

**Table S4** Kinetic parameters measured under *in vivo*-like conditions and implemented in the glycolytic model under the four conditions studied (new measurements).

| Parameter          | D = 0.1 h <sup>-1</sup>  | D = 0.1 h <sup>-1</sup>  | D = 0.35 h <sup>-1</sup> | D = 0.35 h <sup>-1</sup> |                                  |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|----------------------------------|
|                    | Non-starved              | 4h N-starved             | Non-starved              | 4h N-starved             |                                  |
| $V_{max,glc}$      | 220                      | 121                      | 201                      | 95                       | mM.min <sup>-1</sup>             |
| $K_{m,glc,GLC}$    | 1.6                      | 11.0                     | 0.9                      | 7.0                      | mM                               |
| $V_{max,hk}$       | 285                      | 223                      | 258                      | 227                      | mM.min <sup>-1</sup>             |
| $V_{max,pgi}$      | 808                      | 852                      | 903                      | 856                      | mM.min <sup>-1</sup>             |
| $V_{max,pfk}$      | 213                      | 165                      | 179                      | 93                       | mM.min <sup>-1</sup>             |
| $V_{max,ald}$      | 189                      | 153                      | 200                      | 161                      | mM.min <sup>-1</sup>             |
| $V_{max,gapdh}^+$  | 1859 (2090) <sup>a</sup> | 1075 (1514) <sup>a</sup> | 1496                     | 853 (1450) <sup>a</sup>  | mM.min <sup>-1</sup>             |
| $V_{max,gapdh}$    | 1211                     | 877                      | 867                      | 840                      | mM.min <sup>-1</sup>             |
| $K_{m,gapdh,GAP}$  | 2.48                     | 1.15                     | 0.39                     | 1.41                     | mM;<br>taken from [1]            |
| $K_{m,gapdh,NAD}$  | 2.92                     | 2.95                     | 2.85                     | 2.62                     | mM                               |
| $K_{m,gapdh,NADH}$ | 0.022                    | 0.10                     | 0.007                    | 0.014                    | mM;<br>taken from [1]            |
| $K_{m,gapdh,BPG}$  | 1.18 <sup>b</sup>        | 0.15 <sup>b</sup>        | 0.51 <sup>b</sup>        | 1.43 <sup>b</sup>        | mM; calculated                   |
| $K_{eq,gapdh}$     | 0.0056                   | 0.0056                   | 0.0056                   | 0.0056                   | Dimensionless;<br>taken from [1] |
| $V_{max,pgk}$      | 2670                     | 3030                     | 2416                     | 1962                     | mM.min <sup>-1</sup>             |
| $V_{max,gpm}$      | 856                      | 748                      | 871                      | 403                      | mM.min <sup>-1</sup>             |
| $V_{max,eno}$      | 357                      | 285                      | 485                      | 272                      | mM.min <sup>-1</sup>             |
| $V_{max,pyk}$      | 559                      | 636                      | 677                      | 480                      | mM.min <sup>-1</sup>             |
| $V_{max,fdc}$      | 248                      | 297                      | 335                      | 172                      | mM.min <sup>-1</sup>             |
| $V_{max,adh}$      | 817                      | 744                      | 856                      | 744                      | mM.min <sup>-1</sup>             |

<sup>a</sup> The value between brackets is the  $V_{max}$  value calculated using the Haldane relationship, the  $K_{eq}$ , the reverse  $V_{max}$  of the specific condition and the  $K_m$  values of the non-starved cells from the respirofermentative culture ( $D = 0.35 \text{ h}^{-1}$ )

<sup>b</sup> The  $K_m$  for BPG was calculated using the Haldane relationship, the measured  $V_{max}$  values, the  $K_m$  values for the other substrates and products, and the  $K_{eq}$ .

## References

1. Teusink B, Passarge J, Reijenga CA, Esgalhado E, van der Weijden CC, et al. (2000) Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? Testing biochemistry. *Eur J Biochem* 267: 5313-5329.