Supplementary Text: Kinetic Modelling of β-cell metabolism reveals control points in the insulin-regulating pyruvate cycling pathways.

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1 Kinetic Mechanism Details

1.1 Abbreviations

Subscripts c, m, and s denote cytoplasmic, mitochondrial, and transport enzymes respectively. Species without subscripts are not separated into compartments.

Table 1: Abbreviations for Metabolites/Enzymes

| Glycolysis Metabolites/Enzymes | Compound/EC number |
|-------------------------------|--------------------|
| Abbreviation                  | Substance name     |
| GT_s                          | Glucose Transporter|
| GK_c                          | Glucokinase        |
| PFK_c                         | 6-phosphofructokinase|
| FBA_c                         | Fructose-bisphosphate Aldolase |
| GAPD_c                        | Glyceraldehyde 3-phosphate Dehydrogenase |
| PGP_c                         | Bisphosphoglycerate Phosphatase |
| PK_c                          | Pyruvate Kinase    |
| GLC_c                         | Glucose            |
| F6P_c                         | Fructose-6-phosphate |
| FBP_c                         | Fructose-1,6-bisphosphate |
| GAP_c                         | Glyceraldehyde 3-phosphate |
| DPG_c                         | 1,3-bisphospho-D-glycerate |
| PEP_c                         | Phosphoenol Pyruvate |

| Cytosolic Metabolites/Enzymes  | Compound/EC number |
|-------------------------------|--------------------|
| Abbreviation                  | Substance name     |
| LDH_c                         | Lactate Dehydrogenase |
| ME_c                          | Malic Enzyme Cytoplolic |
| ICD_c                         | Isocitrate Dehydrogenase (NADP+) Cytoplolic |
| MDH_c                         | Malate Dehydrogenase |
| CITL_c                        | Citrate Lyase      |
| LAC_c                         | Lactate            |
| PYR_c                         | Pyruvate           |
| MAL_c                         | Malate             |
| CIT_c                         | Citrate            |
| ICIT_c                        | Isocitrate         |
| AKG_c                         | α-keto-Glutarate   |
| OAA_c                         | Oxaloacetate       |

| TCA cycle mitochondrial: Metabolites/Enzymes | Compound/EC number |
|----------------------------------------------|--------------------|
| Abbreviation                                | Substance name     |
| PC_m                                        | Pyruvate Carboxylase |
| PDH_m                                       | Pyruvate Dehydrogenase Complex |

| Abbreviation                  | Substance name     |
|-------------------------------|--------------------|
| PC_m                          | Pyruvate Carboxylase |
| PDH_m                         | Pyruvate Dehydrogenase Complex |
| Abbreviation | Substance name                      | Compound/EC number |
|--------------|-------------------------------------|--------------------|
| CS_m         | Citrate Synthase                    | EC 4.1.3.7         |
| ACO_m        | Aconitase                           | EC 4.2.1.3         |
| ICD_m        | Isocitrate Dehydrogenase Mitochondrial | EC 1.1.1.41       |
| AKD_m        | α-Ketoglutarate Dehydrogenase       | EC 1.2.4.2 etc.    |
| SCS_m        | Succinyl-CoA synthetase              | EC 6.2.1.4         |
| SDH_m        | Succinate Dehydrogenase             | EC 1.3.5.1         |
| FM_m         | Fumarase                            | EC 4.2.1.2         |
| MDH_m        | Malate Dehydrogenase                | EC 1.1.1.37        |
| ME_m         | Malic Enzyme Mitochondrial          | EC 1.1.1.39        |
| PYR_m        | Pyruvate                            | C00022             |
| ACOA_m       | Acetyl-CoA                           | C00024             |
| CIT_m        | Citrate                             | C00158             |
| ICIT_m       | Isocitrate                           | C00311             |
| AKG_m        | α-keto-Glutaraate                    | C00026             |
| SCOA_m       | Succinyl-CoA                         | C00091             |
| SUC_m        | Succinate                            | C00042             |
| FUM_m        | Fumarate                             | C00122             |
| MAL_m        | Malate                               | C00149             |
| OAA_m        | Oxaloacetate                         | C000036            |

### Transporter Enzymes

| Abbreviation | Substance name | Compound/EC number |
|--------------|----------------|--------------------|
| CIC_s        | Citrate Carrier |                    |
| DIC_s        | Dicarboxylate Carrier |          |
| PYC_s        | Pyruvate Carrier |                    |
| OGC_s        | Oxoglutarate Carrier |               |

### Co-Factors

| Abbreviation | Substance name                      | Compound/EC number |
|--------------|-------------------------------------|--------------------|
| NADPH_c      | Nicotinamide Adenine Dinucleotide Phosphate | C00005             |
| NADP_c       | Nicotinamide Adenine Dinucleotide Phosphate (Oxidized) | C00006             |
| Pi           | Phosphate                            | C00009             |
| Q            | Ubiquinone                           | C00399             |
| QH2          | Ubiquinol                            | C00390             |
| CO2          | Carbon Dioxide                       | C00011             |
| ATP          | Adenosine Triphosphate               | C00002             |
| ADP          | Adenosine Diphosphate                | C00008             |
| NAD*         | Nicotinamide Adenine Dinucleotides (Oxidized) | C00003             |
| NADH         | Nicotinamide Adenine Dinucleotides   | C00004             |
| CoA          | Coenzyme A                           | C00010             |
### 1.2 Model Reactions

Table 2: Model Reactions

#### Glycolysis Reactions

| Flux  | Enzyme | Reaction                                    |
|-------|--------|---------------------------------------------|
| J0entry_s | GT    | InputGlucose ⇄ GLC_c                        |
| J1gk_c  | GK     | GLC_c + ATP → F6P_c + ADP                  |
| J2pfk_c | PFK    | F6P + ATP → FBP + ADP                      |
| J3fba_c | FBA    | FBP ⇄ 2GAP                                  |
| J4gapd_c| GAPD   | GAP + NAD → DPG + NADH                     |
| J5pgp_c | PGP    | DPG + ADP ⇄ PEP + ATP                      |
| J6pk_c  | PK     | PEP + ADP → PYR + ATP                      |

#### Transporter Reactions

| Flux  | Enzyme | Reaction                                    |
|-------|--------|---------------------------------------------|
| J9pyr_s | PYC    | PYR_c + H_m ⇄ PYR_m + H_c                  |
| J10cit_s| CIC    | CIT_c + MAL_m ⇄ CIT_m + MAL_c              |
| J11icit_s| CIC   | ICIT_c + MAL_m ⇄ ICIT_m + MAL_c           |
| J12akg_s| OGC    | AKG_c + MAL_m ⇄ AKG_m + MAL_c             |
| akgflow_c |       | AKG_c → φ                                 |
| J13malh_s| DIC   | MAL_c + Pi_m ⇄ MAL_m + Pi_c               |

