



CRISPRi-omics regulation of free fatty acids production in *Escherichia coli*

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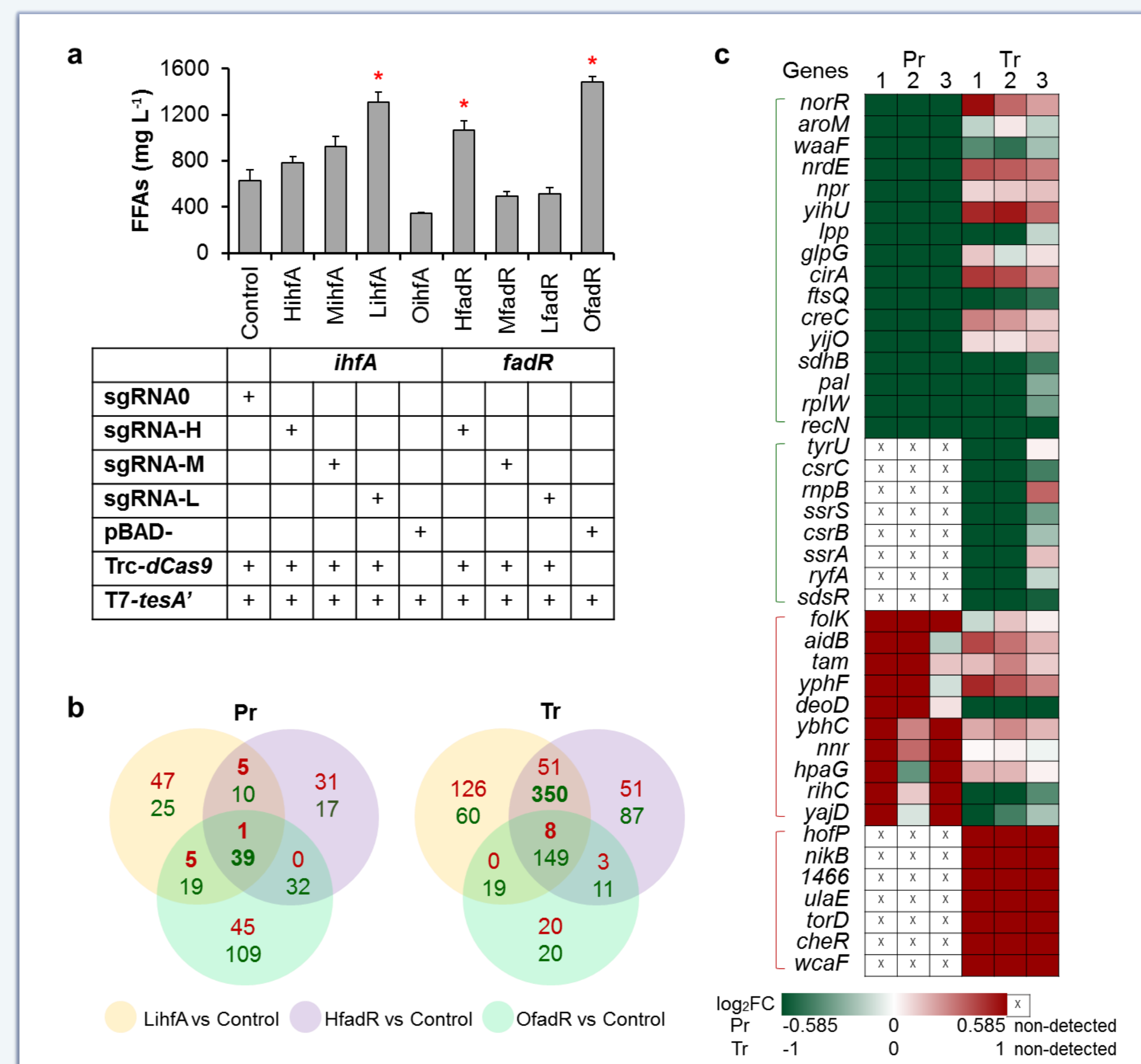
Abstract

- Identification of beneficial genetic targets and fine-tuning of their expression are critical for improving the production of the desired metabolites.
- Because of the laboriousness of conventional metabolic engineering methods and the complexity of genetic and metabolic networks, this is challenging for genome-scale identification of the chromosomal genes.
- Here, we report a combined CRISPRi-omics strategy allowing for rapid, systematical and effective identification of targets that can be engineered to optimize FFAs production in *E. coli*.

