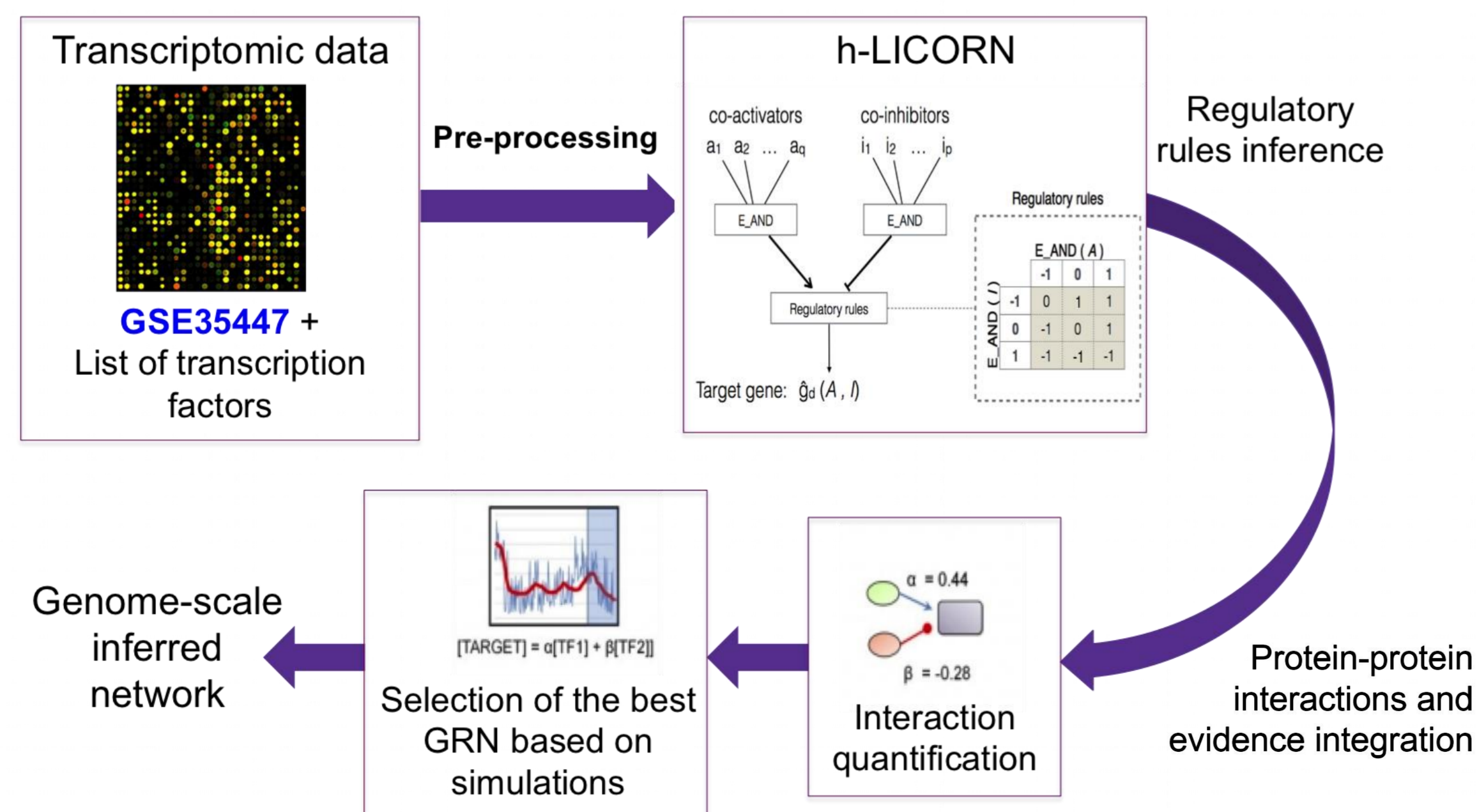


Cooperativity network for *Yarrowia lipolytica* (YL-CoRegNet-1) constructed from YL-GRN-1 during phase II

- Using our GRN, master regulators and most influential TFs of lipid accumulation were identified and mapped on the cooperativity network.
- Cooperativity modules were also revealed among TFs, suggesting non-trivial candidates regulators supporting lipid accumulation.

