

**A modified serine cycle in *Escherichia coli* converts methanol and CO<sub>2</sub> to two-carbon compounds**

Yu *et al*

## Supplementary Note 1

### Construction of the *HY106* strain to evaluate the effect of expressing the complete cycle genes *in vivo*

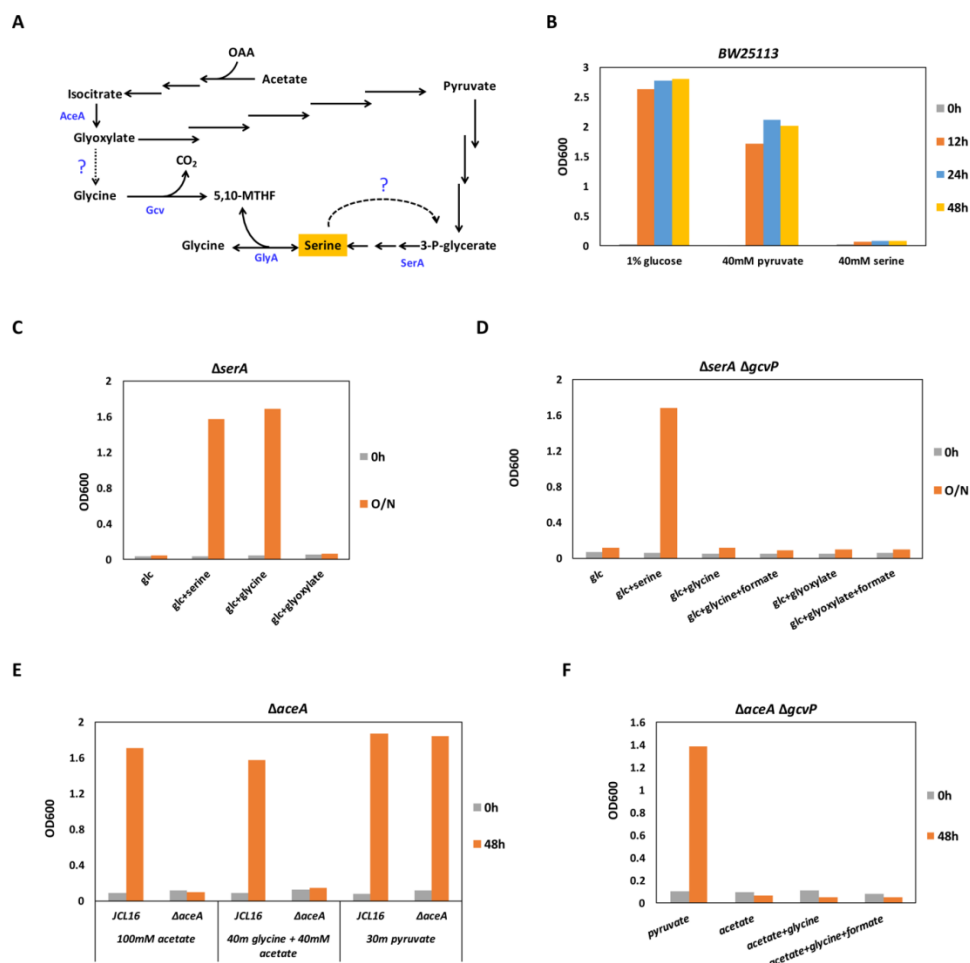
To investigate the effect of the modified serine cycle on C1-carbon assimilation, we first expressed the complete required cycle genes, including *mtk*(*M.c*), *mcl*(*M.e*), *AGX1*(*S.c*), *sdaA*(*C.n*), *fthfl*(*M.t*) and *methfs*(*M.t*), in the strain  $\Delta serA \Delta gcvP$ . However, this *E. coli* strain grew much poorly compared to the previous result under the same condition (Supplementary Fig. 4D), which might be caused by shortage of serine because of the *serA* deletion and the *SdaA*(*C.n*)-mediated serine deamination. Thus, an *E. coli* strain *HY106* ( $\Delta aceB \Delta glcB \Delta gcvP \Delta gcl \Delta frdB \Delta ldhA$ ) was created (Supplementary Fig. 6), which did not show auxotrophic growth defect and was used to investigate the effect of expressing the complete cycle genes. The gene deletions in *HY106* were to avoid byproduct formation and reactions that could counter part of the modified serine cycle, and further channel the metabolic flux towards acetyl-CoA derived C2 compounds (acetate and ethanol) as the main fermentation products<sup>1</sup>. *AceB*(*E.c*) and *GlcB*(*E.c*)<sup>2</sup> are deleted since they act as malate synthases that catalyze the reverse reaction of *Mtk*/*Mcl*. *Gcl*(*E.c*)<sup>3</sup> catalyzes the glyoxylate condensation to form tartronate semialdehyde. *GcvP*(*E.c*) performs the glycine decarboxylation. *LdhA* and *FrdABCD* are lactate dehydrogenase and fumarate reductase, specific for production of D-lactate and succinate, respectively.

## Supplementary Note 2

### Prediction of the acetate labeling pattern produced by the modified serine cycle in *E. coli*

In the engineered *E. coli* strain, methanol can be oxidized to formate by expressing heterologous enzymes *Medh*(CT4-1)/*Faldh*(*P.p*). The intermediate formate is further converted to 5,10-methylene- $H_4F$  catalyzed by *Fthfl*(*M.t*)/*Mthfs*(*M.t*), or to  $CO_2$  equivalent by endogenous formate dehydrogenases *Fdo*(*E.c*) and *Fdn*(*E.c*). As the native pathway produces acetyl-CoA through pyruvate decarboxylation (Supplementary Fig. 8A), *E. coli* strain can only incorporate  $^{13}C$  labeled methanol into acetyl-CoA by

constructing the modified serine cycle, resulting in two forms of acetyl-CoA (M+1 and M+2) from three possibilities. The first possibility is unlabeled pyruvate assimilates  $^{13}\text{C}$  labeled bicarbonate, derived from oxidation of  $^{13}\text{C}$ -methanol, to generate oxaloacetate and further split into single-carbon labeled acetyl-CoA (M+1) and unlabeled glyoxylate (Supplementary Fig. 8B). In the first possibility, acetyl-CoA (M+1) is produced only through the partial cycle, while the remaining possibilities may produce  $^{13}\text{C}$  labeled acetyl-CoA from the complete cycle. The unlabeled glyoxylate (produced via Supplementary Fig. 8B and 8C) assimilates labeled 5,10-methylene- $\text{H}_4\text{F}$ , derived from  $^{13}\text{C}$ -methanol, to produce single-carbon labeled serine. The M+1 serine is converted to pyruvate, and then carboxylated with either unlabeled bicarbonate (Supplementary Fig. 8D) or labeled bicarbonate (Supplementary Fig. 8E) to produce M+1 or M+2 form of oxaloacetate, respectively. The labeled malate can be split into unlabeled glyoxylate and single (or double)-carbon labeled acetyl-CoA (Supplementary Fig. 8D and 8E).



**Supplementary Figure 1. Serine and glycine metabolism in *E. coli*.**

(A) In *E. coli*, serine is synthesized either from two glycine or one 3-P-glycerate. The C1-carbon carrier 5,10-methylene-H<sub>4</sub>F can be produced by glycine-cleavage or glycine synthesis from serine. The questionmarks indicate that *E. coli* wt strain doesn't have such enzymatic activities under normal growth condition, which are further demonstrated in (B) and (C). Gcv: glycine cleavage complex; GlyA: serine hydroxymethyltransferase (SHMT); SerA: D-3-phosphoglycerate dehydrogenase. AceA: isocitrate lyase.