Identification of beneficial targets from pathways related to FFAs metabolism using CRISPRi system

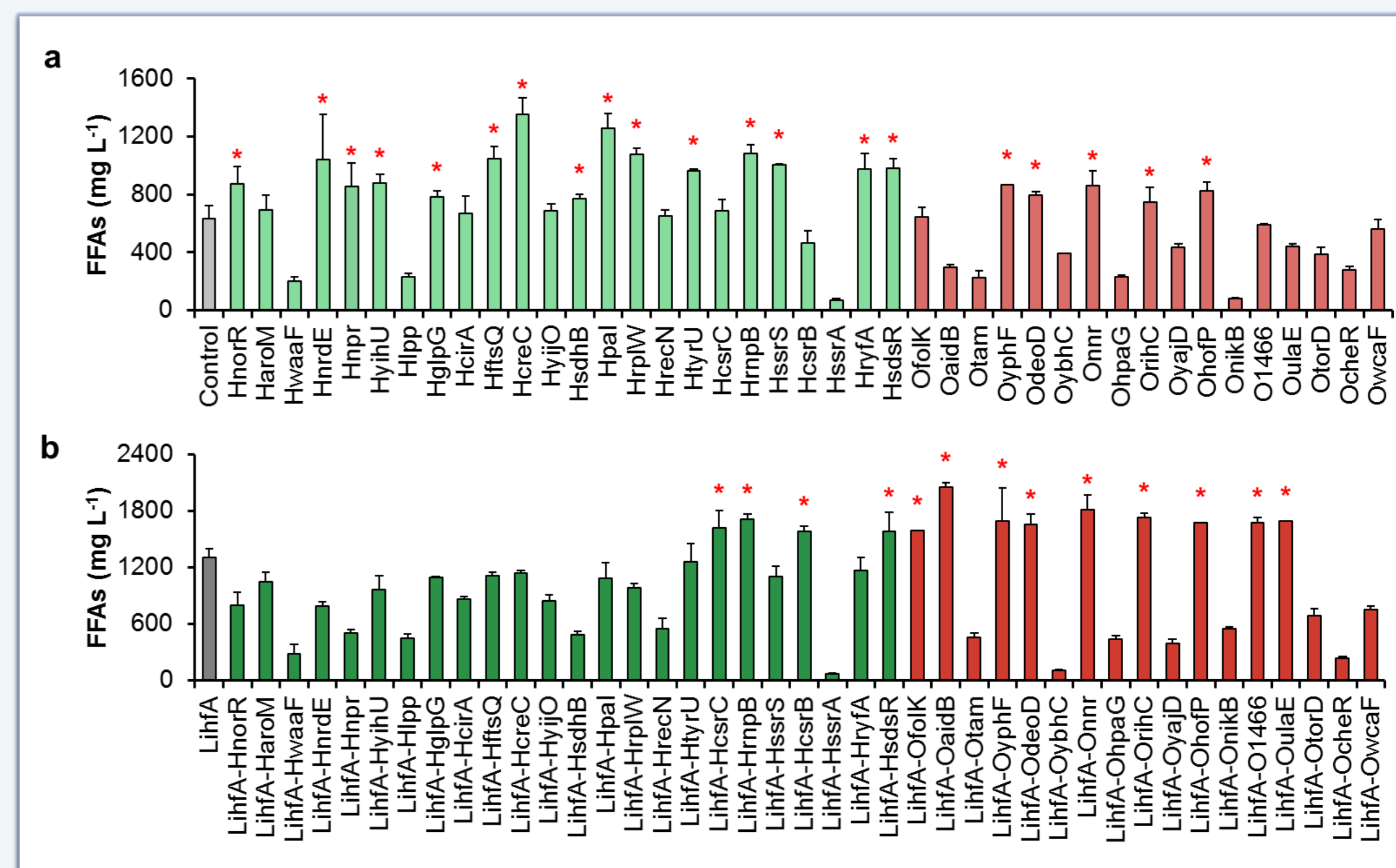
Exploration of potential targets in the cellular network via transcriptomics and proteomics analysis

