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Unraveling the function of the two Entner–Doudoroff branches in the thermoacidophilic Crenarchaeon *Sulfolobus solfataricus* P2

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Supplementary Material

Derivation of GK activity at constant ATP concentration (Eqn.1)

From Fig. 6 the rate equation for forming product P is given as:

$$v = 2k_{cat}[ES_2] + 3k_2[ES_3] \tag{S1}$$

Affinity constant (K_S) and inhibitor constant (K_i) are defined as (assuming rapid equilibria):

$$K_S = \frac{[E][S]^2}{[ES_2]}; \qquad K_I = \frac{[ES_2][S]}{[ES_3]} \tag{S2}$$

The total enzyme concentration $[E]_0$ is given as:

$$[E]_0 = [E] + [ES_2] + [ES_3] \tag{S3}$$

Solving for [E] and $[ES_3]$ from the equations defining K_S and K_i, and inserting these expressions into Eq. S3, leads to:

$$[E]_0 = \frac{K_S[ES_2]}{[S]^2} + [ES_2] + \frac{[ES_2][S]}{K_i} = [ES_2]\left(\frac{K_S}{[S]^2} + 1 + \frac{[S]}{K_i}\right) \tag{S4}$$

Solving for $[ES_2]$ and $[ES_3]$ from Eqs. S4 and S2, respectively, gives:

$$[ES_2] = \frac{[E]_0}{\frac{K_S}{[S]^2} + 1 + \frac{[S]}{K_i}}; \qquad [ES_3] = \left(\frac{[S]}{K_i}\right)[ES_2] \tag{S5}$$

Inserting the expressions for $[ES_2]$ and $[ES_3]$ from Eq. S5 into Eq. S1 gives Eq. 1:

$$\begin{aligned} v &= 2k_{cat}[ES_2] + 3k_2\left(\frac{[S]}{K_i}\right)[ES_2] = 2k_{cat}[ES_2]\left(1 + \frac{3k_2}{2k_{cat}} \cdot \frac{[S]}{K_i}\right) \\ &= \frac{2k_{cat}[E]_0}{\left(\frac{K_S}{[S]^2} + 1 + \frac{[S]}{K_i}\right)}\left(1 + \frac{3k_2}{2k_{cat}} \cdot \frac{[S]}{K_i}\right) = \frac{V_{\max}[S]^2}{\left(K_S + [S]^2 + \frac{[S]^3}{K_i}\right)}\left(1 + \alpha \cdot \frac{[S]}{K_i}\right) \end{aligned} \tag{S6}$$

where $V_{max} = 2k_{cat}[E]_0$, and $\alpha = 3k_2 / 2k_{cat}$

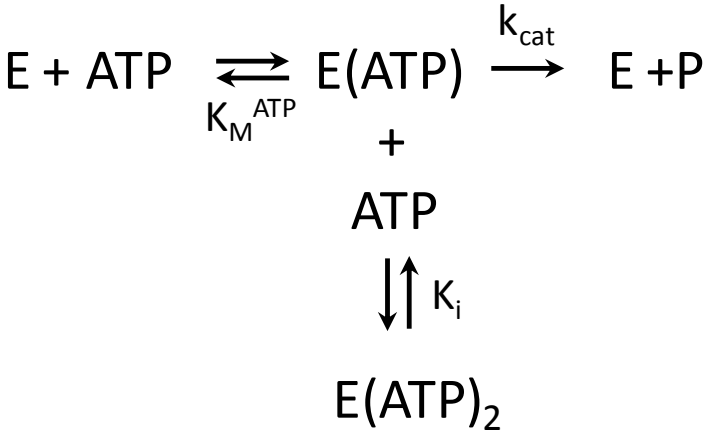

Figure S1. Possible catalytic mechanism at 80°C for GK (represented as E) at constant glycerate concentration leading to the rate equation $v = \frac{V_{\max}[ATP]}{K_S^{ATP} + [ATP] + \frac{[ATP]^2}{K_i}}$ by binding a second ATP to the enzyme-substrate complex E(ATP), and leading to an inactive (dead-end) complex $E(ATP)_2$. The following (rapid) equilibria are assumed with the constants $K_M^{ATP} = \frac{[E][ATP]}{[E \times ATP]}$ (Michaelis constant) and $K_i = \frac{[E \cdot (ATP)][ATP]}{[E \cdot (ATP)_2]}$. The reaction rate is defined as $v = k_{cat}[E \times ATP]$.

