Supplementary Table S8:

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| **Parameters of the kinetic model of the PYK from *Lactococcus lactis*** |
| Parameter | Model parameter value | Experimentally determined value | Reference |
| *Km*(PEP) [mM] | 6.432 | 4 | [32] |
| *Km*(ADP) [mM] | 0.022 | 2.1 \* | [10] |
| *Km*(PYR) [mM] | 0.593 | n.d. |  |
| *Km*(ATP) [mM] | 7.105 | n.d. |  |
| *V* [U/mg] | 0.403 | n.d. |  |
| *n* | 2.173 | 3.3 (PEP) 2.1 (ADP) | [32] |
| *ka*$ [mM] | 1.707 | (0.07- 0.2) | [10,32] |
| *k*i$ [mM] | 0.124 | (0.65 -1.3 ) | [10,32] |
| *β1* | 246.077 |  |  |
| *β2* | 0.0036 |  |  |
| *kout*§ [1/min] | 176381 |  |  |

\*in the presence of 0.1 mM FBP

$ the model parameters describe the ratio of ligand binding and ligand dissociation and are therefore not directly comparable with experimentally measured ka0.5v and ki0.5v values.

§ represents the second reaction that consumes the product pyruvate (PYR) of the PYK reaction, following the experimental set-up; the reaction is described by mass action.