#### Cytosolic Reactions

| Flux  | Enzyme | Reaction                                    |
|-------|--------|---------------------------------------------|
| J7ldh_c | LDH    | PYR_c ⇄ LAC_c                              |
| lacsink_c |       | LAC_c → φ                                 |
| J14nad_c |        | NADPH_c → φ                               |
| J15citl_c| CITL   | CIT_c → OXA_c                              |
| J16mdh_c| MDHc   | MAL_c + NAD ⇄ OXA_c + NADH                 |
| J17acon_c| ACOc   | CIT_c ⇄ ICIT_c                            |
| J18isod_c| ICDc   | ICIT_c + NADP ⇄ AKG_c + NADPH             |
| J19me_c  | MEc    | MAL_c + NADP ⇄ PYR_c + NADPH              |

#### Mitochondrial Reactions

| Flux  | Enzyme | Reaction                                    |
|-------|--------|---------------------------------------------|
| J20pdh_m | PDH   | PYR_m + NAD + CoA → ACO_m + NADH + CO2     |
| J21pc_m | PC     | PYR_m + ATP_m + CO₂ ⇄ OXA + ADP + Pi       |
| J22cs_m | CS     | OXA_m + ACOA_m → CIT_m + CoA               |
| J23ac_m | ACOm   | CIT_m ⇄ ICIT_m                             |
| J24icd_m| ICDm   | ICIT_m + NAD → AKG_m + NADH               |
| J25akg_m| AKD    | AKG_m + NAD + CoA → SCOA_m + NADH + CO2    |
1.3 Fixed Species Concentrations and Biophysical Parameters

**ATP and ADP** Cellular ATP and ADP concentrations are assumed to be directly dependent on the glucose concentration. This dependence is assumed to be piece-wise linear [1, 2, 3]. The ATP concentration is taken to increase linearly between 3mM and 7mM as glucose increases from 1mM to 10mM, with saturation at [ATP]= 7mM [2]. Similarly, the ADP concentration is taken to decrease linearly between 1.2mM and 0.6mM as glucose increases from 1mM to 10mM, with saturation at [ADP]= 0.6mM [2]. Finally, glucose serves as an input to the model and the input value is decided based on the simulation objective.

| Species   | Constant value         | Reference |
|-----------|------------------------|-----------|
| COA_m     | 3.0 × 10^{-3} M        | [22]      |
| Q_m       | 9.5 × 10^{-4} M        | [22]      |
| QH2_m     | 4.1 × 10−4 M           | [22]      |
| CO2       | 3.0 × 10^{-6} M        | [22]      |
| pH        | 8.0                    | [11]      |
| Pi_c      | 1.0 × 10^{-3} M        | [17]      |
| Pi_m      | 1.0 × 10^{-3} M        | [22]      |
| NADPtot   | 5.0 × 10^{-4} M        | [11]      |
| inglc (Input Glucose Range.) | 0.001 ≤ inglc ≤ 0.016 M | [11] |

1.4 Rate Expressions and Model Organization

The model kinetics and differential equations are provided below. Here we provide an overview of the model structure, which is divided in two parts:

- glycolysis
- the TCA cycle, including the pyruvate cycling pathways

Glucose entry into the glycolytic pathway is modelled as per Sweet and Matschinsky using second order Michaelis-Menten Kinetics [17]. Details are provided in Table 4.
Glycolysis is described by a six-step pathway based on the work of Jiang et.al. [6]. We simplified the description by removing feedback regulation and fixing the concentration of co-factors. The reaction kinetics and parameters are taken from SABIO-RK [20]. (Glycolysis is treated as an influx model; the associated kinetics parameters are not investigated in our sensitivity analysis.)

The ATP concentration is described by a piece-wise linear function of glucose influx, as described above. NADP is described through conservation.

Glycolysis-produced pyruvate enters the pyruvate cycling pathways (our main subject of study) through pyruvate dehydrogenase and pyruvate carboxylase. Pyruvate is transported between the cytosol and mitochondria by the pyruvate transporter (PYC). The three main pyruvate cycle pathways are the pyruvate-malate, pyruvate-citrate, and pyruvate-isocitrate pathways.

The pyruvate-malate cycle is described by the conversion of mitochondrial oxaloacetate (OAAm) to malate via mitochondrial malate dehydrogenase (MDHm). Mitochondrial malate takes two routes: either (i) transported to the cytosol via the dicarboxylate carrier (DIC) and then converted back to pyruvate via cytosolic malic enzyme, or (ii) converted to pyruvate via mitochondrial malic enzyme.

The pyruvate-citrate cycle involves oxaloacetate combining with acetyl-coA to form citrate via citrate synthase. Citrate is then converted to isocitrate by mitochondrial aconitase or transported to the cytosol via the citrate isocitrate carrier (CIC). Isocitrate is converted to citrate by cytosolic aconitase (ACOc). Acetyl-CoA is produced when citrate lyase (CLc) converts citrate to oxaloacetate. Cytosolic malate dehydrogenase (MDHe) converts cytoplasmic oxaloacetate (OAAc) to malate. Malic enzyme completes the cycle by converting malate to pyruvate.

The pyruvate-isocitrate cycle shares common steps with the pyruvate-citrate cycle up to conversion of citrate to isocitrate. Isocitrate is transferred to the cytosol by the citrate isocitrate carrier (CIC) or converted to α-ketoglutarate, which is then transported to the cytosol via the oxoglutarate carrier (OGC). In the cytosol, isocitrate is converted to α-ketoglutarate by cytosolic NADP+-dependent-isocitrate dehydrogenase (ICDc).

Reaction kinetics for the enzymes in the TCA cycle model and the pyruvate cycling enzymes are reported in Table 4. The model structure is based primarily on that of Yugi and Tomita [23]. The cytosolic citrate lyase rate is described by reversible Michaelis-Menten kinetics [19]. The outflow models for lactate and α-ketoglutarate are likewise adapted from Westermark et.al. [19], with parameters estimated by fitting to the experimental data of Ronnebaum et. al. [11] as described in Section 2.2. We lumped NADPH consumption into a single reaction with a rate described by irreversible Michaelis-Menten kinetics [17, 19], with fitted parameter values (Section 2.2). Lactate dehydrogenase kinetics are adapted from Hoefnagel et.al. [5]. We adapted the rapid equilibrium random bi-bi mechanism kinetics of cytosolic malic enzyme from Westermark et.al. [19] to incorporate NADPH as a dynamic variable. Similarly, we modified the kinetics of mitochondrial malic enzyme (from Jiang et.al. [6]) to reversible Michaelis-Menten kinetics. The pyruvate dehydrogenase complex is subject to regulatory patterns involving Ca²⁺, NADH/NAD, acetyl-coA, and phosphorylation-dephosphorylation [19]. It was not feasible to incorporate all of these interactions. Instead, we simplified the kinetics from [19] to incorporate product inhibition by acetyl-CoA. All the parameters for these modified kinetics are taken from the BRENDA and SABIO-RK databases [20, 13]. Finally, for cytosolic isocitrate dehydrogenase, we set the parameters from the BRENDA database [13] without modifying the kinetics.

