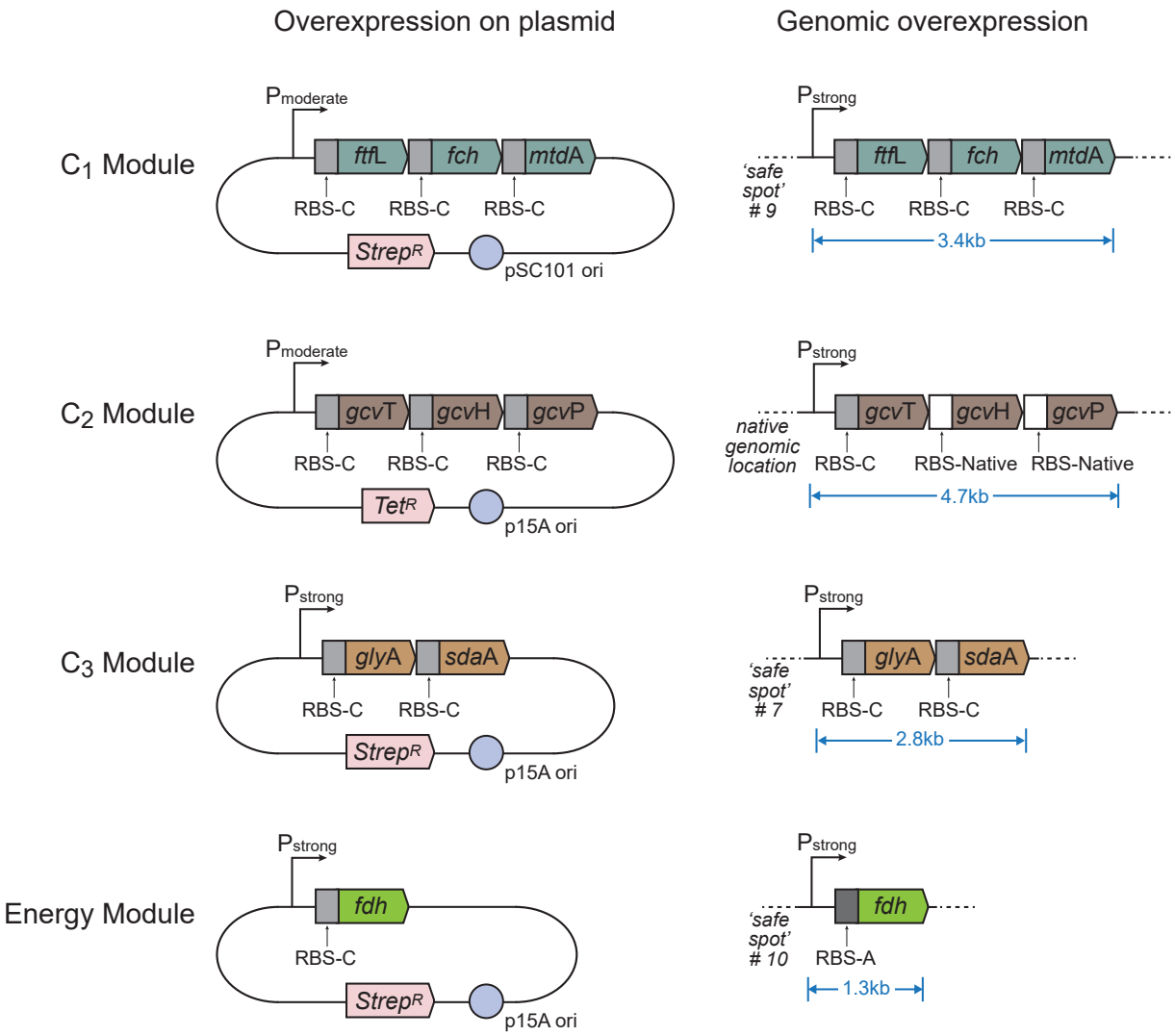
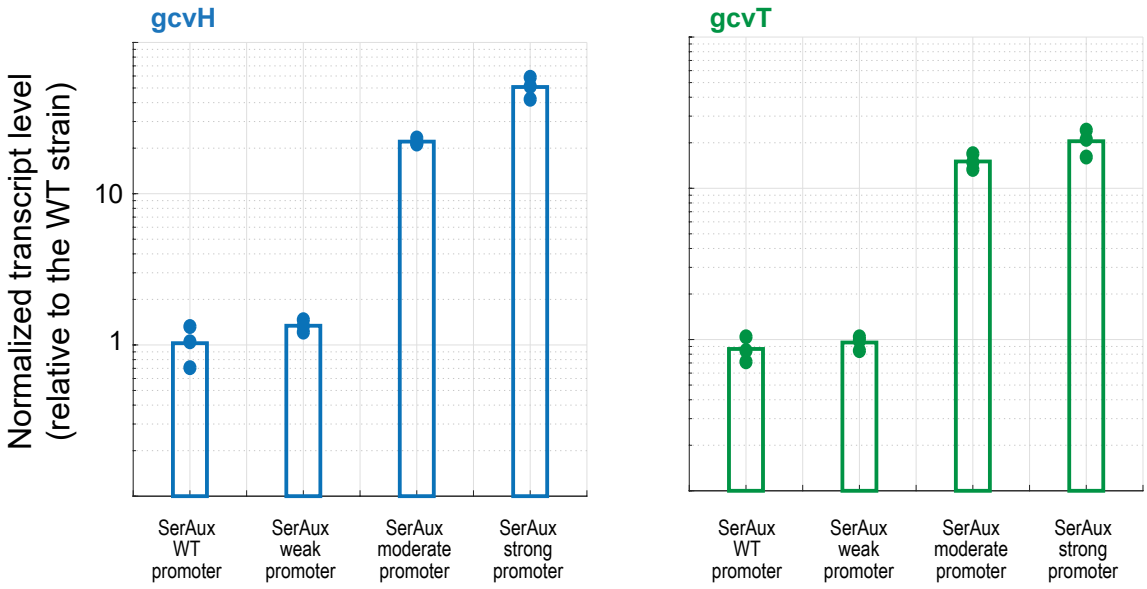