- 41 different expressed genes (DEGs) were selected from comparative transcriptomic and proteomic analysis of three high-yield strains LihfA, HfadR, OfadR and the control strain.



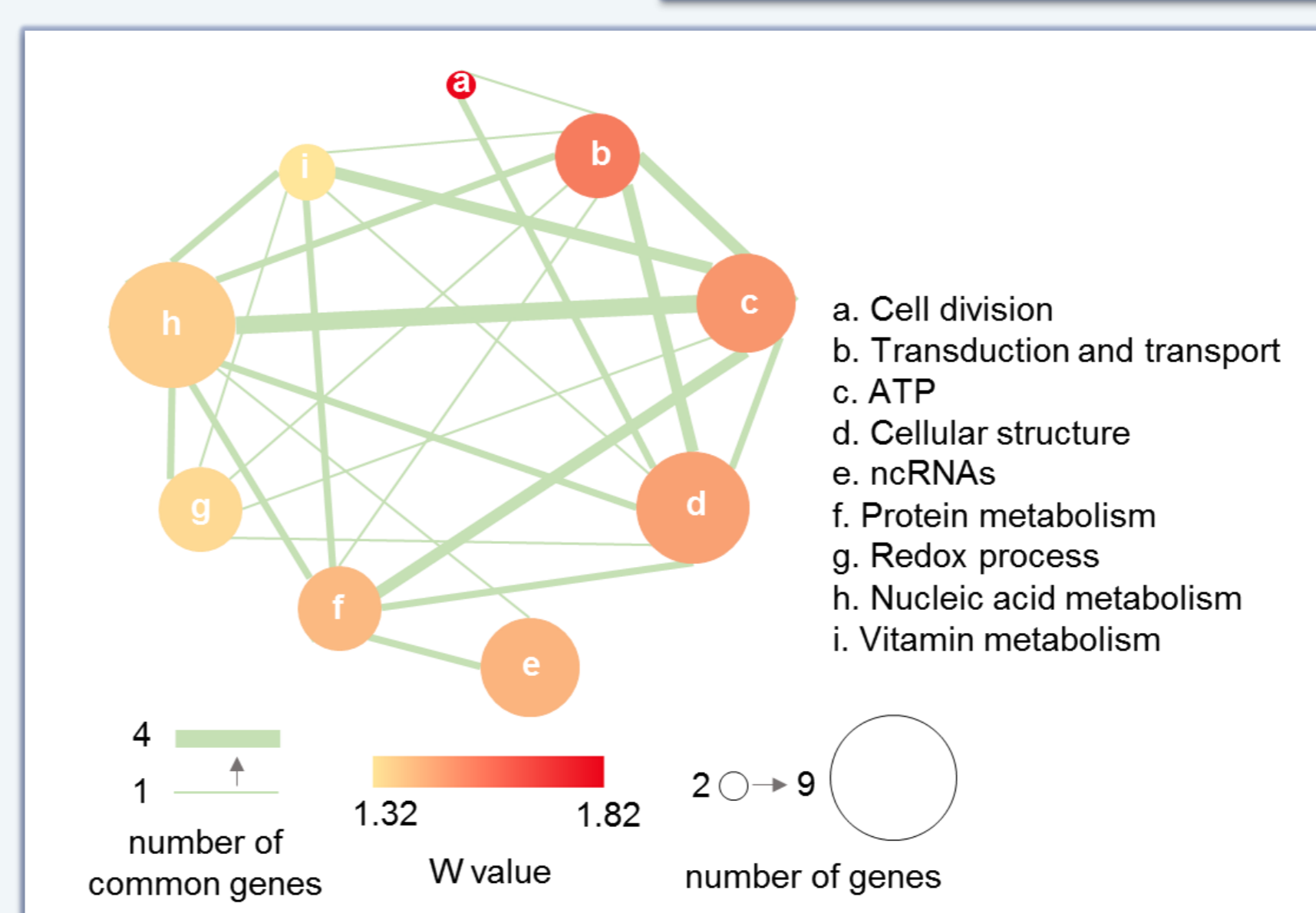
Reverse engineering

- 26 beneficial targets were identified from the 41 DEGs.
- The best engineered strain LihfA-OaidB produced 2052.46 mg L⁻¹ FFAs.