To simplify the model description, we held the concentrations of ions and some cofactors constant (Table 1.3). Rate expressions that involve these factors were simplified by defining effective rate constants.
incorporating the fixed concentrations. Such simplifications were applied to the rate equations for the pyruvate transporter \((\text{J9pyr}_s)\), the DIC carrier \((\text{J13malh}_s)\), succinyl-coA synthetase \((\text{J26sco}_m)\), and succinate dehydrogenase \((\text{J27sdh}_m)\).

### Table 4: Kinetic Expressions

**Glycolysis kinetics**

| Reaction    | Flux expression                                                                 | Reference                        |
|-------------|--------------------------------------------------------------------------------|----------------------------------|
| \(\text{J0entry}_s\) | \(v_0 \cdot 1 + v_0 \cdot 1 + (\text{GLC}_c \cdot \text{GLC}_c) + v_0 \cdot 2 + (\text{GLC}_c \cdot \text{GLC}_c)\) | Sweet and Matschinsky [17]       |
| \(\text{J1gk}_c\)       | \(v_1 \cdot 1 + (\text{GLC}_c \cdot \text{GLC}_c) + v_1 \cdot 2 + (\text{GLC}_c \cdot \text{GLC}_c)\) | SABIO-RK [20, 21]               |
| \(\text{J2pfk}_c\)       | \(v_2 \cdot 1 + (\text{F6P}_c \cdot \text{F6P}_c) + v_2 \cdot 2 + (\text{F6P}_c \cdot \text{F6P}_c)\) | SABIO-RK [20, 21]               |
| \(\text{J3fba}_c\)       | \(v_3 \cdot 1 + (\text{F6P}_c \cdot \text{F6P}_c) + v_3 \cdot 2 + (\text{F6P}_c \cdot \text{F6P}_c)\) | SABIO-RK [20, 21]               |
| \(\text{J4gapd}_c\)      | \(v_4 \cdot 1 + (\text{GAP}_c \cdot \text{GAP}_c) + v_4 \cdot 2 + (\text{GAP}_c \cdot \text{GAP}_c)\) | SABIO-RK [20, 21]               |
| \(\text{J5pgp}_c\)       | \(v_5 \cdot 1 + (\text{DPG}_c \cdot \text{DPG}_c) + v_5 \cdot 2 + (\text{DPG}_c \cdot \text{DPG}_c)\) | SABIO-RK [20, 21]               |
| \(\text{J6pk}_c\)        | \(v_6 \cdot 1 + (\text{PEP}_c \cdot \text{PEP}_c) + v_6 \cdot 2 + (\text{PEP}_c \cdot \text{PEP}_c)\) | SABIO-RK [20, 21]               |

**Transporter kinetics**

| Reaction   | Flux expression                                                                                                                                                                                                 | Reference               |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| \(\text{J9pyr}_s\) | \(v_9 \cdot 1 + (\text{PYR}_m \cdot \text{PYR}_m) + v_9 \cdot 2 + (\text{PYR}_c \cdot \text{PYR}_c)\)                                                                                                   | Yugi and Tomita [23]    |
| \(\text{J10cit}_s\) | \(\text{CIT}_c \cdot \text{MAL}_m \cdot v_{10} \cdot 3 + \text{MAL}_c \cdot \text{CIT}_m \cdot v_{10} \cdot 2\)                                                                                           | Yugi and Tomita [23]    |

\[\text{denom} = 1 + \text{CIT}_c \cdot v_{10} \cdot 3 + \text{MAL}_m \cdot v_{10} \cdot 4 + \text{MAL}_c \cdot v_{10} \cdot 5 + \text{CIT}_m \cdot v_{10} \cdot 6 \]

\[\text{denom} + \text{CIT}_c \cdot \text{MAL}_m \cdot v_{10} \cdot 4 + \text{MAL}_c \cdot \text{CIT}_m \cdot v_{10} \cdot 5 + \text{CIT}_m \cdot \text{MAL}_c \cdot v_{10} \cdot 6\]
| Reaction     | Flux expression                                                                 | Reference                      |
|--------------|---------------------------------------------------------------------------------|--------------------------------|
| J11icit_s    | \[ \text{denom} = 1 + \frac{|\text{ICIT}_c|}{v_{10.3}} + \frac{|\text{MAL}_m|}{v_{10.4}} + \frac{|\text{ICIT}_m|}{v_{10.5}} + \frac{|\text{ICIT}_m|}{v_{10.6}} + \frac{|\text{MAL}_c|}{v_{10.3}v_{10.4}} + \frac{|\text{ICIT}_m|}{v_{10.5}v_{10.6}} + \frac{|\text{MAL}_m|}{v_{10.4}v_{10.6}} + \frac{|\text{ICIT}_m|}{v_{10.5}v_{10.6}} \] | Yugi and Tomita [23]          |
| J12akg_s     | \[ \text{denom} = 1 + \frac{|\text{AKG}_m|}{v_{12.3}} + \frac{|\text{MAL}_c|}{v_{12.4}} + \frac{|\text{MAL}_m|}{v_{12.5}} + \frac{|\text{AKG}_c|}{v_{12.6}} + \frac{|\text{MAL}_m|}{v_{12.3}v_{12.4}} + \frac{|\text{AKG}_c|}{v_{12.5}v_{12.6}} + \frac{|\text{MAL}_m|}{v_{12.4}v_{12.6}} + \frac{|\text{AKG}_c|}{v_{12.3}v_{12.5}} \] | Yugi and Tomita [23]          |
| J13malh_s    | \[ v_{13.1}|\text{MAL}_m| - v_{13.2}|\text{MAL}_m| = v_{13.3}|\text{MAL}_m|=v_{13.4}|\text{MAL}_m| \] | Yugi and Tomita [23]          |