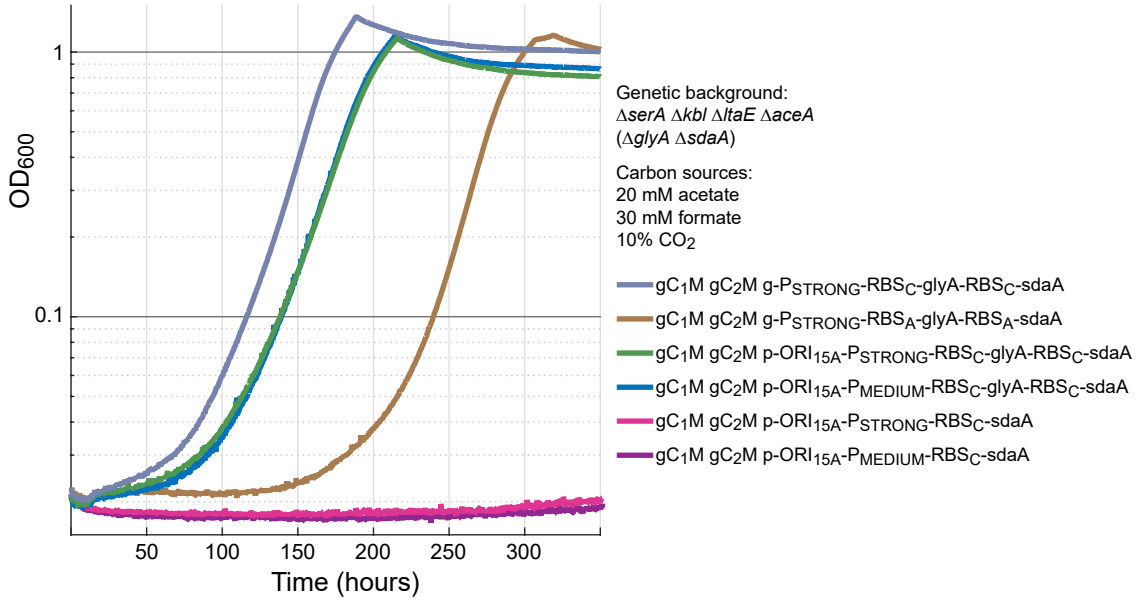
Supplementary Figure 1

Schematic overview of overexpression strategy. Gene overexpression from plasmid is shown in the left column while genomic overexpression is shown in the right column. Promoter and ribosome binding sites are as described in a previous manuscript [5]. Genomic 'safe spots' were described previously [6].