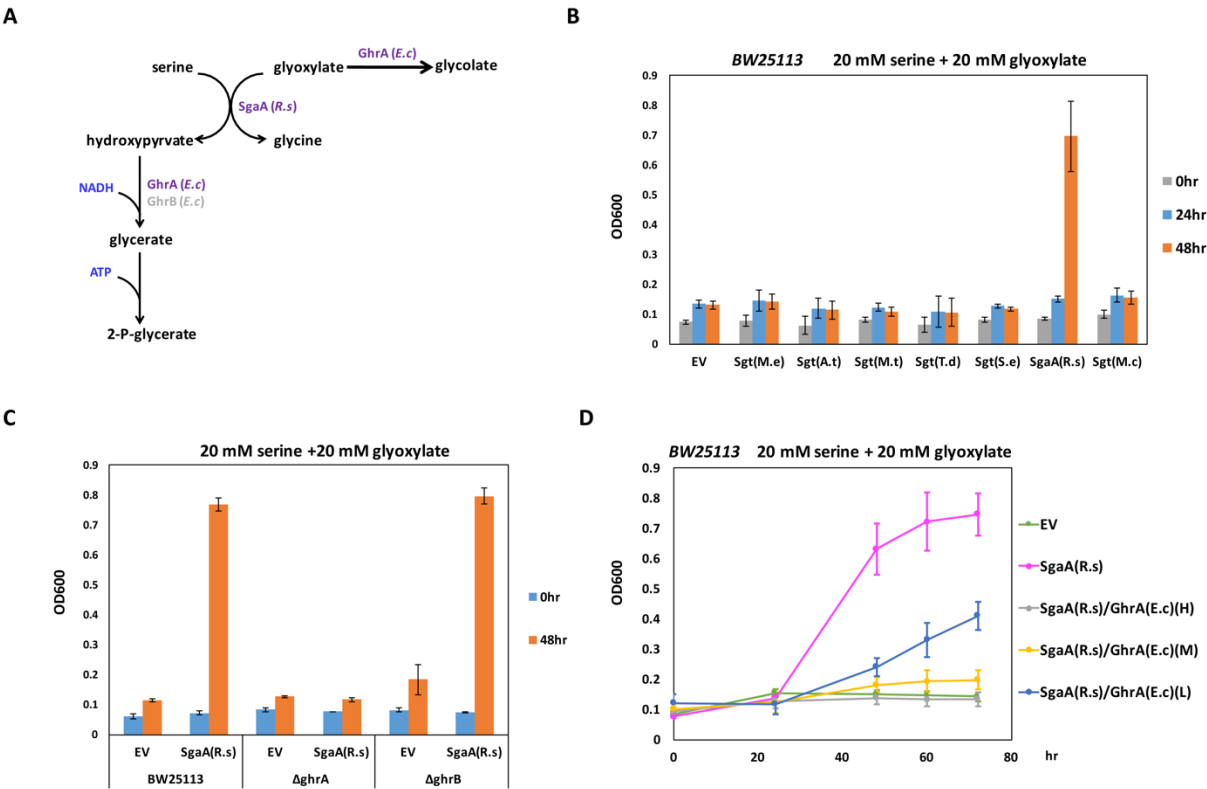
(B) The wt strain BW25113 cannot grow in minimal medium with serine as the sole carbon source.

(C) The *serA* deletion causes *E. coli* strain to be a serine auxotroph, which can be rescued by supplement of serine or glycine in glucose minimal medium. However, addition of glyoxylate cannot rescue the growth of the *ΔserA* strain, suggesting that conversion of glyoxylate to glycine is inefficient. O/N: overnight, 0 h: time zero.

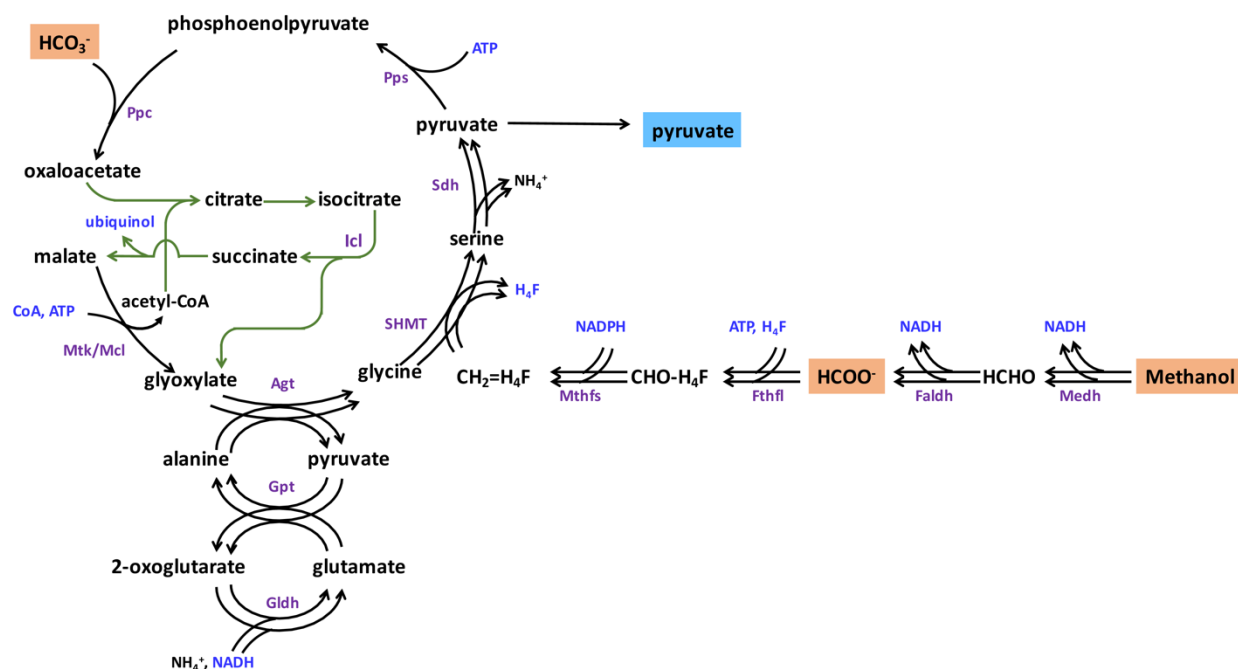
(D) The *ΔserA ΔgcvP* strain can grow in glucose minimal medium with only serine addition, but not glycine or glyoxylate addition, compared to the single *serA* deletion (C). This result indicates the role of GcvP in the serine synthesis.

(E) AceA(*E.c*) acts as an isocitrate lyase, which catalyzes the key step of the glyoxylate cycle. Its deletion causes *E. coli* strain cannot grow in minimal medium with acetate (C2) as the sole carbon source, but can grow on pyruvate (C3) or succinate (C4).

(F) The *ΔaceA ΔgcvP* strain cannot grow in minimal medium by using acetate/glycine or acetate/glycine/formate as carbon sources.