**Cytosolic Fluxes**

| Reaction     | Flux expression                                                                 | Reference                      |
|--------------|---------------------------------------------------------------------------------|--------------------------------|
| J71dh_c      | \[ v_{7.1}(|\text{PYR}_m| - \frac{|\text{LAC}_c|}{v_{7.1}}) \]                     | Reversible Michaelis Menten Kinetics. Parameters from Hoefnagel et al. [5] |
| lacsink_c    | sink \cdot \text{LAC}_c                                                         | Westermark et al. [19]         |
| J14nad_c     | \[ v_{14.1}|\text{NADPH}_c| \] \quad v_{14.2}+|\text{NADPH}_c| \] | Lumped process                |
| J15citl_c    | \[ v_{15.1}(|\text{CIT}_c| - v_{15.2}|\text{OAA}_c|) \]                         | Westermark et al. [19]         |
| J16mdh_c     | \[ v_{16.1}|\text{MAL}_c| - v_{16.2}|\text{OAA}_c| \]                          | Yugi and Tomita [23]          |
| J17acon_c    | \[ v_{17.1}(|\text{CIT}_c| - v_{17.2}|\text{CIT}_c|) \]                         | Yugi and Tomita [23]          |
| J18isod_c    | \[ v_{18.1} - v_{18.2} \] \quad v_{18.1}(|\text{ICIT}_c| - |\text{NADP}_c|) \] + \frac{v_{18.4}|\text{NADP}_c| - v_{18.5}|\text{ICIT}_c|}{v_{18.6}|\text{AKG}_c| + v_{18.7}|\text{NADPH}_c| + v_{18.8}|\text{AKG}_c|} + 1 | Yugi and Tomita [23]          |
| akgflow_c    | flow \cdot \text{AKG}_c                                                         | Westermark et al. [19]         |
| J19me_c      | \[ \text{denom} = 1 + \frac{|\text{MAL}_m|}{v_{19.2}v_{19.3}} + \frac{|\text{NADP}_c|}{v_{19.4}v_{19.5}} + \frac{|\text{PYR}_c|}{v_{19.2}v_{19.4}} + \frac{|\text{PYR}_c|}{v_{19.2}v_{19.4}} \] | Rapid Equilibrium Random Bi Bi. All parameters from Brenda [13]. |

**Mitochondrial Kinetics**
| Reaction     | Flux expression                                                                 | Reference                      |
|--------------|--------------------------------------------------------------------------------|--------------------------------|
| J20pdh_m     | $\frac{v_{20}}{v_{20} \cdot 1} \cdot \frac{[\text{PYR} \_m]}{[\text{ADP} \cdot [\text{OAA} \_m]]} + [\text{PYR} \_m]$ | Irreversible Michaelis-Menten with product inhibition. All parameters from BRENDA[13] |
| J21pc_m      | $v_{21} \cdot 1 \cdot (\text{ATP} \cdot [\text{PYR} \_m]) - v_{21} \cdot 2 \cdot \text{ADP} \cdot [\text{OAA} \_m] + [\text{PYR} \_m]$ | Yugi and Tomita [23]          |
| J22cs_m      | $v_{22} \cdot 1 \cdot [\text{ACO} \_m] \cdot [\text{OAA} \_m] + v_{22} \cdot 2 \cdot [\text{ACO} \_m] + v_{22} \cdot 3 \cdot [\text{OAA} \_m] + v_{22} \cdot 4 \cdot [\text{ACO} \_m] + 1$ | Yugi and Tomita [23]          |
| J23ac_m      | $v_{23} \cdot 1 \cdot [\text{CIT} \_m] - v_{23} \cdot 2 \cdot [\text{CIT} \_m] + v_{23} \cdot 3 \cdot [\text{CIT} \_m] + v_{23} \cdot 4 \cdot [\text{CIT} \_m] + 1$ | Yugi and Tomita [23]          |
| J24icd_m     | $v_{24} \cdot 1 \cdot (\text{ICIT} \_m)^{2} + v_{24} \cdot 2 \cdot [\text{ICIT} \_m]$ | Yugi and Tomita [23]          |
| J25akg_m     | $v_{25} \cdot 1 \cdot [\text{AKG} \_m] + v_{25} \cdot 2 \cdot [\text{SCOA} \_m] + v_{25} \cdot 3 \cdot [\text{SCOA} \_m] + v_{25} \cdot 4 \cdot [\text{SCOA} \_m] + v_{25} \cdot 5 \cdot [\text{SCOA} \_m]$ | Yugi and Tomita [23]          |
| J26sco_m     | $v_{26} \cdot 1 \cdot [\text{SCOA} \_m] \cdot [\text{SUC} \_m] + [\text{SCOA} \_m] + v_{26} \cdot 2 \cdot [\text{SCOA} \_m] \cdot [\text{SUC} \_m] + v_{26} \cdot 3 \cdot [\text{SCOA} \_m] \cdot [\text{SUC} \_m]$ | Yugi and Tomita [23]          |
| J27sdh_m     | $v_{27} \cdot 1 \cdot [\text{SUC} \_m] - [\text{FUM} \_m] + v_{27} \cdot 2$ | Yugi and Tomita [23]          |
| J28fum_m     | $v_{28} \cdot 1 \cdot [\text{FUM} \_m] - v_{28} \cdot 2 \cdot [\text{MAL} \_m] + v_{28} \cdot 3 \cdot [\text{MAL} \_m] + v_{28} \cdot 4 \cdot [\text{FUM} \_m] + 1$ | Yugi and Tomita [23]          |
| J29mdh_m     | $v_{29} \cdot 1 \cdot [\text{MAL} \_m] - v_{29} \cdot 2 \cdot [\text{OAA} \_m] + v_{29} \cdot 3 \cdot [\text{MAL} \_m] + v_{29} \cdot 4 \cdot [\text{OAA} \_m] + v_{29} \cdot 5 \cdot [\text{OAA} \_m] + v_{29} \cdot 6 \cdot [\text{MAL} \_m] + v_{29} \cdot 7 \cdot [\text{OAA} \_m] + v_{29} \cdot 8 \cdot [\text{MAL} \_m]$ | Yugi and Tomita [23]          |
| J30me_m      | $v_{30} \cdot 1 \cdot (\text{MAL} \_m)^{-1} \cdot [\text{PYR} \_m] + v_{30} \cdot 2 \cdot [\text{MAL} \_m] + v_{30} \cdot 3 \cdot [\text{PYR} \_m]$ | Reversible Michaelis-Menten. All parameters from BRENDA[13] |

### 1.5 Differential Equations

The model is described by the following equations. Rate equations from 1.1-6 describe the glycolytic influx model, which serves as the glucose entry point into the TCA cycle. Rate equations from 1.7-26 describes the pyruvate cycling pathways, including the TCA cycle. The concentration of NADP_c is determined through
conservation. ATP and ADP are described as a piecewise linear function of glucose abundance [1, 2, 3].