Materials and methods

Network inference (CoRegNet [4])

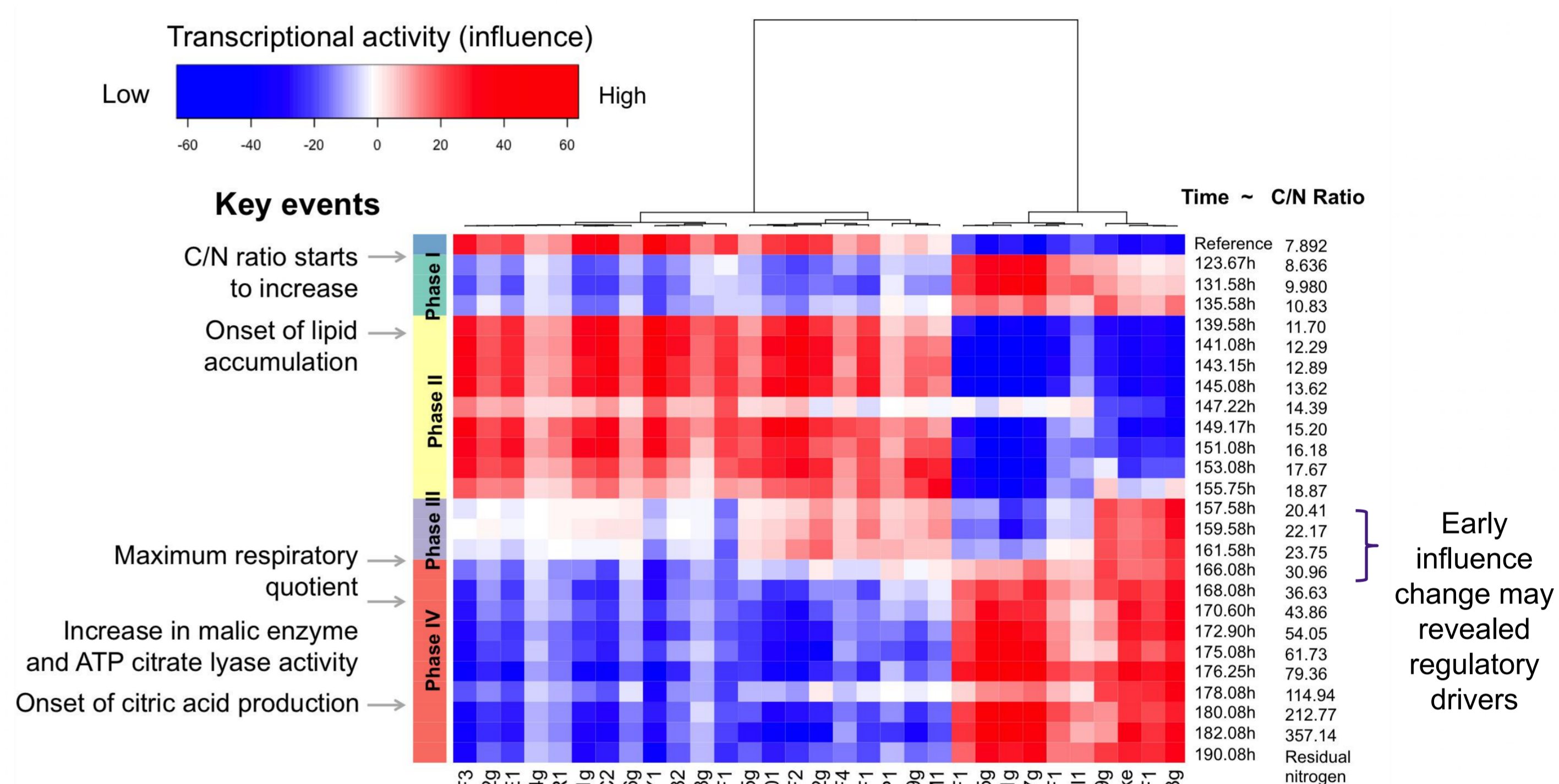


Construction of TFs overexpressing mutants

- Mutants were constructed by inserting the TF expression cassette (*URA3ex-pTEF-TF*) into *JMY2566* using the Gateway system [3]. The wild-type strain *JMY2810* was used as the wild-type control.
- YNB medium, 3% glucose or glycerol, C/N ratio=30, 28°C.
- GC & HPLC for lipids and metabolites analysis.