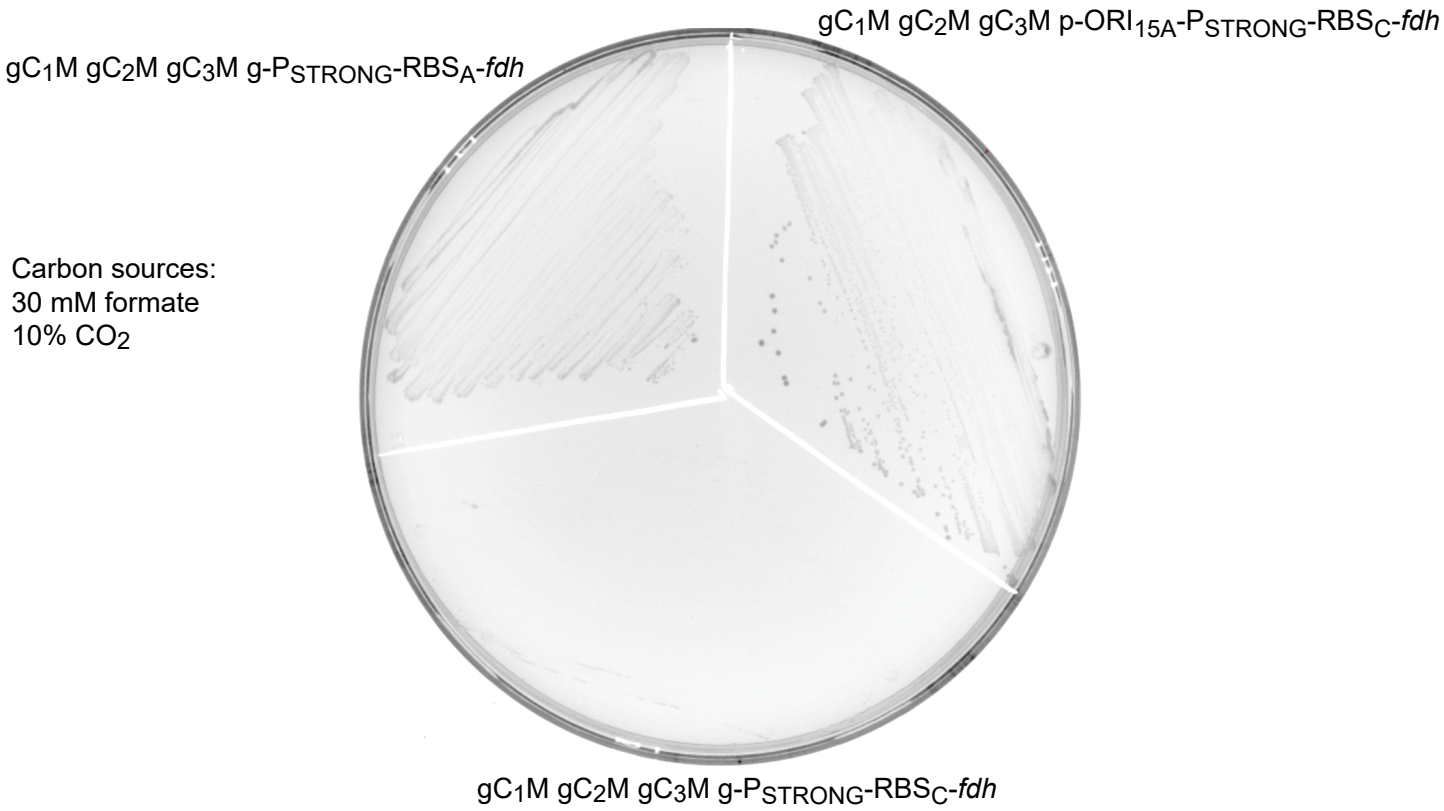
Supplementary Figure 2

Replacement of the native promoter of the GCV operon with a strong constitutive promoter increases gene expression 20-50 fold in a serine auxotroph (SerAux) strain (Δ*serA* Δ*kbl* Δ*ltaE* Δ*aceA*). Transcript levels were normalized to the expression of the *rrsA* gene and are shown relative to the expression of a WT (non-serine auxotroph) strain. As a comparison, the transcript levels induced by a weak constitutive promoter and moderate constitutive promoter are shown [5]. Experiments were performed in (biological) triplicates. Dots correspond to the measured values and bars to their average.



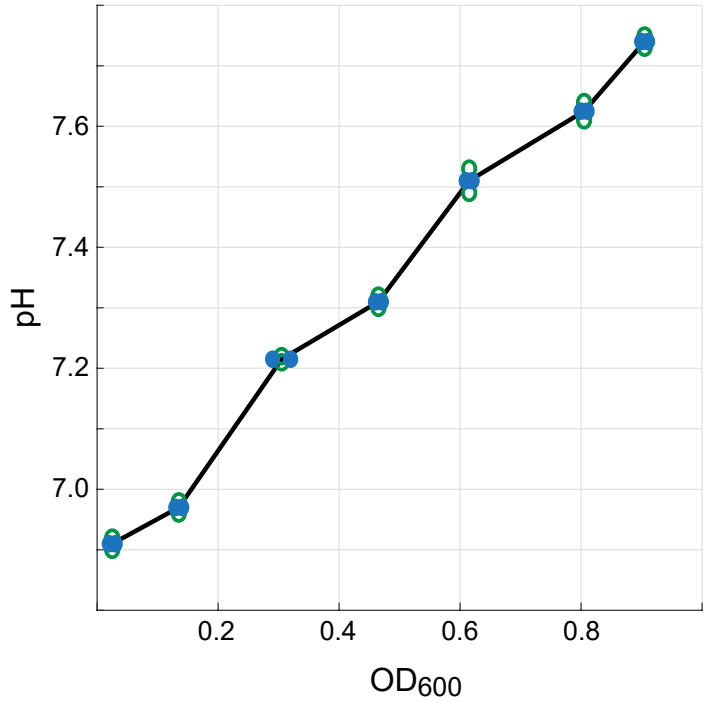
Supplementary Figure 3

Different expression approaches of the genes of C_3M – *glyA* and *sdaA* – affect growth via the reductive glycine pathway, with acetate serving as an energy source. Expression on a plasmid resulted in an identical growth regardless of the promoter strength (green and blue lines). Overexpression of *sdaA* alone failed to achieve growth (pink and purple lines). Genomic expression (after deletion of endogenous glyA and sdaA) resulted in better growth when gene expression was controlled by a medium strength ribosome binding site ('C', pale blue line) than by a strong ribosome binding site ('A', brown line). 'g' corresponds to genomic expression and 'p' to expression on a plasmid. Origin and replication, promoters, and ribosome binding sites are described in a previous study [5]. All experiments (in triplicates) were repeated five times, which showed highly similar growth behavior.



Supplementary Figure 4

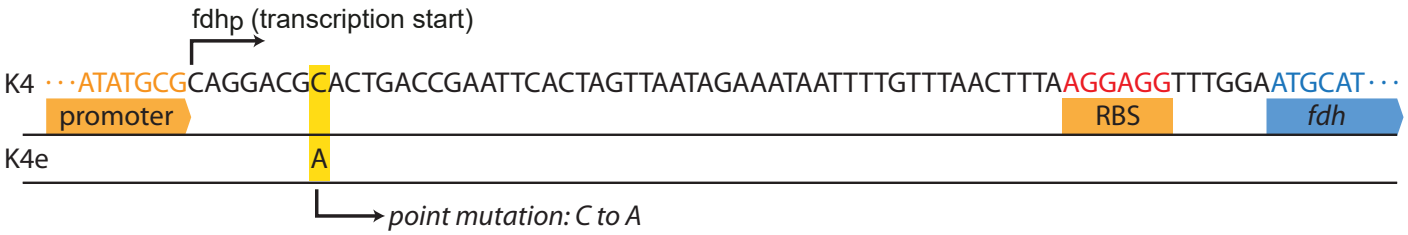
Different expression approaches of the genes of EM – formate dehydrogenase – affect growth via the reductive glycine pathway. Expression on a plasmid supported growth. Genomic overexpression supported growth only when the ribosome binding site was of the highest strength ('A'). 'g' corresponds to genomic expression and 'p' to expression on a plasmid. Origin and replication, promoters, and ribosome binding sites are described in a previous study [5]. All experiments were repeated four times, which showed identical growth behavior.