\[ \frac{d[\text{glc}_c]}{dt} = J_{0\text{entry}_c} - J_{1\text{gk}_c} \] (1.1)
\[ \frac{d[\text{F6P}_c]}{dt} = J_{1\text{gk}_c} - J_{2\text{pfk}_c} \] (1.2)
\[ \frac{d[\text{FBP}_c]}{dt} = J_{2\text{pfk}_c} - J_{3\text{fba}_c} \] (1.3)
\[ \frac{d[\text{GAP}_c]}{dt} = 2 \cdot J_{3\text{fba}_c} - J_{4\text{gapd}_c} \] (1.4)
\[ \frac{d[\text{DPG}_c]}{dt} = J_{4\text{gapd}_c} - J_{5\text{pgp}_c} \] (1.5)
\[ \frac{d[\text{PEP}_c]}{dt} = J_{5\text{pgp}_c} - J_{6\text{pk}_c} \] (1.6)
\[ \frac{d[\text{LAC}_c]}{dt} = J_{7\text{ldh}_c} - \text{lac}_{\text{sink}}_c \] (1.7)
\[ \frac{d[\text{PYR}_c]}{dt} = J_{6\text{pk}_c} - J_{7\text{ldh}_c} + V_r \cdot J_{9\text{pyr}_s} + J_{19\text{me}_c} \] (1.8)
\[ \frac{d[\text{MAL}_c]}{dt} = -J_{16\text{mdh}_c} + V_r \cdot (J_{13\text{malh}_s} + J_{10\text{cit}_s} - J_{12\text{akhmal}_s} + J_{11\text{icit}_s}) \] (1.9)
\[ - J_{19\text{me}_c} \] (1.10)
\[ \frac{d[\text{CIT}_c]}{dt} = -J_{17\text{acon}_c} - J_{15\text{citl}_c} - V_r \cdot J_{10\text{cit}_s} \] (1.11)
\[ \frac{d[\text{ICIT}_c]}{dt} = -J_{18\text{isosd}_c} + J_{17\text{acon}_c} - V_r \cdot J_{11\text{icit}_s} \] (1.12)
\[ \frac{d[\text{AKG}_c]}{dt} = J_{18\text{isosd}_c} + V_r \cdot J_{12\text{akhmal}_s} - \text{akg}_{\text{flow}}_c \] (1.13)
\[ \frac{d[\text{OAA}_c]}{dt} = J_{15\text{citl}_c} + J_{16\text{mdh}_c} \] (1.14)
\[ \frac{d[\text{NADPH}_c]}{dt} = J_{18\text{isosd}_c} + J_{19\text{me}_c} - J_{14\text{nadph}_c} \] (1.15)
\[ \frac{d[\text{PYR}_m]}{dt} = -J_{21\text{pc}_m} + J_{30\text{me}_m} - J_{9\text{pyr}_s} - J_{20\text{pdh}_m} \] (1.16)
\[ \frac{d[\text{ACO}_m]}{dt} = J_{20\text{pdh}_m} - J_{22\text{cs}_m} \] (1.17)
\[ \frac{d[\text{CIT}_m]}{dt} = J_{22\text{cs}_m} - J_{23\text{ac}_m} + J_{10\text{cit}_s} \] (1.18)
\[ \frac{d[\text{ICIT}_m]}{dt} = J_{23\text{ac}_m} - J_{24\text{icd}_m} + J_{11\text{icit}_s} \] (1.19)
\[ \frac{d[\text{AKG}_m]}{dt} = J_{24\text{icd}_m} - J_{25\text{akg}_m} - J_{12\text{akhmal}_s} \] (1.20)
\[ \frac{d[\text{SCO}_m]}{dt} = J_{25\text{akg}_m} - J_{26\text{co}_m} \] (1.21)
\[ \frac{d[\text{SUC}_m]}{dt} = J_{26\text{co}_m} - J_{27\text{sdh}_m} \] (1.22)
\[ \frac{d[\text{FUM}_m]}{dt} = J_{27\text{sdh}_m} - J_{28\text{fum}_m} \] (1.23)
\[ \frac{d[\text{MAL}_m]}{dt} = -J_{29\text{mdh}_m} + J_{28\text{fum}_m} - J_{10\text{cit}_s} - J_{11\text{icit}_s} - J_{13\text{malh}_s} + J_{12\text{akhmal}_s} - J_{30\text{me}_m} \] (1.24)
\[
\frac{d[\text{OAA}_m]}{dt} = -J_{22\text{cs}_m} + J_{29\text{mdh}_m} + J_{21\text{pc}_m}
\]

\[
\text{NADP}_c = \text{NADP}_{\text{tot}} - \text{NADPH}_c
\]

\[
\text{ATP} = \begin{cases} 
0.44 \ast (\text{inglc} - 0.001) + 0.003 & 0.001 \leq \text{inglc} < 0.01 \\
0.007 & \text{inglc} \geq 0.01 
\end{cases}
\]

\[
\text{ADP} = \begin{cases} 
-0.067 \ast (\text{inglc} - 0.001) + 0.0012 & 0.001 \leq \text{inglc} < 0.01 \\
0.0006 & \text{inglc} \geq 0.01 
\end{cases}
\]

1.6 Initial Conditions

The initial conditions for all simulations were obtained by integrating the system from the zero states for 4hrs (14400s) under appropriate glucose conditions.

2 Computational Settings for Solvers

2.1 Steady State Calculation

To calculate the steady state we used the ode15s and fsolve functions of MATLAB®. The system of differential equations was first integrated up to $10^7$ seconds. The resulting state was then passed to fsolve to confirm the steady state conditions had been achieved. In order to increase the stability and robustness of solvers, we generated a symbolic Jacobian using SBTOOLBOX2 [16, 15]. Since only 18% of the Jacobian coefficients were non-zero, we utilized the sparse storage mechanism as described in the ode15s and fsolve manual in order to increase the efficiency of solvers. We fixed the relative tolerance of ode15s at $10^{-3}$ and the absolute tolerance at $10^{-6}$, except for the states [F6P] and [G6P] for which we set the absolute tolerance at $10^{-12}$. For fsolve we used the default values of TolX and TolF ($10^{-6}$).

2.2 Parameter Optimization

The least-squares objective for parameter optimization is

\[
f(x) = \frac{1}{N} \sum_{i=1}^{N} \frac{1}{\sigma_i} \left( \frac{x_{\text{obs}}(x)_i - x_{\text{sim}}(x)_i}{x_{\text{obs}}(x)_i} \right)^2,
\]

in which $N$ is the number of experimental time-points, $x_{\text{obs}}(x)_i$ is the observed value of $i^{\text{th}}$ state, $x_{\text{sim}}(x)_i$ is the corresponding $i^{\text{th}}$ simulated state and $\sigma_i$ is the standard error of the mean (SEM) of the $i^{\text{th}}$ state. When the error was unknown, it was assumed to be 10%. All the state values are log scaled during optimization. The parameters were optimized in the range $0.01p_{\text{nom}} \leq p_{\text{nom}} \leq 100p_{\text{nom}}$, where $p_{\text{nom}}$ is the nominal parameter value pulled from literature and databases. The optimization was carried out by a combination of the downhill simplex method in multiple dimensions and simulated annealing, implemented in the system biology toolbox [16].