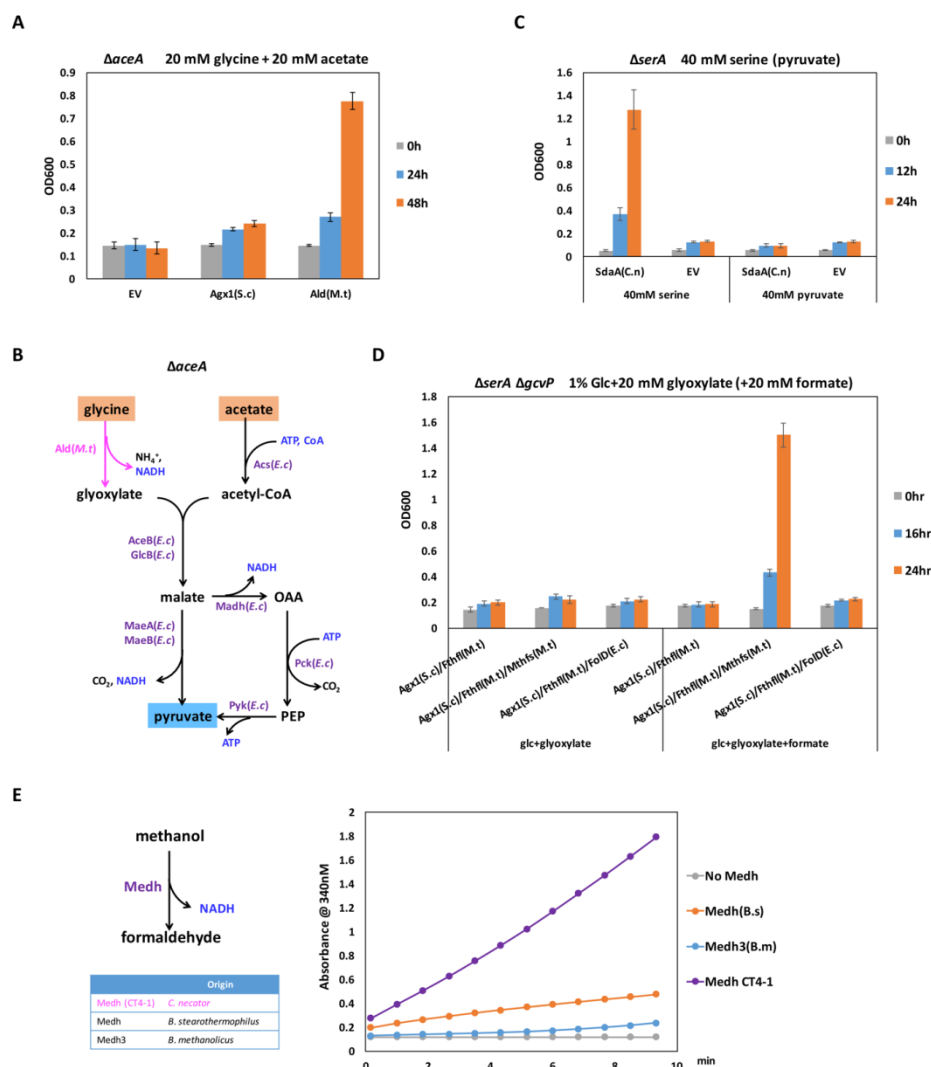


**Supplementary Figure 2.** Investigation of the GhrA function in *E. coli*.  
(A) Pathway from serine and glyoxylate to produce 2-phosphoglycerate for *E. coli* growth. SgaA: serine-glyoxylate transaminase. GhrA, GhrB: glyoxylate (hydroxypyruvate) reductase.  
(B) Expression of *sgaA* (coding for serine-glyoxylate transaminase) from *Rhodobacter sphaeroides* allowed *E. coli* wt strain to grow in minimal medium with serine and glyoxylate as carbon sources. It suggested that SgaA(R.s) was functional in converting serine and glyoxylate to produce hydroxypyruvate and glycine, hydroxypyruvate could be further reduced to glycerate by endogenous hydroxypyruvate reductase to support growth, as shown in (A).  
*M.e*: *Methylobacterium extorquens*. *A.t*: *Arabidopsis thaliana*. *M.t*: *Moorella thermoacetica*. *T.d*: *Treponema denticola*. *S.e*: *Synechococcus elongatus* PCC 7942. *R.s*: *Rhodobacter sphaeroides*. *M.c*: *Methylococcus capsulatus* (Bath).  
(C) Deletion of *ghrA*, but not *ghrB*, abolished the phenotype observed in (B), suggesting that GhrA was the major hydroxypyruvate reductase in *E. coli*.  
(D) Increased expression levels of *ghrA(E.c)* gradually slowed down the growth rate of *E. coli* wt in minimal medium with serine and glyoxylate as carbon sources. The expression level of *ghrA(E.c)* was controlled by synthetic ribosome binding site (RBS), designed by the RBS calculator (<https://salislab.net/software/>). GhrA(E.c)(H), GhrA(E.c)(M) and GhrA(E.c)(L) represented high, medium and low expression levels of *ghrA(E.c)*. Each RBS was designed with 10-fold difference on the translation rate. The RBS sequences were listed in Supplementary Table 6. EV means empty vector.  
Error bars are s.d., n=3.



**Supplementary Figure 3.** Illustration of the modified serine cycle for pyruvate synthesis.

Compared to Fig. 1B, this form of the modified serine cycle integrates with the partial glyoxylate cycle (labeled as green lines), which allows the pathway to assimilate two C1-carbon units, derived from formic acid (or methanol), and one bicarbonate to produce one pyruvate. Icl: isocitrate lyase.



**Supplementary Figure 4.** Investigation of suitable enzymes for the modified serine cycle.

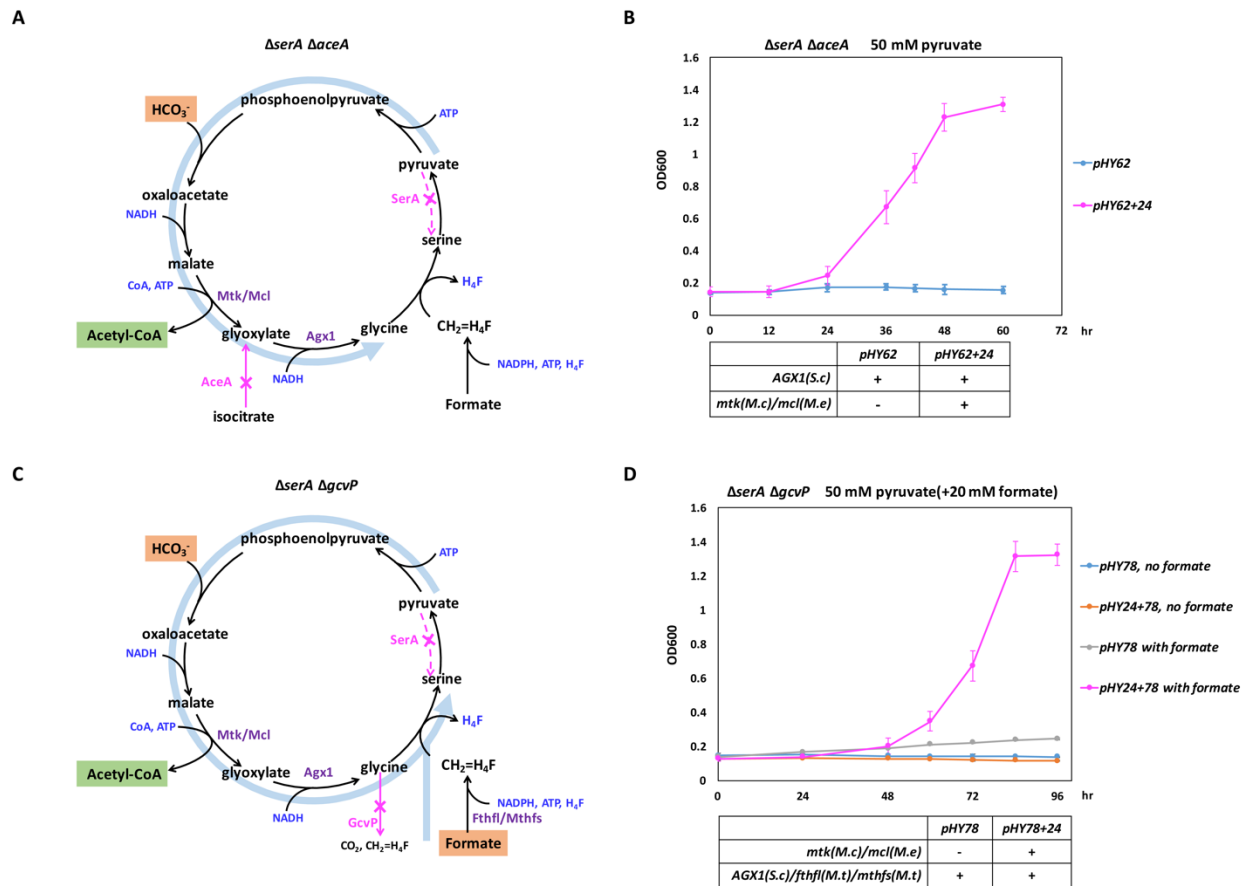
(A,B) Expression of *ald(M.t)* allowed the  $\Delta aceA$  strain to grow in minimal medium with acetate and glycine as carbon sources (A). Ald(M.t) catalyzed the reaction to convert glycine to glyoxylate, which could be condensed with acetyl-CoA to generate malate for growth-supporting, as shown in (B). Enzymes labeled in purple are endogenous enzymes in *E. coli*. Together with the result of Fig. 2A, it suggested that the function of Ald(M.t) preferred to catalyze the reaction of converting glycine to glyoxylate in *E. coli*. Acs: acetyl-CoA synthase. AceB, GlcB: malate synthase. MaeA, MaeB: malate dehydrogenase. Pck: phosphoenolpyruvate carboxykinase. Pyk: pyruvate kinase.

(C) Expression of *sdaA(C.n)* could support the  $\Delta serA$  strain to grow in minimal medium with serine addition, but not pyruvate, which indicated that SdaA(C.n) catalyzed the serine deamination irreversibly *in vivo*.