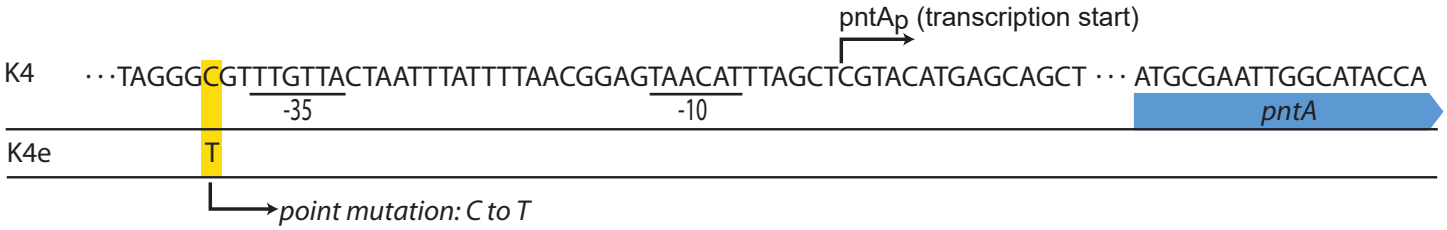
Supplementary Figure 5

Cell growth on formate directly correlates with increased medium pH due to the accumulation of OH^-. Dots correspond to the measured values of biological replicates (blue dots to measured OD and green open circles to measured pH). Line corresponds to the average of values.