2.3 Global Sensitivity Analysis Settings

We used the variance-based Global Sensitivity Analysis (GSA, extended Fourier amplitude sensitivity test (eFAST) and the partial rank correlation method (PRCC) implemented in SBTOOLBOX2 [16, 15]. The the
analysis excluded the glycolysis influx mode but treated all other model parameters. The relative parameter range variation was selected to be 100%. For the total sensitivity analysis, the objective function was defined as the sum of the squared errors between the observed and perturbed system output values:

\[ f_{\text{obj}} = \sum_{i=1}^{n} (x_{\text{nom}}^i - x_{\text{pert}}^i)^2, \]

in which \( x_{\text{nom}} \) is the steady state nominal model output, \( x_{\text{pert}} \) is the steady state perturbed model output and \( n \) is the number of state variables. In addition, we calculated the sensitivities of individual state variables as a squared error:

\[ f_{\text{ind}}^i = (x_{\text{nom}}^i - x_{\text{pert}}^i)^2 \quad \text{for } i = 1, \ldots, n. \]

The pyruvate cycling rate is defined as the ratio of PC flux to the sum of the TCA cycle fluxes [8]

\[ f_{\text{pr}} = \left( \frac{J_{\text{pc,nom}}}{J_{\text{tca,nom}}} - \frac{J_{\text{pc,pert}}}{J_{\text{tca,pert}}} \right)^2. \]

Here, \( f_{\text{pr}} \) denotes the perturbation in the pyruvate cycling rate, \( J_{\text{pc}} \) is a flux through the pyruvate carboxylase enzyme, and \( J_{\text{tca}} \) is a sum of all TCA cycle fluxes; nom and pert denote the nominal and perturbed values respectively.

The total number of model simulations was selected to be \( 10^5 \), based on the suggestion of Saltelli [12] (\( 2 \times 512 \times \) total number of parameters).

### Integration Settings for Global Optimization

For simulating the model for global sensitivity analysis we used the SUNDIALS [4] package (MATLAB® interface) in order to reduce the simulation time [16]. The final state was checked for steady-state as described previously using fsolve. The system was integrated using a relative tolerance of \( 10^{-4} \) and an absolute tolerance of \( 10^{-14} \) for all species concentrations. With these settings, the output agreed with the MATLAB® ode15s solver.

#### 2.4 Model Codes

The model is built in the MATLAB® environment. The model codes are available at https://github.com/r2rahul/pyruvatecycle. The model is developed using SBToolbox2 [16, 15]. The code repository contains detailed instructions on executing the model and reproducing the figures. In addition, the model is available in the system biology markup language (SBML) format to support model interchange [? , 18, 10, 9, 7].

### 3 Parameters

The model incorporates 129 parameters. Of these, 125 parameter values were sourced from the literature, beginning with values reported in the models of Yugi and Tomita, and Westermark et al. [23, 19]. Other estimates were drawn from the BRENDA and SABIO-RK databases [21, 21, 14]. For the cases where multiple sources provided conflicting values, we gave preference to estimates from sources most closely resembling INS-1 cell lines.

In the table below, parameter values pulled from the literature are labelled as ‘Lit.’ Those that were fit in this study are labelled ’Fit’, along with the source of the nominal values used as an initial estimate in the
Table 5: Model parameters.