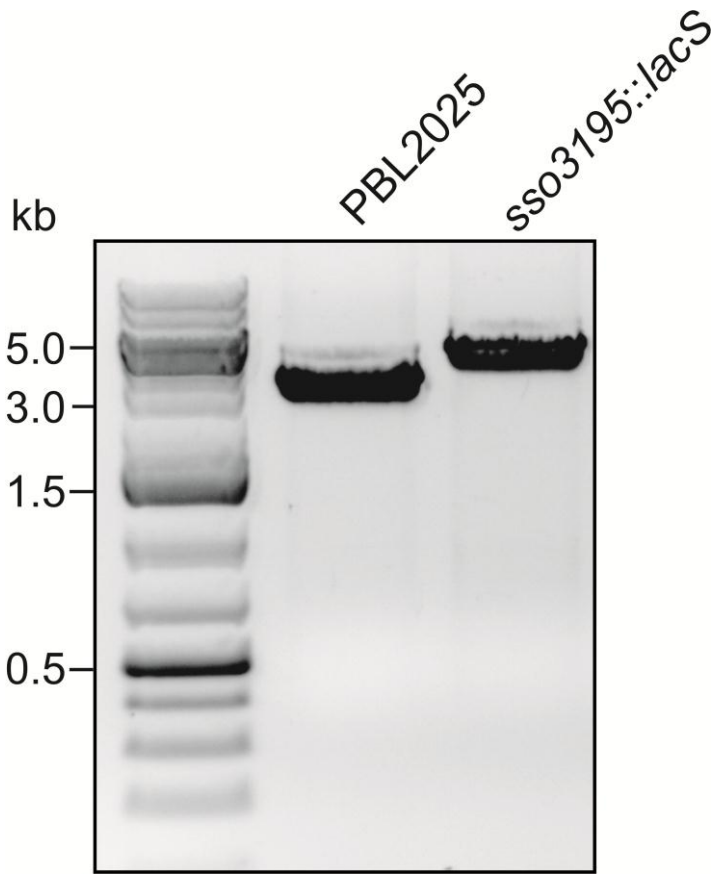

Figure S2. Construction of the PBL2025Δ3195 mutant. Exchange of the KDGK encoding gene SSO3195 with the marker gene *lacS* was used for blue/white screening with X-gal. The correct mutant was confirmed by PCR and sequencing with the primers 1557 and 1558. The mutant product runs slower on agarose gel, since the *lacS* gene is larger than SSO3195.

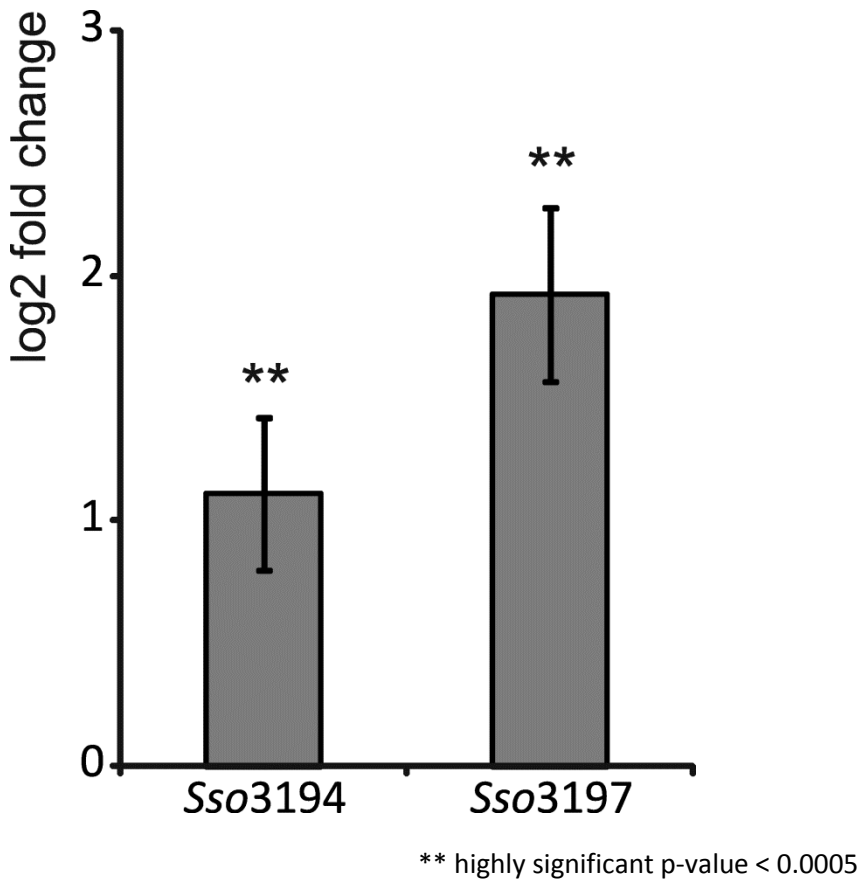

Figure S3. qRT-PCR analysis of PBL2025Δ3195. The C_t values of SSO3194 and SSO3197 were standardized to the C_t values of SSO0685 (*secY*) for each biological replica.