(D) Comparison of the activity between Fthfl(M.t)/Mthfs(M.t) and Fthfl(M.e)/FolD(E.c). The results showed that Fthfl(M.t)/Mthfs(M.t) displayed higher activities to rescue the growth defect of the  $\Delta serA \Delta gcvP$  strain in glucose minimal medium with glyoxylate/formate supplements.

(E) *In vitro* enzymatic assay showed that Medh(CT4-1) displayed the highest enzymatic activity on methanol oxidation among the enzymes tested at 37°C. NADH formation was recorded at 340 nm. 20  $\mu$ g of each purified protein was used.

EV means empty vector. Error bars are s.d., n=3.



**Supplementary Figure 5.** Construction of the modified serine subpathway in *E. coli*.

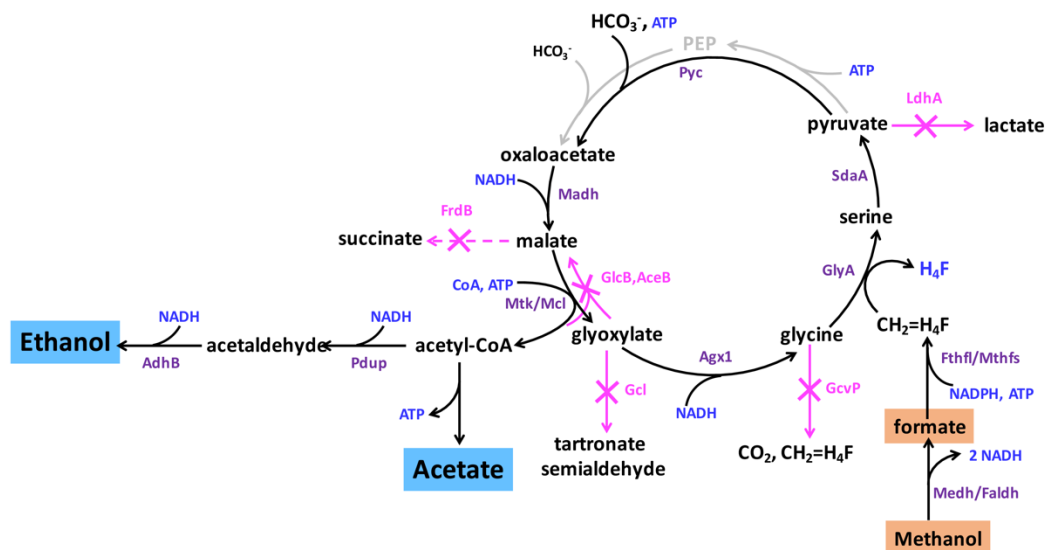
(A,B) Introduction of Mtk(M.c), Mcl(M.e) and Agx1(S.c) allowed the  $\Delta serA \Delta aceA$  strain to grow in minimal medium with 50 mM pyruvate as the sole carbon source (B), however, expression of AGX1(S.c) alone did not show similar effect under the same conditions, which demonstrated the function of the modified serine subpathway marked as blue arrow in (A).

(C,D) Expression of a set of heterologous genes, including mtk(M.c), mcl(M.e), AGX1(S.c), fthfl(M.t) and mthfs(M.t), allowed the  $\Delta serA \Delta gcvP$  strain to grow in minimal medium with pyruvate and formate as carbon sources (D), while the controls without formate addition or omitting mtk/mcl expression did not show obvious growth, which indicated the function of the modified serine subpathway marked as blue arrow in (C).

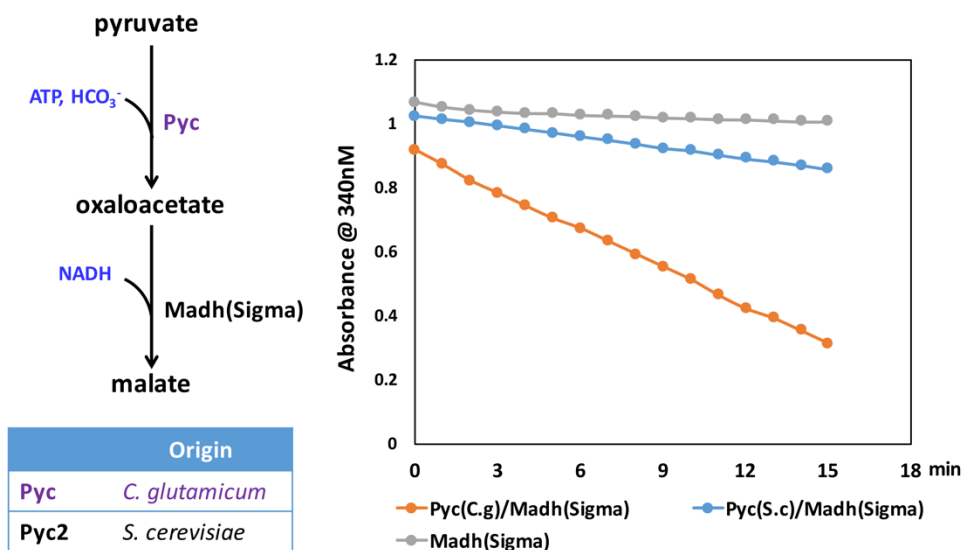
Error bars are s.d., n=3.



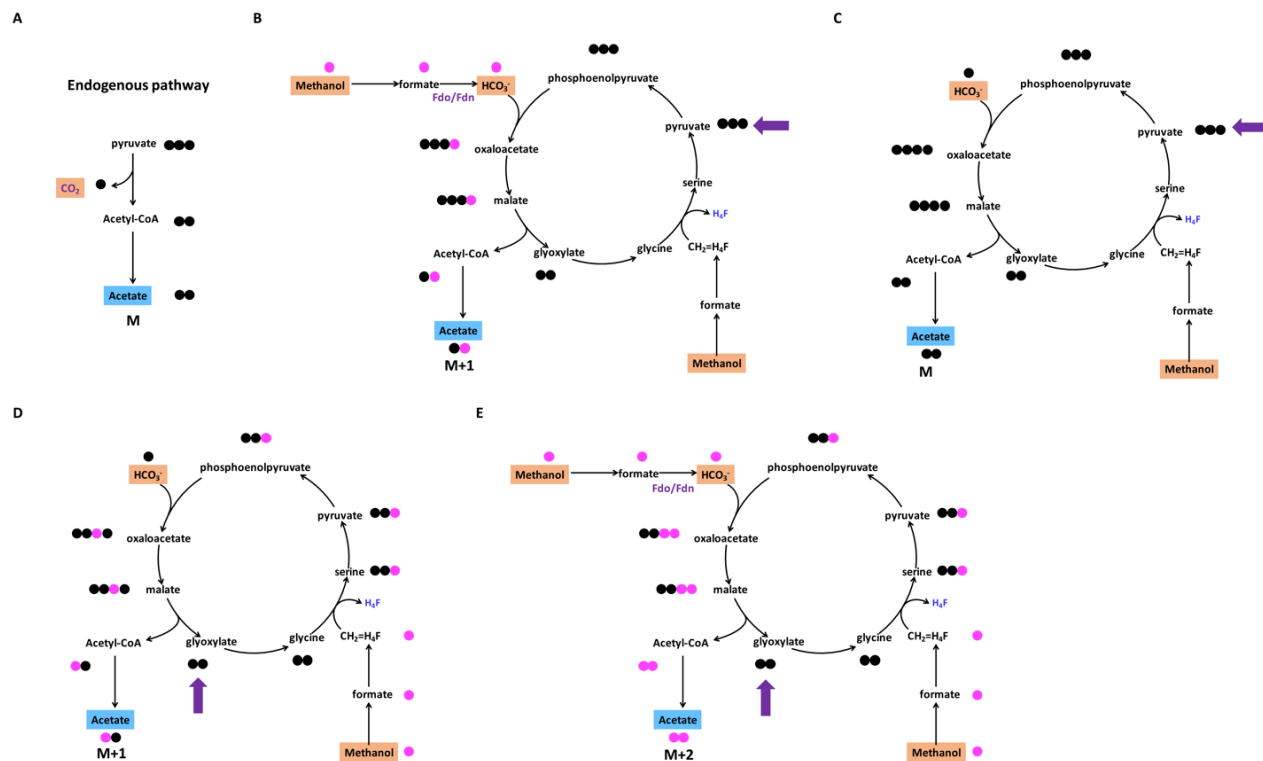
**HY106 ( $\Delta aceB$   $\Delta glcB$   $\Delta gcvP$   $\Delta gcl$   $\Delta frdB$   $\Delta ldhA$ )**



**Supplementary Figure 6.** Rationale of using the *E. coli* strain HY106 ( $\Delta aceB$   $\Delta glcB$   $\Delta gcvP$   $\Delta gcl$   $\Delta frdB$   $\Delta ldhA$ ) to investigate the effect of expressing the modified serine cycle *in vivo* (Supplementary Note 1). The genes *pyc*(*C.g*) and *madh*(*E.c*) were overexpressed in order to enhance the carbon flux from pyruvate to malate, *pdup*(*S.e*) and *adhB*(*Z.m*) were expressed to facilitate ethanol production.