| Parameter | Name (c:cytosol, m:mitochondria) | Value | Units | Fit or Lit. [References] |
|-----------|----------------------------------|-------|-------|--------------------------|
| v0_1      | Vmax(GLCc)                       | 2.7271·10^{-6} | s^{-1} | Fit [17]               |
| v0_2      | Km(GLCc)                         | 101.0544 | M^{-1}  | Fit [17]               |
| v1_1      | Vmax(GKc)                        | 1.3424·10^{-4} | M·s^{-1} | Lit. [20, 21]         |
| v1_2      | n(GKc)                           | 1.34 |       | Lit. [20, 21]         |
| v1_3      | Km(GKc)                          | 3.0118·10^{-4} | M     | Lit. [20, 21]         |
| v2_1      | Vmax(PFKc)                       | 3.16667·10^{-5} | M·s^{-1} | Lit. [20, 21]        |
| v2_2      | n(PFKc)                          | 0.9 |       | Lit. [20, 21]        |
| v2_3      | Km(PFKc)                         | 0.0089 | M     | Lit. [20, 21]       |
| v3_1      | Vmax(FBAc)                       | 7.9783·10^{-5} | M·s^{-1} | Lit. [20, 21]     |
| v3_2      | Km(FBAc)                         | 4 · 10^{-6} | M     | Lit. [20, 21]       |
| v4_1      | Vmax(GAPc)                       | 0.001 |       | Lit. [20, 21]      |
| v4_2      | n(GAPc)                          | 1.5 |       | Lit. [20, 21]     |
| v4_3      | Km(GAPc)                         | 3.2 · 10^{-4} | M     | Lit. [20, 21]     |
| v5_1      | Vmax(DPGc)                       | 3.33 · 10^{-5} | M·s^{-1} | Lit. [20, 21]  |
| v5_2      | Km(DPGc)                         | 8 · 10^{-6} | M     | Lit. [20, 21]   |
| v6_1      | Vmax(PKc)                        | 5.33 · 10^{-5} | M·s^{-1} | Lit. [20, 21]  |
| v6_2      | n(PKc)                           | 2.9 |       | Lit. [20, 21]    |
| v6_3      | Km(PKc)                          | 1.5 · 10^{-4} | M     | Lit. [20, 21]    |
| v7_1      | Vmax(LDHc)                       | 29.0969 | s^{-1} | Fit [5]           |
| v7_eq     | Keq(LDHc)                        | 21.121 |       | Lit. [5]          |
| v7_2      | Km1(LDHc)                       | 448491.0 | M^{-1}  | Fit [5]            |
| v7_3      | Km2(LDHc)                       | 449.0936 | M^{-1}  | Fit [5]           |
| sink      | Lactate Sink                    | 8.89·10^{-8} | M·s^{-1} | Fit                 |
| v9_1      | Vmax(PYC, forward)              | 3.7674·10^{-8} | s^{-1} | Lit. [23]        |
| v9_2      | Vmax(PYC, reverse)              | 0.004 | s^{-1} | Fit [23]          |
| v9_3      | Km1(PYC)                        | 49.2637 | M^{-1}  | Lit. [23]        |
| v9_4      | Km2(PYC)                        | 187.3789 | M^{-1}  | Lit. [23]       |
| v10_1     | Vmax(CIC, forward)              | 32514.0 | M^{-1} · s^{-1} | Lit. [23]    |
| v10_2     | Vmax(CIC, reverse)              | 84267.0 | M^{-1} · s^{-1} | Lit. [23]  |
| v10_3     | Km1(CIC)                        | 1.3 · 10^{-4} | M     | Lit. [23]        |
| v10_4     | Km2(CIC)                        | 4.4 · 10^{-4} | M     | Lit. [23]    |
| v10_5     | Km3(CIC)                        | 3.3 · 10^{-4} | M     | Lit. [23]    |
| v10_6     | Km4(CIC)                        | 4.18 · 10^{-5} | M     | Lit. [23]   |
| v12_1     | Vmax(OGC, forward)              | 5811.9 | M^{-1} · s^{-1} | Lit. [23]  |
| v12_2     | Vmax(OGC, reverse)              | 6739.9 | M^{-1} · s^{-1} | Lit. [23]  |
| v12_3 | Km1(OGC) | $3 \cdot 10^{-4}$ | M | Lit. [23] |
| v12_4 | Km2(OGC) | $7 \cdot 10^{-4}$ | M | Lit. [23] |
| v12_5 | Km3(OGC) | 0.0014 | M | Lit. [23] |
| v12_6 | Km4(OGC) | $1.7 \cdot 10^{-4}$ | M | Lit. [23] |
| v13_1 | Vmax(DIC, forward) | 0.8512 | s$^{-1}$ | Fit [23] |
| v13_2 | Vmax(DIC, reverse) | 0.2721 | s$^{-1}$ | Fit [23] |
| v13_3 | Km1(DIC) | 1388.9 | M$^{-1}$ | Lit. [23] |
| v13_4 | Vmax(DIC) | 1111.1 | M$^{-1}$ | Lit. [23] |
| v14_1 | Vmax(NADPH, sink) | 0.0025598 | M$\cdot$s$^{-1}$ | Fit |
| v14_2 | Km(NADPH, sink) | 0.98653 | M | Fit |
| v15_1 | Vmax(CITLc, forward) | $4.0008 \cdot 10^{-7}$ | s$^{-1}$ | Fit [19] |
| v15_2 | Vmax(CITLc, reverse) | $1.2 \cdot 10^{-4}$ | M$^{-1}$ | Lit. [19] |
| v15_3 | Km1(CITLc) | $1.2 \cdot 10^{-4}$ | M$^{-1}$ | Lit. [19] |
| v15_4 | Km2(CITLc) | $1.2 \cdot 10^{-4}$ | M$^{-1}$ | Lit. [19] |
| v16_1 | Vmax(MDHc, forward) | 0.0036 | s$^{-1}$ | Lit. [23] |
| v16_2 | Vmax(MDHc, reverse) | 5000 | s$^{-1}$ | Lit. [23] |
| v16_3 | Km1(MDHc) | $8 \cdot 10^{-6}$ | M$^{-1}$ | Lit. [23] |
| v16_4 | Km2(MDHc) | 16667 | M$^{-1}$ | Lit. [23] |
| v17_1 | Vmax(ACONc, forward) | 0.0518 | s$^{-1}$ | Lit. [23] |
| v17_2 | Vmax(ACONc, forward) | 0.1104 | s$^{-1}$ | Fit [23] |
| v17_3 | Km2(ACONc) | 9090 | M$^{-1}$ | Lit. [23] |
| v17_4 | Km2(ACONc) | 2000 | M$^{-1}$ | Lit. [23] |
| v18_1 | Vmax(ISODc, forward) | 1152100 | M$^{-1}\cdot$s$^{-1}$ | Lit. [13] |
| v18_2 | Vmax(ISODc, reverse) | 5482500 | M$^{-1}\cdot$s$^{-1}$ | Lit. [13] |
| v18_3 | Km1(ISODc) | $2.3042 \cdot 10^{10}$ | M$^{-1}$ | Lit. [13] |
| v18_4 | Km2(ISODc) | 142857.1 | M$^{-1}$ | Fit [13] |
| v18_5 | Km3(ISODc) | 161290.3 | M$^{-1}$ | Lit. [13] |
| v18_6 | Km4(ISODc) | $1.0965 \cdot 10^{11}$ | M$^{-1}$ | Lit. [13] |
| v18_7 | Km5(ISODc) | 416666.7 | M$^{-1}$ | Lit. [13] |
| v18_8 | Km6(ISODc) | 263157.9 | M$^{-1}$ | Lit. [13] |
| flow | AKGc Sink | $1.46 \cdot 10^{-9}$ | M$\cdot$s$^{-1}$ | Fit |
| v19_1 | Vmax(MEc) | 913070 | M$^{-1}\cdot$s$^{-1}$ | Lit. [13] |
| v19_eq | Keq(MEc) | 1000 | | Lit. [19] |
| v19_2 | Km1(MEc) | $1.2 \cdot 10^{-4}$ | M | Lit. [13] |
| v19_3 | Km2(MEc) | $1.39 \cdot 10^{-6}$ | M | Lit. [13] |
| v19_4 | Km3(MEc) | 0.0048 | M | Lit. [13] |
| v19_5 | Km4(MEc) | $5.3 \cdot 10^{-6}$ | M | Lit. [13] |
| v20_1 | Vmax(PDHm) | $1.417 \cdot 10^{-7}$ | M$\cdot$s$^{-1}$ | Fit [13] |
| v20_2 | Km(PDHm) | $3.5 \cdot 10^{-5}$ | M | Lit. [13] |
| v20_3 | Ki(PDHm) | $2 \cdot 10^{-5}$ | M | Lit. [13] |
| v21_1 | Vmax(PCm) | 0.1057 | M$^{-1} \cdot $s$^{-1}$ | Fit [23] |
| v21_2 | Keq(PCm) | 0.54 | | Lit. [23] |
| v21_3 | Km1(PCm) | 4424100.0 | M$^{-1}$ | Lit. [23] |
| v21_4 | Km2(PCm) | $4.8528 \cdot 10^7$ | M$^{-1}$ | Lit. [23] |
| v21_5 | Km3(PCm) | 0.1057 | M$^{-1}$ | Fit [23] |
| v21_6 | Km4(PCm) | 0.54 | M$^{-1}$ | Lit. [23] |
| v21_7 | Km5(PCm) | 4424100.0 | M$^{-2}$ | Lit. [23] |
| v21_8 | Km6(PCm) | $4.8528 \cdot 10^7$ | M$^{-2}$ | Lit. [23] |
| v21_9 | Km7(PCm) | 0.1057 | M$^{-2}$ | Fit [23] |
| v21_10 | Km8(PCm) | 0.54 | M$^{-2}$ | Lit. [23] |
| v22_1 | Vmax(CSm) | 50.85 | M$^{-1} \cdot $s$^{-1}$ | Fit [23] |
| v22_2 | Km1(CSm) | $2.5 \cdot 10^{10}$ | M$^{-2}$ | Fit [23] |
| v22_3 | Km2(CSm) | 295000.0 | M$^{-1}$ | Lit. [23] |
| v22_4 | Km3(CSm) | 120000.0 | M$^{-1}$ | Lit. [23] |
| v23_1 | Vmax(ACOm, forward) | 0.0518 | s$^{-1}$ | Lit. [23] |
| v23_2 | Vmax(ACOm, reverse) | 0.1104 | s$^{-1}$ | Lit. [23] |
| v23_3 | Km1(ACOm) | 9090.9 | M$^{-1}$ | Lit. [23] |
| v23_4 | Km2(ACOm) | 2000.0 | M$^{-1}$ | Lit. [23] |
| v24_1 | Vmax1(ICDm) | 0.1126 | M$^{-1} \cdot $s$^{-1}$ | Fit [23] |
| v24_2 | Vmax2(ICDm) | 0.0148 | M | Lit. [23] |
| v24_3 | Km1(ICDm) | 2777.5 | M$^{-2}$ | Lit. [23] |
| v24_4 | Km2(ICDm) | 0.63969 | M$^{-1}$ | Lit. [23] |
| v24_5 | Km3(ICDm) | 0.1126 | M$^{-2}$ | Fit [23] |
| v24_6 | Km4(ICDm) | 0.0148 | M$^{-2}$ | Lit. [23] |
| v25_1 | Vmax(AKGm) | 0.0311 | s$^{-1}$ | Fit [23] |
| v25_2 | Km1(AKGm) | $1.456 \cdot 10^9$ | M$^{-1}$ | Lit. [23] |
| v25_3 | Km2(AKGm) | $1.4546 \cdot 10^9$ | M$^{-1}$ | Fit [23] |
| v25_4 | Km3(AKGm) | 24691.0 | M$^{-2}$ | Lit. [23] |
| v26_1 | Vmax(SCOdm) | $3.32 \cdot 10^{-5}$ | s$^{-1}$ | Lit. [23] |
| v26_2 | Keq1(SCOdm) | 6.4 | | Lit. [23] |
| v26_3 | Ki(SCOdm) | 4.1876 | M$^{-1}$ | Lit. [23] |
| v26_4 | Km1(SCOdm) | 37.5348 | M$^{-1}$ | Lit. [23] |
| v26_5 | Km2(SCOdm) | 1478.2 | M$^{-1}$ | Lit. [23] |
| v26_6 | Km3(SCOdm) | 9509.6 | M$^{-2}$ | Lit. [23] |
| v26_7 | Km4(SCOdm) | 236.7114 | M$^{-2}$ | Lit. [23] |
| v27_1 | Vmax(SDHm, forward) | 2.941 | s$^{-1}$ | Lit. [23] |
| v27_2 | Vmax(SDHm, reverse) | 11.5344 | | Lit. [23] |
| v27_3 | Km1(SDHm) | 449.6613 | M$^{-1}$ | Lit. [23] |
4 Supplementary Results