(A) Mutation in the 5'UTR of FDH

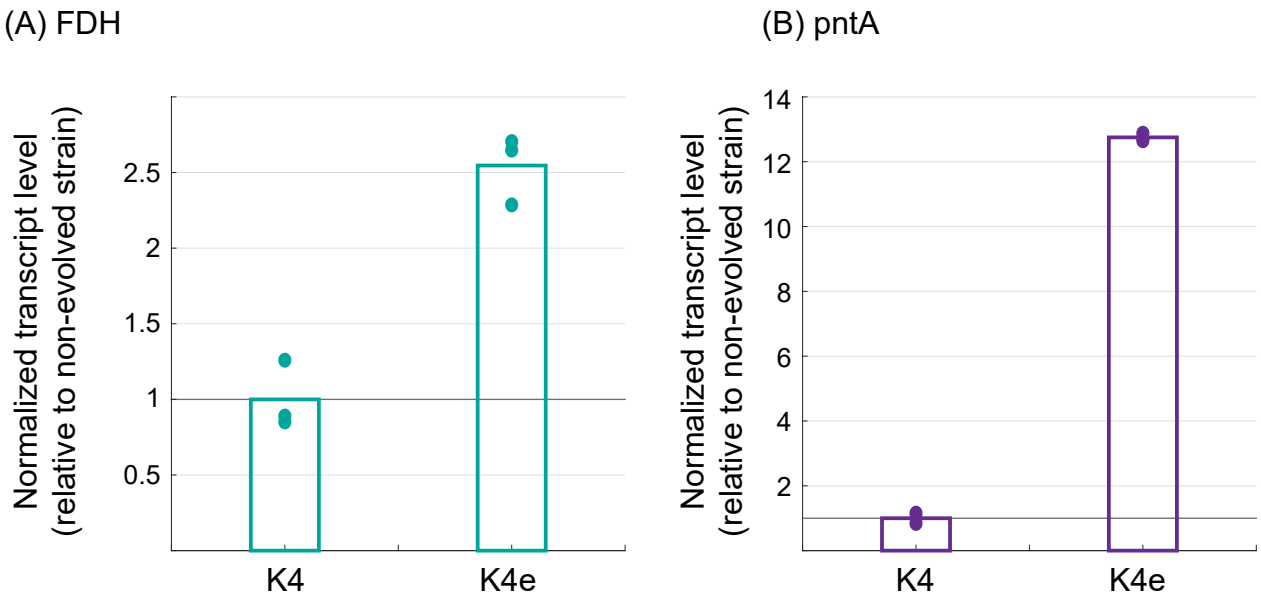


(B) Mutation in the promoter of pntAB



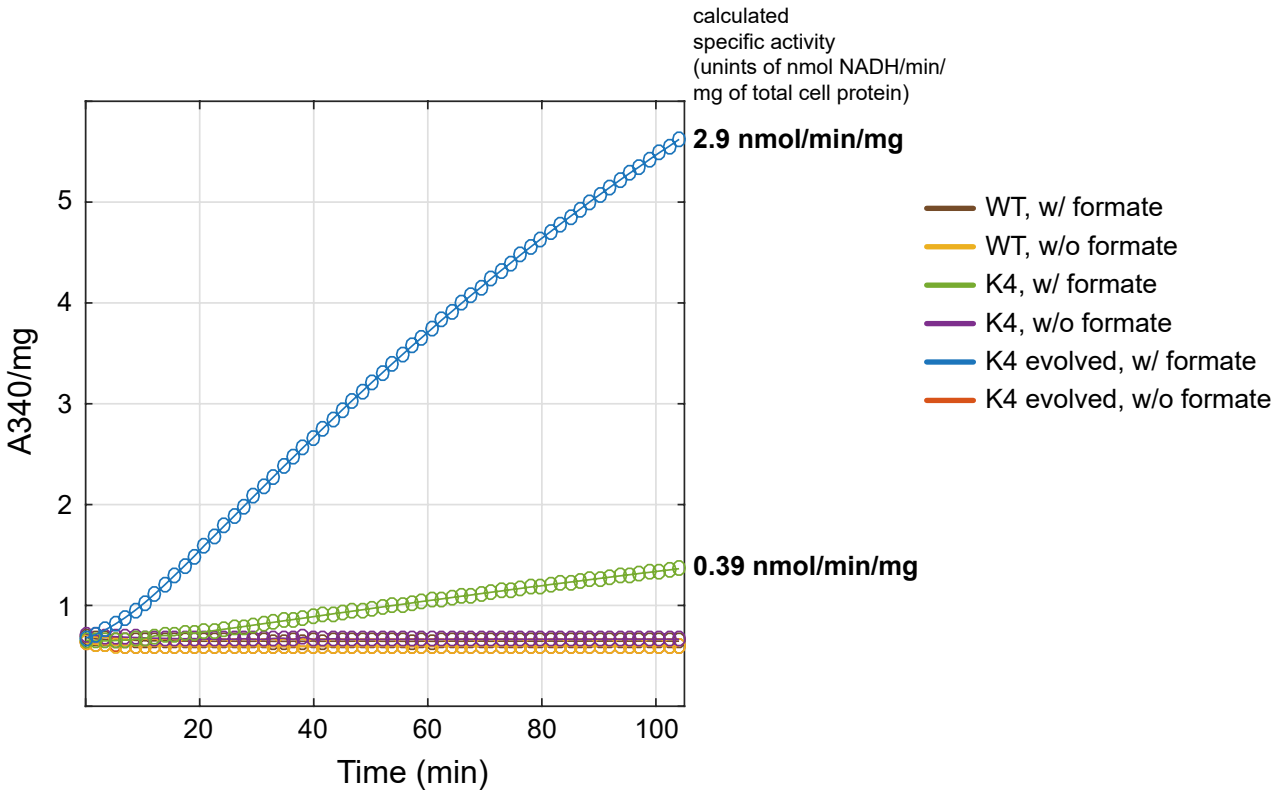
Supplementary Figure 6

Two mutations emerged within the formatotrophic strain after a short period of evolution. (A) A point mutation in the 5'-UTR of the FDH gene. (B) A point mutation in the promoter of the *pntAB* gene. Strain K4 corresponds to a strain in which the four modules of the reductive glycine pathway were introduced into its genome, that is, gC_1M gC_2M gC_3M gEM, while strain K4e the same strain after short term evolution.