**Supplementary Figure 7.** Determination of the activity of Pyc(*C.g*) *in vitro*. Pyc(*C.g*) displayed much higher enzymatic activity than the one from *S. cerevisiae*. NADH consumption was recorded at 340 nm. 10 ug of each purified protein was used. Malate dehydrogenase (Mdh) was acquired from Sigma.



**Supplementary Figure 8.** Prediction of the labeling pattern of acetate produced by the engineered *E. coli* strain with expressing the modified serine cycle genes. Magenta dot stands for  $^{13}\text{C}$  labeled methanol or its derived C1-carbon. Black dot indicates unlabeled carbon. Purple arrow means the starting carbon source.

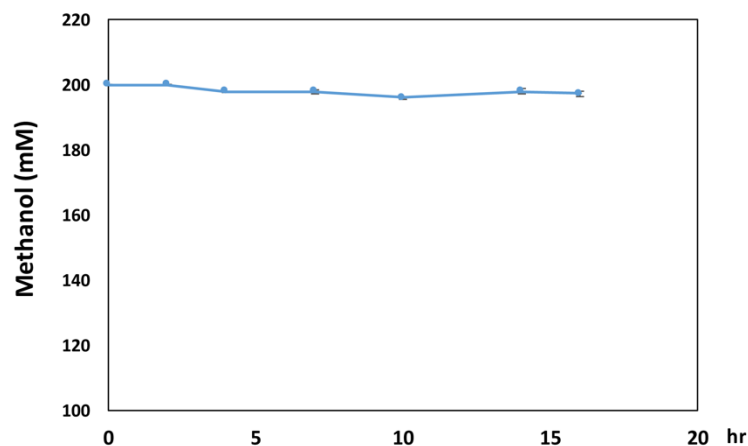
(A) Acetyl-CoA can be produced via pyruvate decarboxylation through endogenous pyruvate dehydrogenase complex.

(B) Unlabeled pyruvate assimilates  $^{13}\text{C}$  labeled bicarbonate, derived from oxidation of  $^{13}\text{C}$ -methanol, to generate labeled malate, and further splits into single-carbon labeled acetyl-CoA (M+1) and unlabeled glyoxylate.

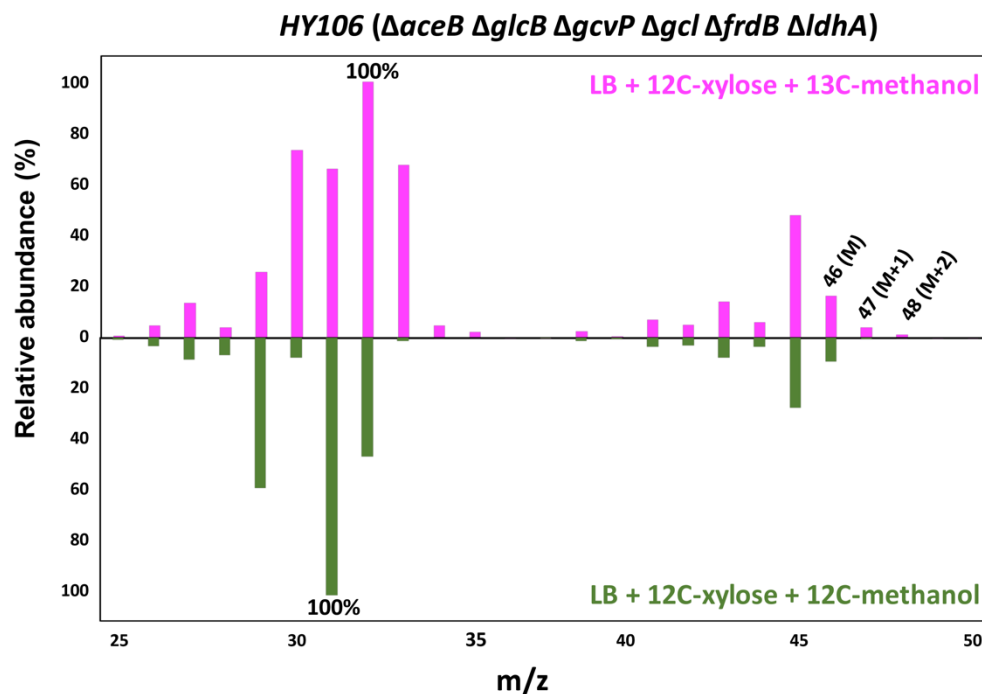
(C) Unlabeled pyruvate assimilates unlabeled bicarbonate to generate malate, and splits into both unlabeled acetyl-CoA and glyoxylate.

(D) Unlabeled glyoxylate (produced from B and C) assimilates labeled 5,10-methylene- $\text{H}_4\text{F}$ , derived from  $^{13}\text{C}$ -methanol, to produce single-carbon labeled serine. The M+1 serine is converted to pyruvate and assimilates unlabeled bicarbonate to produce M+1 form of OAA. The labeled malate can be split into single-carbon labeled acetyl-CoA (M+1) and unlabeled glyoxylate.

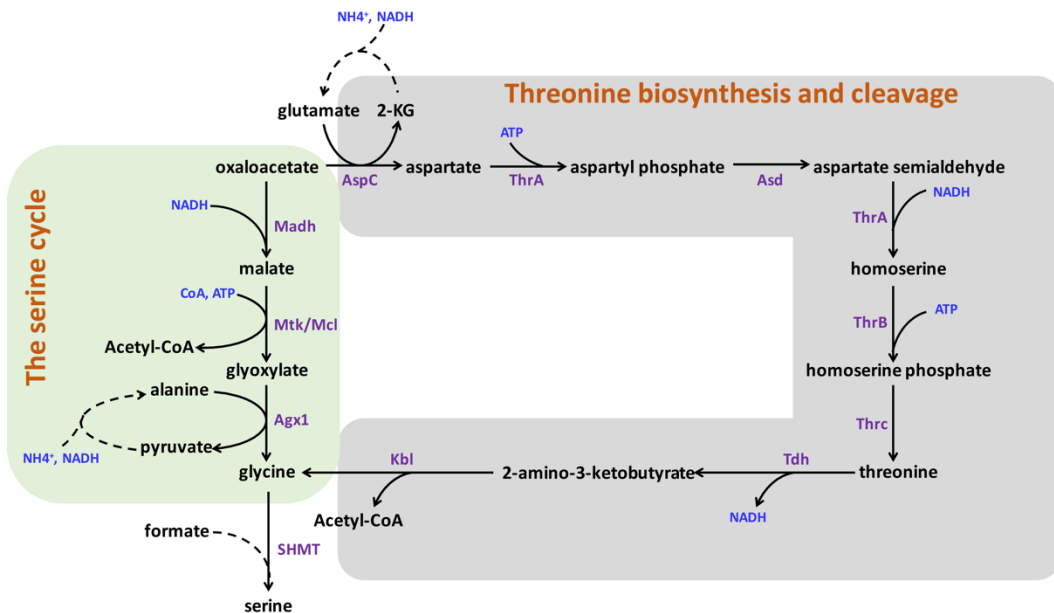
(E) Unlabeled glyoxylate (produced from B and C) assimilates labeled 5,10-methylene- $\text{H}_4\text{F}$ , derived from  $^{13}\text{C}$ -methanol, to produce single-carbon labeled serine. The M+1 pyruvate assimilates  $^{13}\text{C}$  labeled bicarbonate, derived from oxidation of  $^{13}\text{C}$ -methanol, to produce M+2 OAA. The labeled malate can be split into double-carbon labeled acetyl-CoA (M+2) and unlabeled glyoxylate.