4.1 Correlation Between States

To analyse the correlation between metabolite concentrations and the pyruvate cycling ratio, and between the metabolite concentrations and the NADPH level, we sampled the parameter space using Latin hypercube sampling (LHS), recorded the output states and pyruvate cycling ratio, and calculated the correlations.

| v27_4 | Km2(SDHm) | 199130.0 | M$^{-1}$ | Lit. [23] |
|-------|-----------|----------|----------|-----------|
| v27_5 | Km3(SDHm) | 1.7921 \cdot 10^8 | M$^{-2}$ | Lit. [23] |
| v28_1 | Vmax(FUMm, forward) | 13.9024 | s$^{-1}$ | Fit [23] |
| v28_2 | Vmax(FUMm, reverse) | 13.9024 | s$^{-1}$ | Lit. [23] |
| v28_3 | Km1(FUMm) | 4000000 | M$^{-1}$ | Lit. [23] |
| v28_4 | Km2(FUMm) | 880000 | M$^{-1}$ | Lit. [23] |
| v29_1 | Vmax(MDHm, forward) | 0.0016 | s$^{-1}$ | Lit. [23] |
| v29_2 | Vmax(MDHm, reverse) | 2.5563 \cdot 10^{-10} | s$^{-1}$ | Lit. [23] |
| v29_3 | Km1(MDHm) | 33263.0 | M$^{-1}$ | Fit [23] |
| v29_4 | Km2(MDHm) | 14.0754 | M$^{-1}$ | Lit. [23] |
| v29_5 | Km3(MDHm) | 2742900 | M$^{-2}$ | Lit. [23] |
| v30_1 | Vmax(MEm) | 157.8125 | s$^{-1}$ | Fit [13] |
| v30_eq | Keq(MEm) | 1000 | | Lit. [19] |
| v30_2 | Km1(MEm) | 500 | M$^{-1}$ | Fit [13] |
| v30_3 | Km2(MEm) | 227.2727 | M$^{-1}$ | Lit. [13] |
| Vr | Volume Ratio | 20 | | Lit. [19] |

Figure 1: a. Correlation between metabolite concentrations and the NADPH. b. Correlation between metabolite concentrations and the pyruvate cycling ratio.
4.2 Supplementary Sensitivity Figures

This section presents additional sensitivity rankings of the key metabolites cytosolic and mitochondrial Pyruvate. Finally, we show the pyruvate carboxylase flux, and cytosolic isocitrate dehydrogenase flux sensitivity rankings across different methods.
Figure 2: Global and one-at-a-time sensitivity rankings of cytosolic pyruvate concentration across different methods. Panel A one-at-a-time sensitivity, Panel B eFAST total effect, Panel C eFAST first order, and Panel D PRCC.

4.2.1 Cytosolic Pyruvate Sensitivity Rankings
Figure 3: Global and one-at-a-time sensitivity rankings of mitochondrial pyruvate concentration across different methods. Panel A one-at-a-time sensitivity, Panel B eFAST total effect, Panel C eFAST first order, and Panel D PRCC.

4.2.2 Mitochondrial Pyruvate Sensitivity Rankings
4.2.3 Pyruvate Carboxylase Flux Sensitivity Rankings

Figure 4: Global and one-at-a-time sensitivity rankings of pyruvate carboxylase flux across different methods. Panel A one-at-a-time sensitivity, Panel B eFAST total effect, Panel C eFAST first order, and Panel D PRCC.
4.2.4 Cytosolic Isocitrate Dehydrogenase Flux Sensitivity Rankings

Figure 5: Global and one-at-a-time sensitivity rankings of cytosolic isocitrate dehydrogenase flux across different methods. Panel A one-at-a-time sensitivity, Panel B eFAST total effect, Panel C eFAST first order, and Panel D PRCC.
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