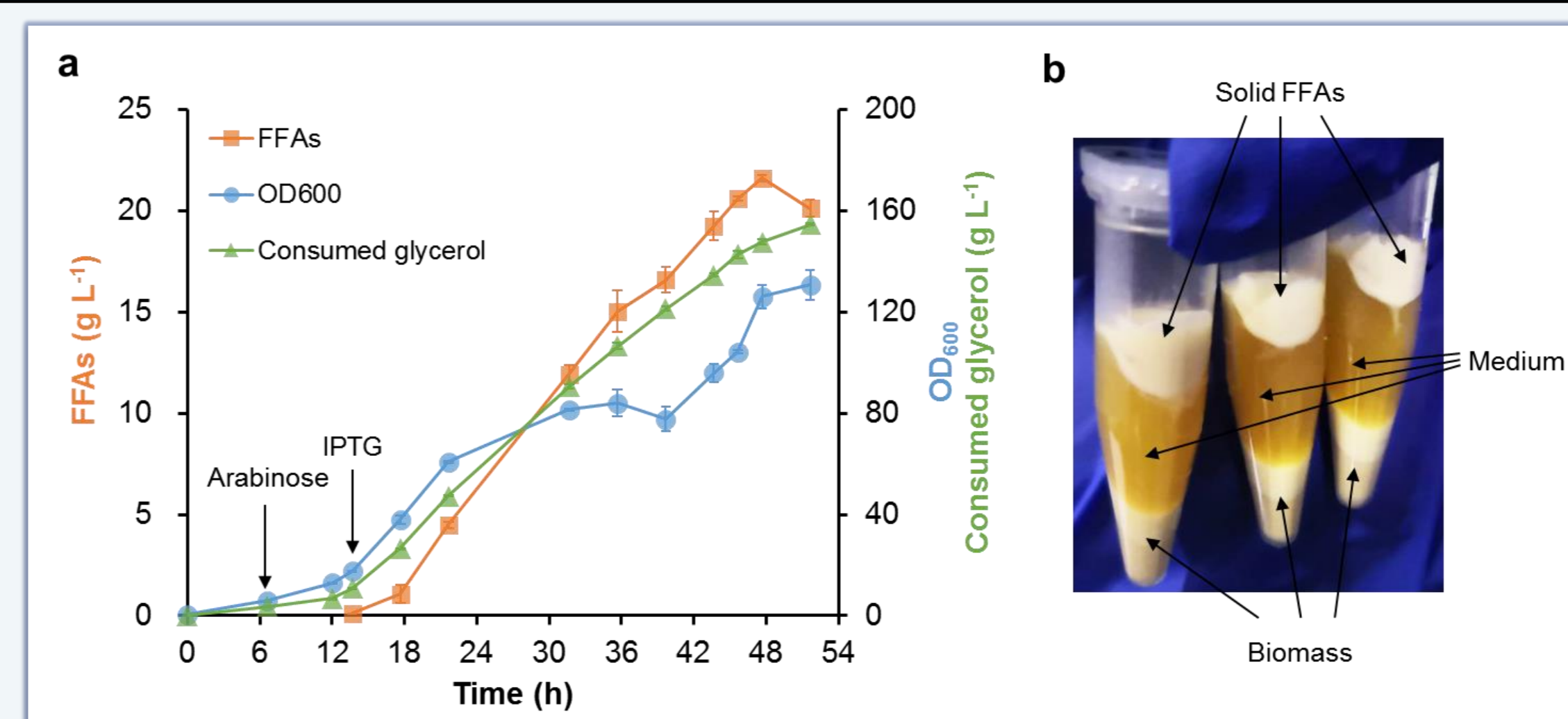


Network plot

- The involved cellular processes of the 26 beneficial targets
- The effect of the cellular processes on FFAs production