**Supplementary Figure 9.** Determination of the methanol evaporation control. To minimize the methanol evaporation at 37°C shaker, we performed the bioproduction experiments under oxygen-limited condition in a sealed tube (BD vacutainer glass tube). The methanol evaporation control was determined in LB medium with xylose/methanol addition at different time points. Error bars are s.d., n=3.



**Supplementary Figure 10.** The ethanol fractional pattern determined by gas chromatography-mass spectrometry (GC-MS). In the up part of the Figure (LB+12C-Xylose+13C-methanol), the fractional peaks of 34, 35, 47 and 48 (m/z) were detected, but were not appeared in the bottom part (LB+12C-Xylose+12C-methanol). A shifting in ethanol fractional peaks was observed in the engineered strain with expressing the complete cycle genes when incubation in 13C-methanol LB medium compared to the same engineered strain but supplied with unlabeled methanol. This result could be explained by 13C-methnaol incorporation into ethanol that caused the shifting in fractional peaks.



| OAA to glycine and Ac-CoA | NADH | ATP | Enzymatic Steps |
|---------------------------|------|-----|-----------------|
| The serine cycle          | 2    | 1   | 4               |
| The threonine route       | 2    | 2   | 8               |

**Supplementary Figure 11.** The threonine biosynthesis and cleavage route can convert oxaloacetate to glycine and acetyl-CoA with the expense of 2 ATP and 2 NADH.

Compared to Mtk/Mcl/Agx1 used in the modified serine cycle, the threonine biosynthesis and cleavage route includes more enzymatic steps and consumes one more ATP to produce glycine from OAA.

AspC: aspartate aminotransferase. ThrA: fused aspartate kinase/homoserine dehydrogenase. ThrB: homoserine kinase. ThrC: threonine synthase. Asd: aspartate-semialdehyde dehydrogenase.

190 **Supplementary Table 1.** Comparison among the C1-carbon assimilation cycles.

| Name                                                  | C1 carbon assimilated                                             | Cycle Product      | Enzymes of the pathway                | ATP equiv. for each acetyl-CoA synthesis | Implement in <i>E. coli</i>         |
|-------------------------------------------------------|-------------------------------------------------------------------|--------------------|---------------------------------------|------------------------------------------|-------------------------------------|
| The modified serine cycle                             | 1. $\text{HCO}_3^-$ /Formic acid<br>2. $\text{HCO}_3^-$ /Methanol | Acetyl-CoA(C2)     | 11 (for formate)<br>13 (for methanol) | 3 ATP                                    | <b>Yes.</b> This study              |
| The natural serine cycle (from <i>M. extorquens</i> ) | 1. $\text{HCO}_3^-$ /Formic acid<br>2. $\text{HCO}_3^-$ /Methanol | Acetyl-CoA(C2)     | 12 (for formate)<br>17 (for methanol) | 2 ATP                                    | No. Smejkalová et al., 2010         |
| The RuMP cycle                                        | Methanol                                                          | DHAP (C3)          | 9                                     | 2 ATP<br>(Type I methanotroph)           | <b>Yes.</b> Whitaker et al., 2017   |
| The MCC                                               | Methanol                                                          | Acetyl-CoA(C2)     | 10                                    | 0 ATP                                    | No. Bogorad et al., 2014            |
| The CBB cycle                                         | $\text{CO}_2$                                                     | 3-p-glycerate (C3) | 13                                    | 8 ATP                                    | <b>Yes.</b> Antonovsky et al., 2016 |
| The 3HP bicycle                                       | $\text{HCO}_3^-$                                                  | Pyruvate (C3)      | 15                                    | 7 ATP                                    | No. Mattozzi et al., 2013           |
| The HP/HB cycle                                       | $\text{HCO}_3^-$                                                  | Acetyl-CoA(C2)     | 13                                    | 6 ATP                                    | No. Könneke et al., 2014            |
| The rTCA cycle                                        | $\text{CO}_2$                                                     | Pyruvate (C3)      | 9                                     | 2 ATP                                    | No. Tang et al., 2010               |
| The Wood-Ljungdahl pathway                            | $\text{CO}_2$                                                     | Pyruvate (C3)      | 9                                     | 1 ATP                                    | No. Ragsdale et al., 2008           |

191 The natural serine cycle is the only pathway that can simultaneously assimilate one reduced C1 compound (formic acid or methanol) and one  $\text{CO}_2$   
192 equivalent to produce an acetyl-CoA (the C2 building block).

193 DHAP: dihydroxyacetone phosphate. The 3HP bicycle: the 3-hydroxypropionate bicycle. The HP/HB cycle: the 3-hydroxypropionate/4-  
194 hydroxybutyrate cycle.

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198 **Supplementary Table 2.** Thermodynamic calculation of the modified serine cycle.

| #            | The modified serine cycle                                                                                                                                              | $\Delta_r G^m$<br>(KJ/mol) |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| 1            | $\text{CO}_2 + \text{Phosphoenolpyruvate} + \text{H}_2\text{O} \rightleftharpoons \text{Orthophosphate} + \text{Oxaloacetate}$                                         | -36.7                      |
| 2            | $\text{NADH} + \text{Oxaloacetate} \rightleftharpoons \text{NAD}^+ + (\text{S})\text{-Malate}$                                                                         | -30.3                      |
| 3            | $\text{ATP} + \text{CoA} + (\text{S})\text{-Malate} \rightleftharpoons \text{ADP} + \text{Orthophosphate} + \text{MalyI-CoA}$                                          | -6.6                       |
| 4            | $\text{MalyI-CoA} \rightleftharpoons \text{Acetyl-CoA} + \text{Glyoxylate}$                                                                                            | -4.2                       |
| 5            | $\text{L-Alanine} + \text{Glyoxylate} \rightleftharpoons \text{Pyruvate} + \text{Glycine}$                                                                             | -8.3                       |
| 6            | $\text{NAD}^+ + \text{Methanol} \rightleftharpoons \text{NADH} + \text{Formaldehyde}$                                                                                  | 34.2                       |
| 7            | $\text{NAD}^+ + \text{Formaldehyde} + \text{H}_2\text{O} \rightleftharpoons \text{NADH} + \text{Formate}$                                                              | -43.0                      |
| 8            | $\text{NAD}^+ + \text{Formate} \rightleftharpoons \text{NADH} + \text{CO}_2$                                                                                           | -14.1                      |
| 9            | $\text{ATP} + \text{Formate} + \text{Tetrahydrofolate} \rightleftharpoons \text{ADP} + \text{Orthophosphate} + 10\text{-Formyltetrahydrofolate}$                       | -4.8                       |
| 10           | $10\text{-Formyltetrahydrofolate} \rightleftharpoons 5,10\text{-Methenyltetrahydrofolate} + \text{H}_2\text{O}$                                                        | 5.0                        |
| 11           | $\text{NADPH} + 5,10\text{-Methenyltetrahydrofolate} \rightleftharpoons \text{NADP}^+ + 5,10\text{-Methylenetetrahydrofolate}$                                         | -9.9                       |
| 12           | $\text{Glycine} + 5,10\text{-Methylenetetrahydrofolate} + \text{H}_2\text{O} \rightleftharpoons \text{L-Serine} + \text{Tetrahydrofolate}$                             | 6.5                        |
| 13           | $\text{L-Serine} \rightleftharpoons \text{NH}_3 + \text{Pyruvate}$                                                                                                     | -45.8                      |
| 14           | $\text{ATP} + \text{Pyruvate} + \text{H}_2\text{O} \rightleftharpoons \text{Orthophosphate} + \text{AMP} + \text{Phosphoenolpyruvate}$                                 | -13.3                      |
| <b>Total</b> | <b><math>\text{HCO}_3^- + \text{Formate} + 3\text{NADH} + 3\text{ATP} + \text{CoA} \rightarrow \text{Acetyl-CoA} + \text{AMP} + 2\text{ADP} + 3\text{NAD}^+</math></b> | <b>-148.4</b>              |
|              | <b><math>\text{HCO}_3^- + \text{Methanol} + \text{NADH} + 3\text{ATP} + \text{CoA} \rightarrow \text{Acetyl-CoA} + \text{AMP} + 2\text{ADP} + \text{NAD}^+</math></b>  | <b>-157.2</b>              |
|              | <b><math>2\text{Formic acid} + \text{CoA} + 3\text{ATP} + 2\text{NADH} \rightarrow \text{Acetyl-CoA} + 3\text{ADP} + 2\text{NAD}^+</math></b>                          | <b>-162.5</b>              |
|              | <b><math>2\text{Methanol} + \text{CoA} + 3\text{ATP} + 2\text{NAD}^+ \rightarrow \text{Acetyl-CoA} + 3\text{ADP} + 2\text{NADH}</math></b>                             | <b>-180.1</b>              |

199 The reaction Gibbs energy was calculated through eQuilibrator software.  $\Delta_r G^m$ (KJ/mol) is the free Gibbs energy when the reactant concentration  
200 was set to be 1 mM under the condition of pH=7 and 0.1 M ionic strength.



