

**Table S16** Comparison of the regulation coefficients presented in [1] ( $V_{max}$  values measured under enzyme-optimized conditions) and those re-calculated with the  $V_{max}$  values measured under *in vivo*-like conditions, but using the same changes in flux. Glucose transport was assessed in whole cells in growth medium, hence only under physiological conditions. The hierarchical regulation coefficient  $\rho_h$  reflects regulation of the flux by altered  $V_{max}$  through the entire gene-expression cascade. The metabolic regulation coefficient  $\rho_m$  reflects regulation through interaction of the enzyme with altered metabolite concentrations. For theory see [2,3].

Enzyme	Chapter 3 Enzyme-optimized $V_{max}$ values						<i>In vivo</i> -like $V_{max}$ values					
	D = 0.1 h <sup>-1</sup>			D = 0.35 h <sup>-1</sup>			D = 0.1 h <sup>-1</sup>			D = 0.35 h <sup>-1</sup>		
	$\rho_h$	SEM	$\rho_m$	$\rho_h$	SEM	$\rho_m$	$\rho_h$	SEM	$\rho_m$	$\rho_h$	SEM	$\rho_m$
<i>HXT</i>							-1.5		2.5	2.0		-1.0
<i>HXK</i>	-1.0	0.3	2.0	0.3	0.2	0.7	-0.6	0.0	1.6	0.3	0.1	0.7
<i>PGI</i>	-0.2	0.2	1.2	0.1	0.3	0.9	0.1	0.1	0.9	0.4	0.1	0.6
<i>PFK</i>	-0.7	0.2	1.7	1.9	0.3	-0.9	-0.7	0.01	1.7	4.4	1.1	-3.4
<i>ALD</i>	-0.8	0.2	1.8	3.5	0.8	-2.5	-0.6	0.5	1.6	1.4	0.2	-0.4
<i>GAPDH</i>	-0.5	0.2	1.5	-0.2	-0.4	1.2	-0.5	0.3	1.5	0.3	0.5	0.7
<i>PGK</i>	0.2	0.3	0.8	1.1	0.3	-0.1	0.3	0.1	0.7	1.2	0.0	-0.2
<i>PYK</i>	-0.1	0.2	1.1	1.3	0.1	-0.3	0.4	0.2	0.6	2.0	0.2	-1.0
<i>PDC</i>	1.2	0.3	-0.2	6.6	1.9	-5.6	0.5	0.1	0.5	3.8	0.1	-2.8
<i>ADH</i>	-0.7	0.1	1.7	2.3	0.4	-1.3	-0.2	0.0	1.2	0.8	0.1	0.2

### References

1. van Eunen K, Dool P, Canelas AB, Kiewiet J, Bouwman J, et al. (2010) Time-dependent regulation of yeast glycolysis upon nitrogen starvation depends on cell history. *IET Syst Biol* 4: 157-168.
2. Rossell S, van der Weijden CC, Lindenberg A, van Tuijl A, Francke C, et al. (2006) Unraveling the complexity of flux regulation: a new method demonstrated for nutrient starvation in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 103: 2166-2171.
3. ter Kuile BH, Westerhoff HV (2001) Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. *FEBS Lett* 500: 169-171.