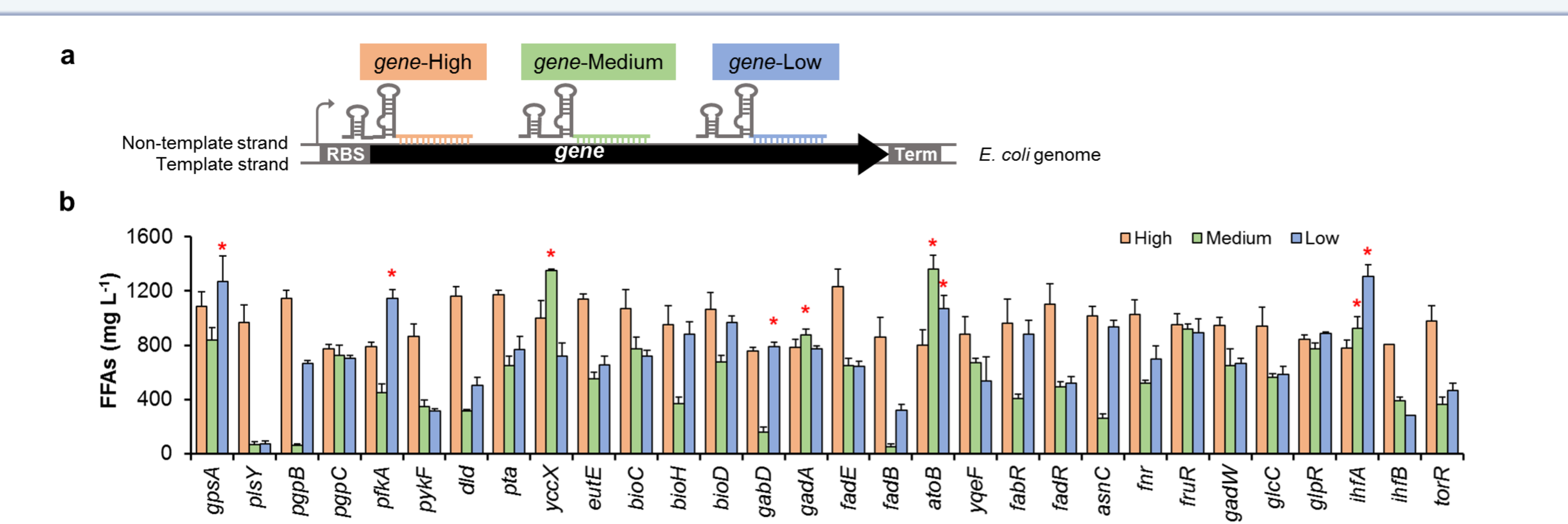
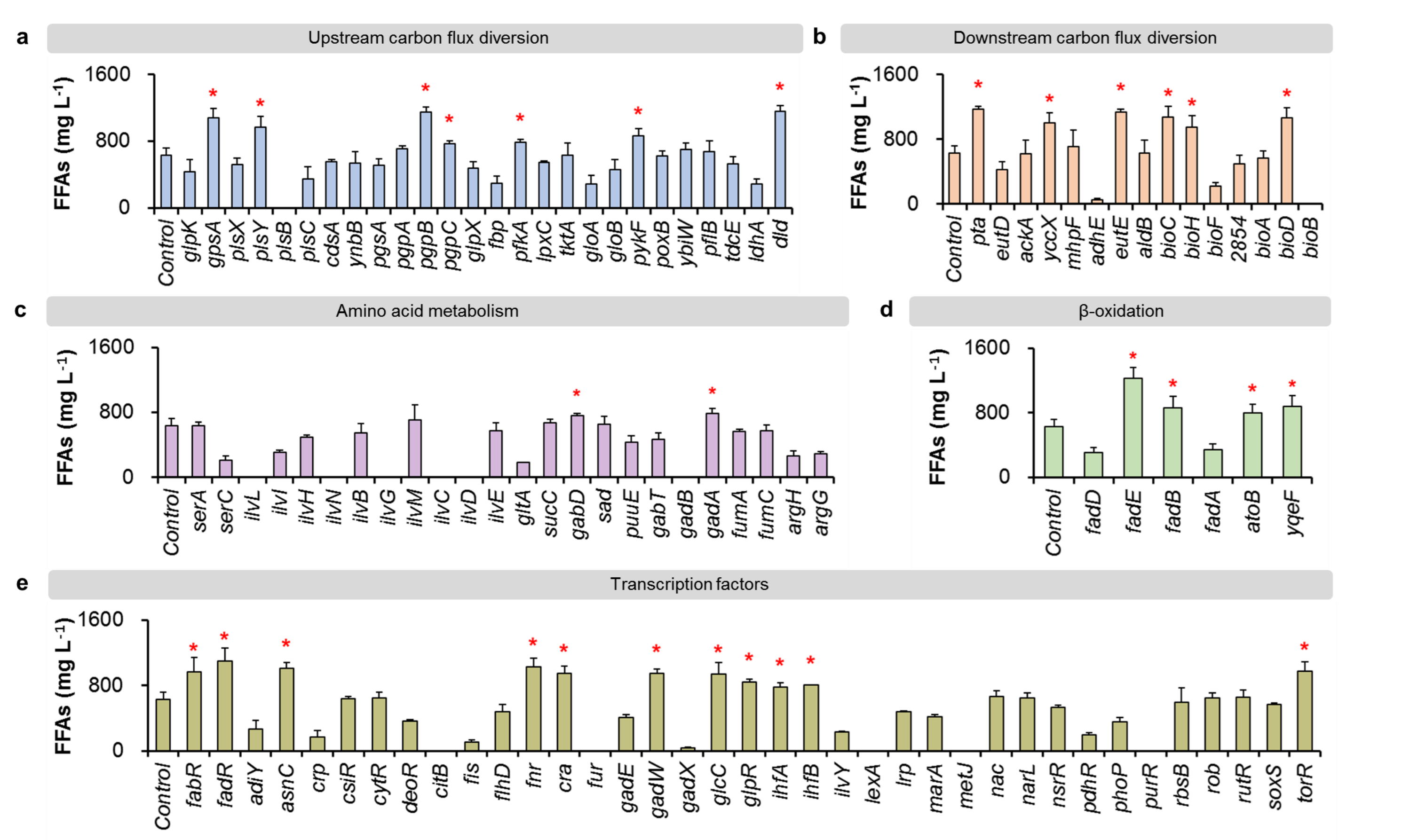
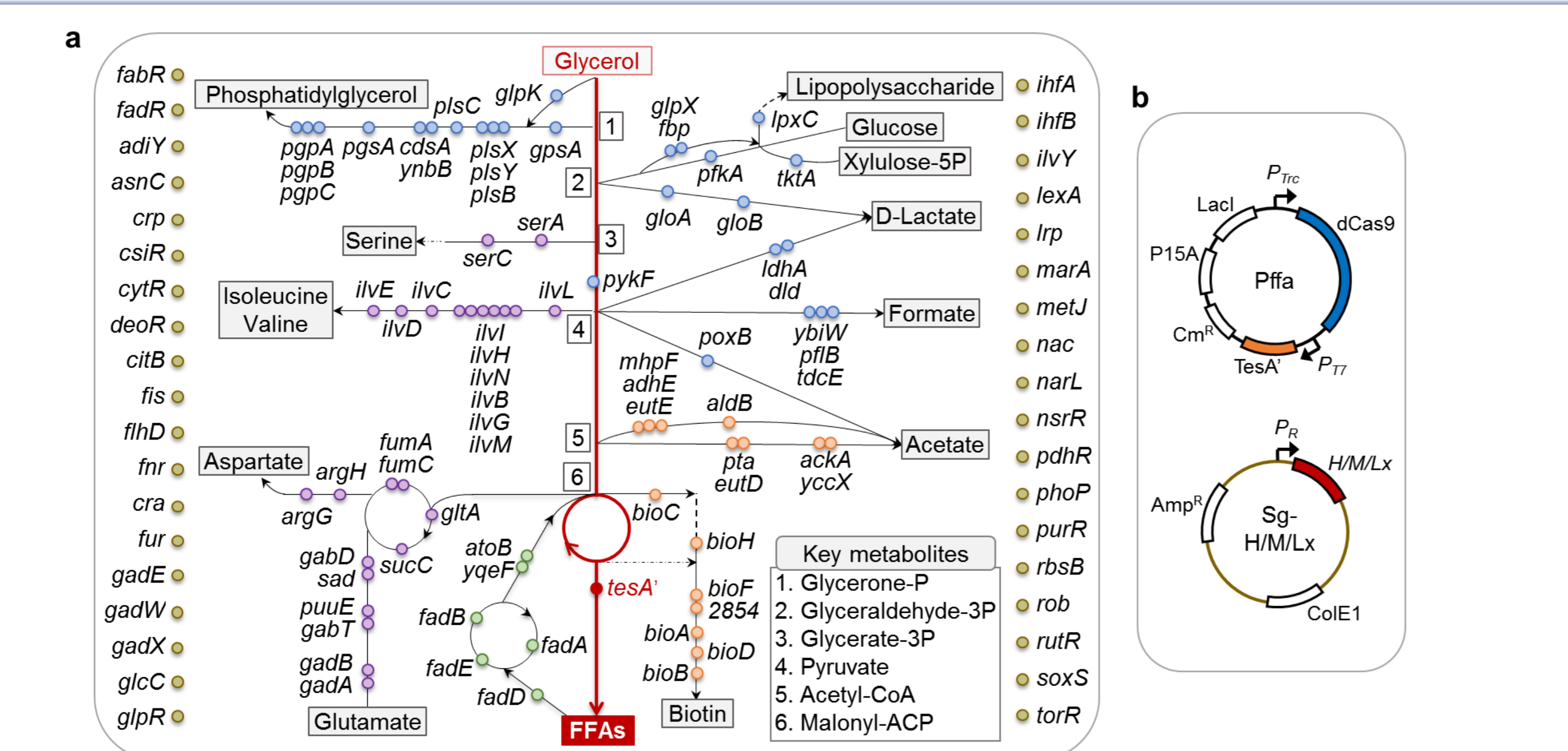
Fed-batch fermentation of the best engineered strain



- Titer: 21.6 g L⁻¹
- Productivity: 0.636 g L⁻¹ h⁻¹
- Yield: 0.146 g / g glycerol

Acknowledgment

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- 30 beneficial targets were identified from the 108 genes in the metabolic or regulatory pathways related to FFAs biosynthesis.
- Tuning the expression of the beneficial targets further enhanced FFAs production.