2 TF activity during nitrogen limitation highlights patterns during lipid accumulation

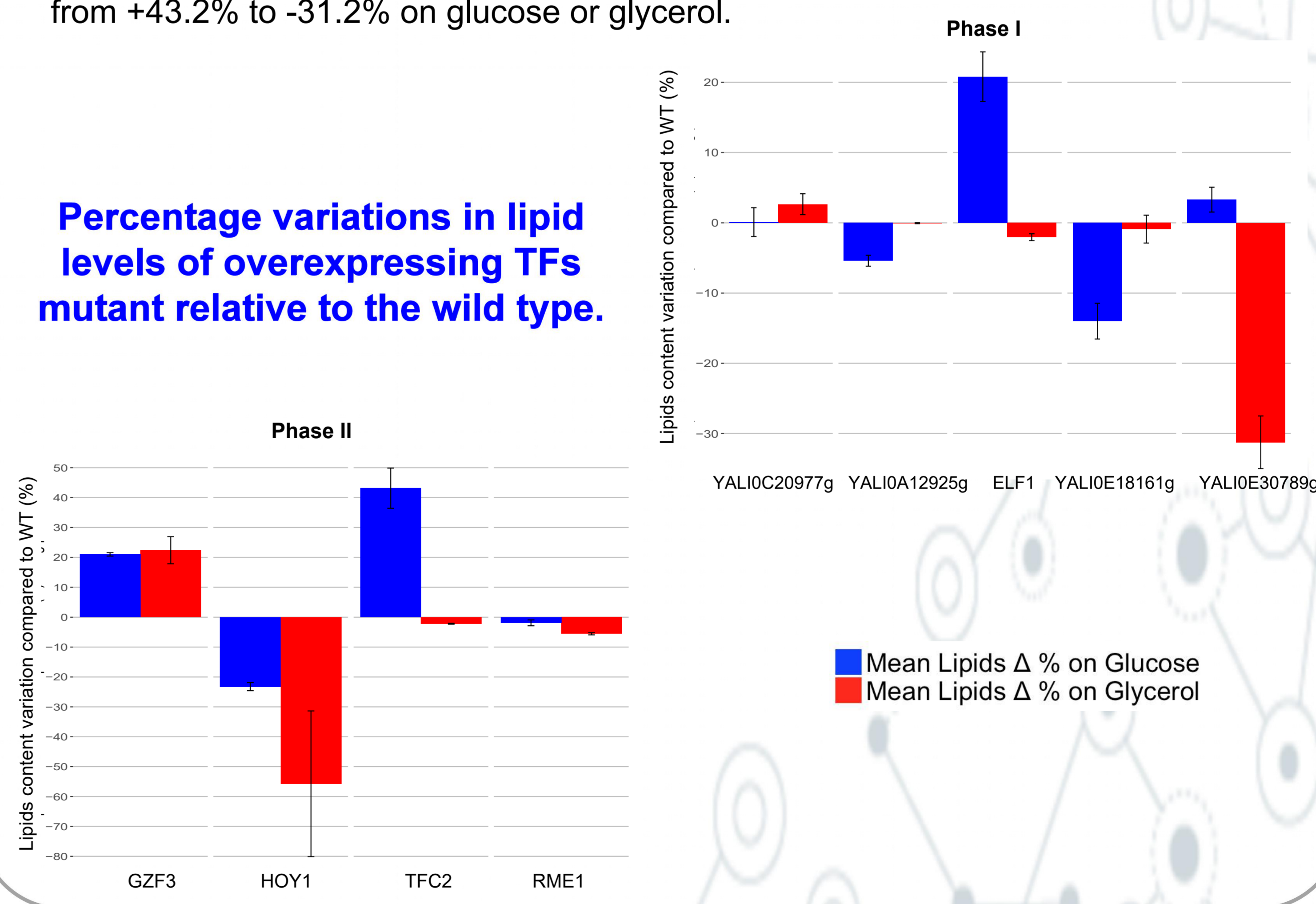
- The influence represents the activity of each transcription factor (TF) in a robust sample-specific manner. It is computed from the GRN and the expression of its targets genes in the transcriptomic data [1,2].
- Influence patterns had allowed the identification of physiological phases without prior knowledge. In particular, earlier change in some TFs influence may point them as drivers of short and long term adaptation to nitrogen limitation and thus as drivers of lipid accumulation.



Heatmap of TF influence over an increasing Carbon / Nitrogen (C/N) ratio

4 Construction of overexpression mutants for experimental validation of the impact of the most influential TFs on lipid accumulation profile

- TFs were ranked according to their influence during phase I (early adaptation to nitrogen limitation) and phase II (onset of biolipid production).
- The most influential TFs in those phases were selected for the construction of overexpressing strains.
- 6 of the 9 regulators identified had an effect on lipid accumulation with variations from +43.2% to -31.2% on glucose or glycerol.



Affiliation, funding and references

- ¹ Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
- ² Université d'Évry, Évry 91000, France
- ³ CNRS-UMR8030 / Laboratoire ISSB, Évry 91000, France
- ⁴ CEA, DRF, IG, Genoscope, Évry 91000, France
- ⁵ Université Paris-Saclay, Évry 91000, France

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- [2] GSE35447 transcriptomic dataset, deposited in NCBI Gene Expression Omnibus database
- [3] Leplat C, et al., High-throughput transformation method for *Yarrowia lipolytica* mutant library screening. FEMS Yeast Res. 2015;15(6).
- [4] Nicolle R, et al., CoRegNet: reconstruction and integrated analysis of co-regulatory networks. Bioinformatics. 2015;31(18)