201 **Supplementary Table 3.** The net reactions of the modified serine cycle.

| # | C1 Substrate(s)                                 | The net reaction of the modified serine cycle                                                   |
|---|-------------------------------------------------|-------------------------------------------------------------------------------------------------|
| 1 | HCO <sub>3</sub> <sup>-</sup> and Formic acid   | HCO <sub>3</sub> <sup>-</sup> + Formic acid + CoA +3ATP +3NADH → Acetyl-CoA + 3ADP + 3NAD       |
| 2 | HCO <sub>3</sub> <sup>-</sup> and Methanol      | HCO <sub>3</sub> <sup>-</sup> + Methanol + CoA +3ATP +NADH → Acetyl-CoA + 3ADP + NAD            |
| 3 | 2 Formic acid                                   | 2Formic acid + CoA +3ATP +2NADH → Acetyl-CoA + 3ADP + 2NAD                                      |
| 4 | 2 Methanol                                      | 2Methanol + CoA +3ATP +2NAD → Acetyl-CoA + 3ADP + 2NADH                                         |
| 5 | 2 Methanol                                      | 2Methanol +3ATP → Ethanol + 3ADP                                                                |
| 6 | HCO <sub>3</sub> <sup>-</sup> and 2 Formic acid | HCO <sub>3</sub> <sup>-</sup> + 2Formic acid + 4NADH + 4ATP + ubiquinone → pyruvate + ubiquinol |
| 7 | HCO <sub>3</sub> <sup>-</sup> and 2 Methanol    | HCO <sub>3</sub> <sup>-</sup> + 2Methanol + 4ATP + ubiquinone → pyruvate + ubiquinol            |
| 8 | 3 Formic acid                                   | 3Formic acid + 3NADH + 4ATP + ubiquinone → pyruvate + ubiquinol                                 |
| 9 | 3 Methanol                                      | 3Methanol + 4ATP + 2NAD + ubiquinone → pyruvate + 2NADH + ubiquinol                             |

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206 **Supplementary Table 4.** Plasmids and strains used in the study.

| Plasmids        | Description                                                                                                                                         | Reference  |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>pCT20_S1</i> | <i>ColE1 ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO::His-medh(CT4-1)</i>                                                                          | Wu, 2016.  |
| <i>pHY24</i>    | <i>ColA ori</i> ; <i>Km<sup>R</sup></i> ; <i>LacI</i> ; <i>PLlacO1::mtkB(M.c) mtkA(M.c) mcl(M.e)</i>                                                | This study |
| <i>pHY49</i>    | <i>ColA ori</i> ; <i>Km<sup>R</sup></i> ; <i>LacI</i> ; <i>PLlacO1::mtkB(M.c) mtkA(M.c) mcl(M.e)</i><br><i>PLlacO1::madh(E.c) PLLacO1::pyc(C.g)</i> | This study |
| <i>pHY56</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::sgaA(R.s)</i>                                                                                | This study |
| <i>pHY62</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::AGX1(S.c)</i>                                                                                | This study |
| <i>pHY64</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::sdaA(C.n)</i>                                                                                | This study |
| <i>pHY68</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::AGX1(S.c) sdaA(C.n)</i>                                                                      | This study |
| <i>pHY78</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::AGX1(S.c) PLLacO1::fthfl(M.t) mthfs(M.t)</i>                                                 | This study |
| <i>pHY79</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::fthfl(M.t) mthfs(M.t)</i>                                                                    | This study |
| <i>pHY80</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::AGX1(S.c) PLLacO1::fthfl(M.t)</i>                                                            | This study |
| <i>pHY81</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::sdaA(C.n) PLLacO1::fthfl(M.t) mthfs(M.t)</i>                                                 | This study |
| <i>pHY84</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::sdaA(C.n) AGX1(S.c) PLLacO1::fthfl(M.t) mthfs(M.t)</i>                                       | This study |
| <i>pHY86</i>    | <i>SCDF ori</i> ; <i>Spec<sup>R</sup></i> ; <i>PLlacO1::medh(CT4-1) faldh(P.p)</i>                                                                  | This study |
| <i>pHY87</i>    | <i>SCDF ori</i> ; <i>Spec<sup>R</sup></i> ; <i>PLlacO1::glyA(E.c) PLLacO1::medh(CT4-1) faldh(P.p)</i>                                               | This study |
| <i>pHY88</i>    | <i>SCDF ori</i> ; <i>Spec<sup>R</sup></i> ; <i>PLlacO1::glyA(E.c)</i>                                                                               | This study |
| <i>pHY89</i>    | <i>ColE1 ori</i> ; <i>Gent<sup>R</sup></i> ; <i>PLlacO1::pdup(S.e) adhB(Z.m)</i>                                                                    | This study |
| <i>pHY95</i>    | <i>P15A ori</i> ; <i>Amp<sup>R</sup></i> ; <i>PLlacO1::AGX1(S.c) PLLacO1::fthfl(M.e) folD(E.c)</i>                                                  | This study |
| Strains         | Relevant Genotype                                                                                                                                   | Reference  |
| BW25113         | <i>rrnBT14 ΔlacZWI16 hsdR514 ΔaraBADAH33 ΔrhaBADLDD78</i>                                                                                           |            |
| HY93            | <i>ΔserA</i> in BW25113                                                                                                                             | This study |
| HY96            | <i>ΔserA ΔaceA</i> in BW25113                                                                                                                       | This study |
| HY94            | <i>ΔserA gcvp::cat</i> in BW25113                                                                                                                   | This study |
| HY99            | <i>ΔaceA Δgcvp</i> in BW25113                                                                                                                       | This study |
| HY106           | <i>ΔaceB ΔglcB Δgcl Δgcvp ΔfrdB ΔldhA</i> in BW25113                                                                                                | This study |

207 *Km<sup>R</sup>*: Kanamycin resistance; *Amp<sup>R</sup>*: Ampicillin resistance; *Spec<sup>R</sup>*: Spectinomycin resistance; *Gent<sup>R</sup>*:  
208 Gentamycin resistance.

211 **Supplementary Table 5.** The ethanol fractional pattern determined by GC-MS.

| m/z      | Relative abundance (%)                            |                                                   |
|----------|---------------------------------------------------|---------------------------------------------------|
|          | <sup>12</sup> C-xylose + <sup>13</sup> C-methanol | <sup>12</sup> C-xylose + <sup>12</sup> C-methanol |
| 25       | 1.01 ± 0.06                                       | 0.62 ± 0.07                                       |
| 26       | 5.16 ± 0.36                                       | 3.23 ± 0.27                                       |
| 27       | 13.88 ± 1.25                                      | 8.39 ± 1                                          |
| 28       | 4.32 ± 0.35                                       | 6.62 ± 0.64                                       |
| 29       | 25.94 ± 0.17                                      | 58.34 ± 5.3                                       |
| 30       | 73.25 ± 5.2                                       | 7.66 ± 0.6                                        |
| 31       | 66.16 ± 3.57                                      | 100                                               |
| 32       | 100                                               | 46.09 ± 3.87                                      |
| 33       | 67.52 ± 4.33                                      | 1.07 ± 0.07                                       |
| 34       | 4.96 ± 0.43                                       | 0                                                 |
| 35       | 2.58 ± 0.11                                       | 0                                                 |
| 37       | 0.16 ± 0.01                                       | 0                                                 |
| 38       | 0.35 ± 0.02                                       | 0.17 ± 0.02                                       |
| 39       | 2.68 ± 0.14                                       | 1.11 ± 0.08                                       |
| 40       | 0.7 ± 0.08                                        | 0.43 ± 0.03                                       |
| 41       | 7.42 ± 0.58                                       | 3.32 ± 0.33                                       |
| 42       | 5.23 ± 0.25                                       | 2.93 ± 0.23                                       |
| 43       | 14.22 ± 1.27                                      | 7.75 ± 0.85                                       |
| 44       | 6.41 ± 0.41                                       | 3.52 ± 0.32                                       |
| 45       | 48.03 ± 4.22                                      | 27.16 ± 1.87                                      |
| 46 (M)   | 16.6 ± 0.9                                        | 12.19 ± 1.13                                      |
| 47 (M+1) | 4.19 ± 0.26                                       | 0.34 ± 0.03                                       |
| 48 (M+2) | 1.59 ± 0.09                                       | 0                                                 |

