

## Supplement

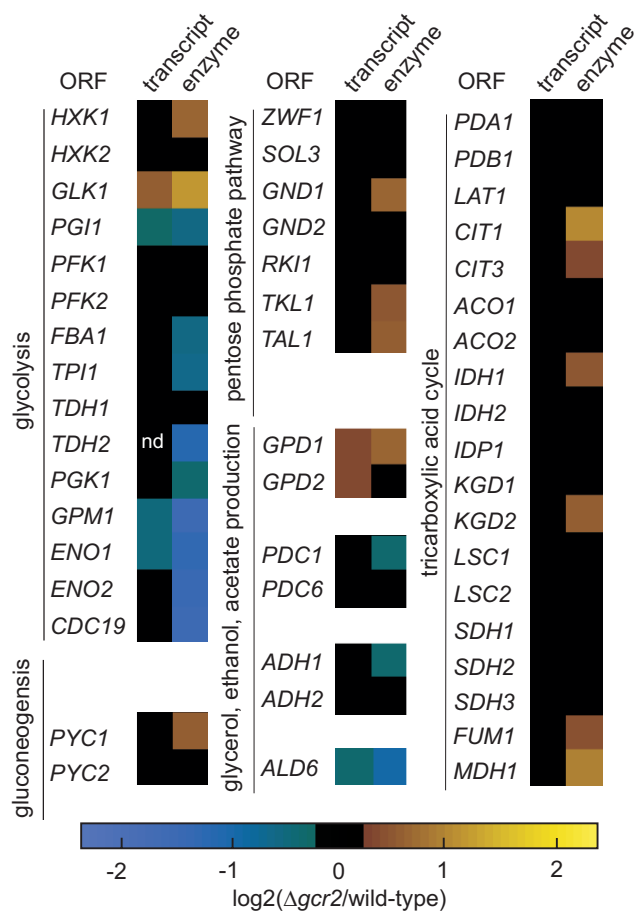


Figure 1: Transcript and enzyme fold changes in exponentially growing *GCR2* mutant and wild-type batch cultures on glucose minimal medium (fold change cutoff of 1.3, p-value cutoff of 0.05). 'nd' stands for not determined. (Data are found in Supplementary Table 1 and 3)

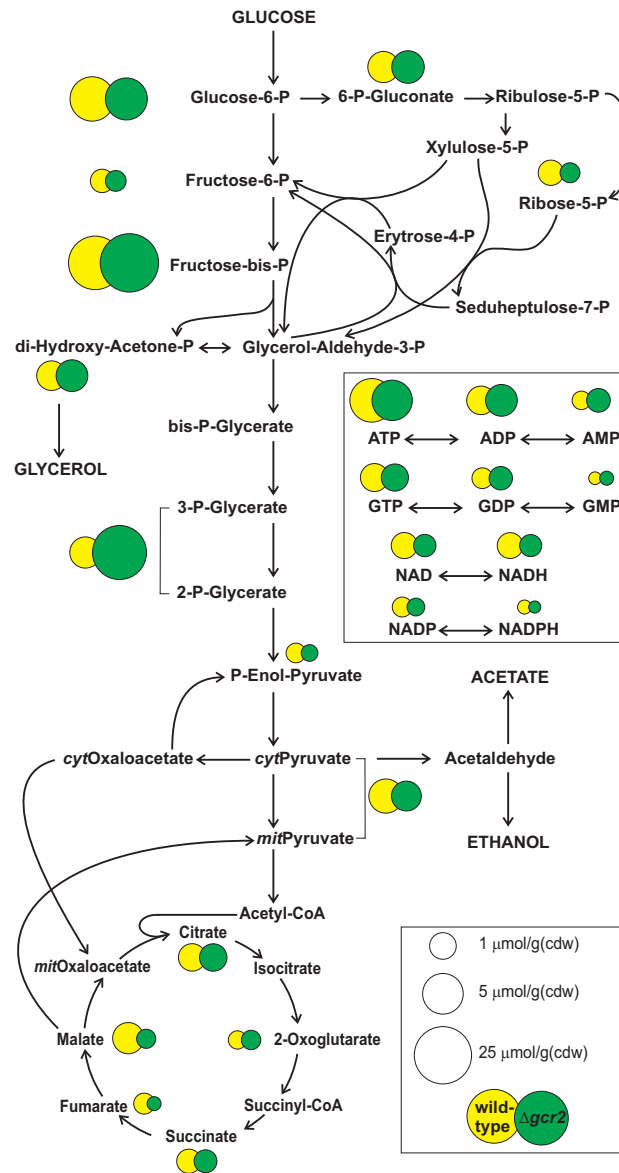


Figure 2: Intracellular metabolite concentrations in exponentially growing *GCR2* mutant (green) and the wild-type (yellow) batch cultures on glucose minimal medium. (Data are found in Supplementary Table 4A)

Table 1: Log2 ratios of transcript fold changes between the *GCR2* mutant and the wild-type. An Average of three biological replicas is reported. (see text file)

Table 2: Physiological data for exponentially growing *GCR2* mutant and wild-type FY4 in batch cultures on glucose minimal medium.

	<b>FY4</b>	<b><math>\Delta gcr2</math></b>
<b>glucose uptake rate</b> [mmol/g/h]	16.94 $\pm$ 3.19	10.46 $\pm$ 0.64
<b>ethanol secretion rate</b> [mmol/g/h]	21.55 $\pm$ 2.10	13.95 $\pm$ 1.67
<b>glycerol secretion rate</b> [mmol/g/h]	2.11 $\pm$ 0.43	1.86 $\pm$ 0.21
<b>acetate secretion rate</b> [mmol/g/h]	1.13 $\pm$ 0.38	0.61 $\pm$ 0.12
<b>pyruvate secretion rate</b> [mmol/g/h]	0.08 $\pm$ 0.01	0.05 $\pm$ 0.01
<b>growth rate</b> [1/h]	0.33 $\pm$ 0.01	0.23 $\pm$ 0.03

Table 3: Log2 ratios of enzyme fold changes between the *GCR2* mutant and the wild-type. An Average of two biological replicas is reported. (see text file)

Table 4: Metabolite concentration. Determined in exponentially grown wild-type FY4 and *GCR2* mutant batch cultures on A) glucose minimal medium and ethanol minimal medium. The *cdw* to OD correlation was determined for wild-type FY4 and applied also to the *GCR2* mutant. B) Metabolite fold changes of four strains with Tet-controlled promotor (Tet-controlled enzymes: Pgi1p, Tpi1p, Eno2p, Cdc19p) determined in glucose minimal medium with amino acids and varying concentrations of doxycycline. Data are normalized to the metabolite concentration determined without the addition doxycycline. (see text files)

Table 5: SRM assays used in this study for measuring enzyme abundance alterations.  $Q_1m/z$ , mass-to-charge ratio for the peptide ion used as  $Q_1$  value;  $Q_3m/z$  mass-to-charge ratio for the fragmentation used as  $Q_3$  value; H/L, heavy/light version of the peptide; CE, collision energy. (see text file)

Table 6: SRM assays used in this study for measuring metabolite concentrations with liquid chromatography mass spectrometry.  $Q_1m/z$ , mass-to-charge ratio for the metabolite ion used as  $Q_1$  value;  $Q_3m/z$  mass-to-charge ratio for the fragmentation used as  $Q_3$  value. (see text file)