| Primer name        | Sequence                                         |
|--------------------|--------------------------------------------------|
| <i>mtkA(M.c)F</i>  | 5'-GATTCTGAAGGAGATATACCATGAATATCCATGAGTACCA-3'   |
| <i>mtkA(M.c)R</i>  | 5'-GCTCATGGTATATCTCCTTTATCCCTTGACGATGGCGA-3'     |
| <i>mtkB(M.c)F</i>  | 5'-AGAGGAGATATACCATGAGCGTATTCGTAAACAAG-3'        |
| <i>mtkB(M.c)R</i>  | 5'-ATATTCATGGTATATCTCCTTCAGAATCTGATTCCGTGTT-3'   |
| <i>mcl(M.e)F</i>   | 5'-CAAGGGATAAAGGAGATATACCATGAGCTTCACCCTGATCCA-3' |
| <i>mcl(M.e)R</i>   | 5'-CAAGCTTCTCGAGTTACTTTCCGCCCATCGCGT-3'          |
| <i>pyc(C.g)F</i>   | 5'-GAGGAGATATACCATGTGCGACTCACACATCTTCAA-3'       |
| <i>pyc(C.g)R</i>   | 5'-CAAGCTTCTCGAGTTAGGAAACGACGACGATCAA-3'         |
| <i>madh(E.c)F</i>  | 5'-GAGGAGATATACCATGAAAGTCGCAGTCCTCGG-3'          |
| <i>madh(E.c)R</i>  | 5'-GGCCTCGTGATACGCCTTACTTATTAACGAACTCTT-3'       |
| <i>sdaA(C.n)F</i>  | 5'-GAGGAGATATACCATGGCAGTCAGCGTCTTTGAT-3'         |
| <i>sdaA(C.n)R</i>  | 5'-CGATACCGTCGACTTAGCATTCCACGATATTCA-3'          |
| <i>AGX1(S.c)F</i>  | 5'-GAGGAGATATACCATGACTAAATCTGTAGATACG-3'         |
| <i>AGX1(S.c)R</i>  | 5'-CTTATCGATACCGTCGACTCACTTTTTCCTCTGAAGAG-3'     |
| <i>fthfl(M.t)F</i> | 5'-GAGGAGATATACCATGTCCAAGGTACCCAGTGATA-3'        |
| <i>fthfl(M.t)R</i> | 5'-CTGGCATGGTATATCTCCTCTAGAAAAGACCGGTAATGA-3'    |
| <i>mthfs(M.t)F</i> | 5'-CTAGAGGAGATATACCATGCCAGCCCAGATCCTCGAC-3'      |
| <i>mthfs(M.t)R</i> | 5'-CGATACCGTCGACCTAGCGCCGGGCCGCCTCGACT-3'        |
| <i>glyA(E.c)F</i>  | 5'-AAGAGGAGATATACCATGTAAAGCGTGAAATGAA-3'         |
| <i>glyA(E.c)R</i>  | 5'-GATACCGTCGACTTATGCGTAAACCGGGTAAC-3'           |

|                    |                                               |
|--------------------|-----------------------------------------------|
| <i>medh F</i>      | 5'-GAGGAGATATACCATGACCCACCTGAACATCGCTA-3'     |
| <i>medh R</i>      | 5'-CATGGTATATCTCCTTTACATCGCCGCAGCGAAGAT-3'    |
| <i>faldh(P.p)F</i> | 5'-GTAAAGGAGATATACCATGTCTGGCAATCGTGGAGTG-3'   |
| <i>faldh(P.p)R</i> | 5'-CGATACCGTCGACTTACGCCGCACCCACATTTT-3'       |
| <i>Pdup(S.e)F</i>  | 5'-GAGGAGATATACCATGAATACTTCTGAACTCGA-3'       |
| <i>Pdup(S.e)R</i>  | 5'-GTATATCTCCTGCATGCTTAGCGAATAGAAAAGCCGTT-3'  |
| <i>adhB(Z.m)F</i>  | 5'-CATGCAGGAGATATACCATGGCTTCTTCAACTTTTTAT-3'  |
| <i>adhB(Z.m)R</i>  | 5'-CGATACCGTCGACTTAGAAAGCGCTCAGGAAGAGT-3'     |
| <i>sgaA(R.s)F</i>  | 5'-GAGGAGATATACCATGTCGCTTGCGCACGGCCGT-3'      |
| <i>sgaA(R.s)R</i>  | 5'-CTTATCGATACCGTCGACTCAGGCTGCCGCGCCGAGGCT-3' |

219 The RBS sequence for expressing gene was 5'-AGGAGATATACC-3'.

220 In Supplementary Figure 2D, to regulate the expression levels of *ghrA(E.c)*, three different RBS  
221 sequences were used as following:

222 RBS(H): 5'-AGGAGATATACC-3',

223 RBS(M): 5'-CATCCGTTAATTACACACCCAGCAAACCTACCTA-3',

224 RBS(L): 5'-CAAGCCTACACAAATAACGACTTACCAACCT-3'.

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230 **Supplementary Table 7.** Summary of enzymes used in the modified serine cycle.

| Enzymes                             | EC #      | Gene               | Accession ID            | Associated database | Origin                  |
|-------------------------------------|-----------|--------------------|-------------------------|---------------------|-------------------------|
| Phosphoenolpyruvate synthetase      | 2.7.9.2   | <i>pps</i>         | P23538                  | Biocyc              | <i>E. coli</i>          |
| Phosphoenolpyruvate carboxylase     | 4.1.1.31  | <i>ppc</i>         | P00864                  | Biocyc              | <i>E. coli</i>          |
| Pyruvate carboxylase                | 6.4.1.1   | <i>pyc</i>         | H7C7K2                  | Biocyc              | <i>C. glutamicum</i>    |
| Malate dehydrogenase                | 1.1.1.37  | <i>mdh</i>         | P61889                  | Biocyc              | <i>E. coli</i>          |
| Malate thiokinase                   | 6.2.1.9   | <i>sucCD2</i>      | MCA1740<br>MCA1741      | Biocyc              | <i>M. capsulatus</i>    |
| Methyl-CoA lyase                    | 4.1.3.24  | <i>mcl</i>         | MexAM1_META1p1733       | Biocyc              | <i>M. extorquens</i>    |
| Alanine-glyoxylate transaminase     | 2.6.1.44  | <i>AGX1</i>        | P43567                  | Biocyc              | <i>S. cerevisiae</i>    |
| Serine hydroxymethyltransferase     | 2.1.2.1   | <i>glyA</i>        | P0A825                  | Biocyc              | <i>E. coli</i>          |
| Serine dehydratase                  | 4.3.1.17  | <i>sdaA</i>        | H16_A3622               | Biocyc              | <i>C. necator</i>       |
| Formate tetrahydrofolate ligase     | 6.3.4.3   | <i>moth_0109</i>   | GH0A-119                | Biocyc              | <i>M. thermoacetica</i> |
| Methylene-tetrahydrofolate synthase | 3.5.4.9   | <i>moth_1516</i>   | GH0A-1544               | Biocyc              | <i>M. thermoacetica</i> |
| Formaldehyde dehydrogenase          | 1.2.1.46  | <i>fdhA</i>        | PP_0328                 | Biocyc              | <i>P. putida kt2440</i> |
| Methanol dehydrogenase              | 1.1.1.244 | <i>medh(CT4-1)</i> | Wu <i>et al.</i> , 2016 |                     | <i>C. necator</i>       |

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