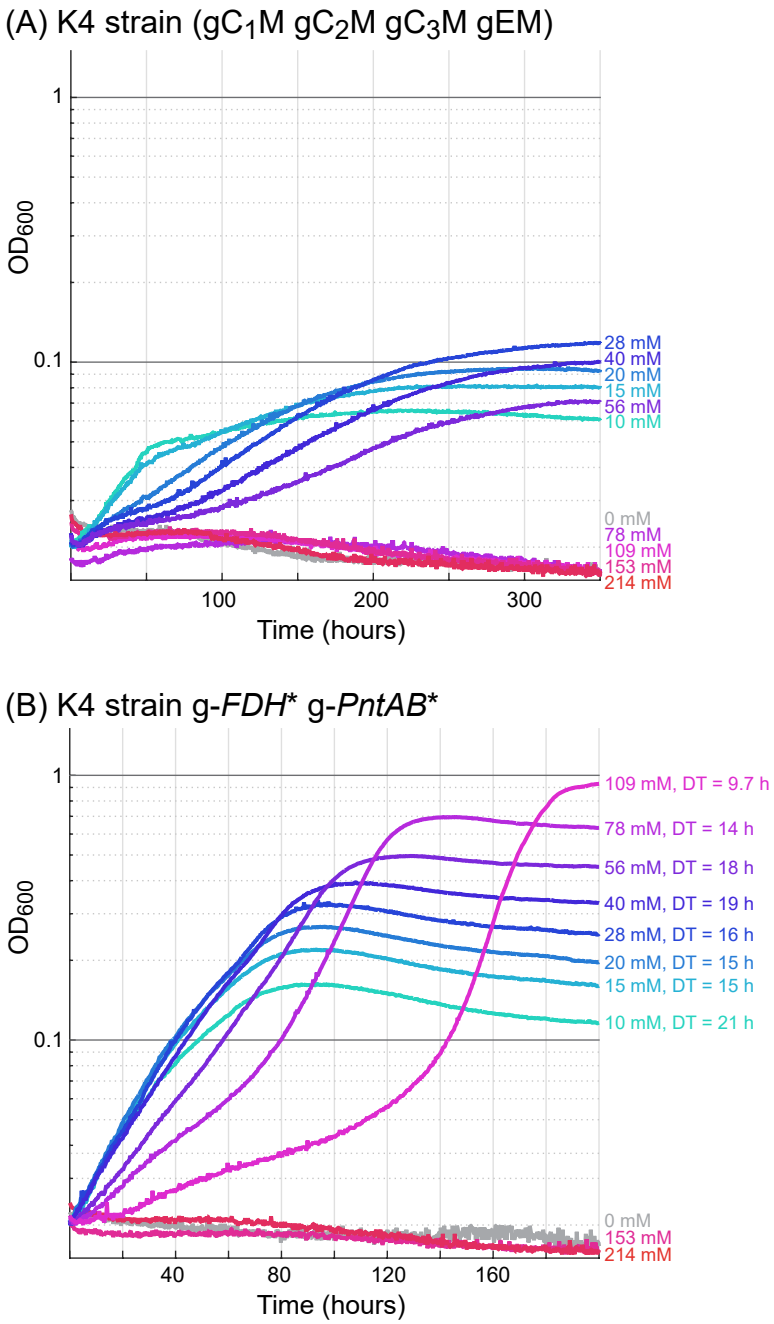
Supplementary Figure 7

Change in transcript level in the evolved strain. (A) Levels of FDH transcript increased 2.7-fold in the evolved strain. (B) Levels of *pntAB* transcript increased by ~14-fold in the evolved strain. In both cases transcript levels were normalized to the rrsA gene and are shown relative to the expression within a non-evolved strain. Experiments were performed in triplicate. Strain K4 corresponds to a strain in which the four modules of the reductive glycine pathway were introduced into its genome, that is, gC_1M gC_2M gC_3M gEM, while strain K4e the same strain after short term evolution. Experiments were performed in (biological) triplicates. Dots correspond to the measured values and bars to their average.



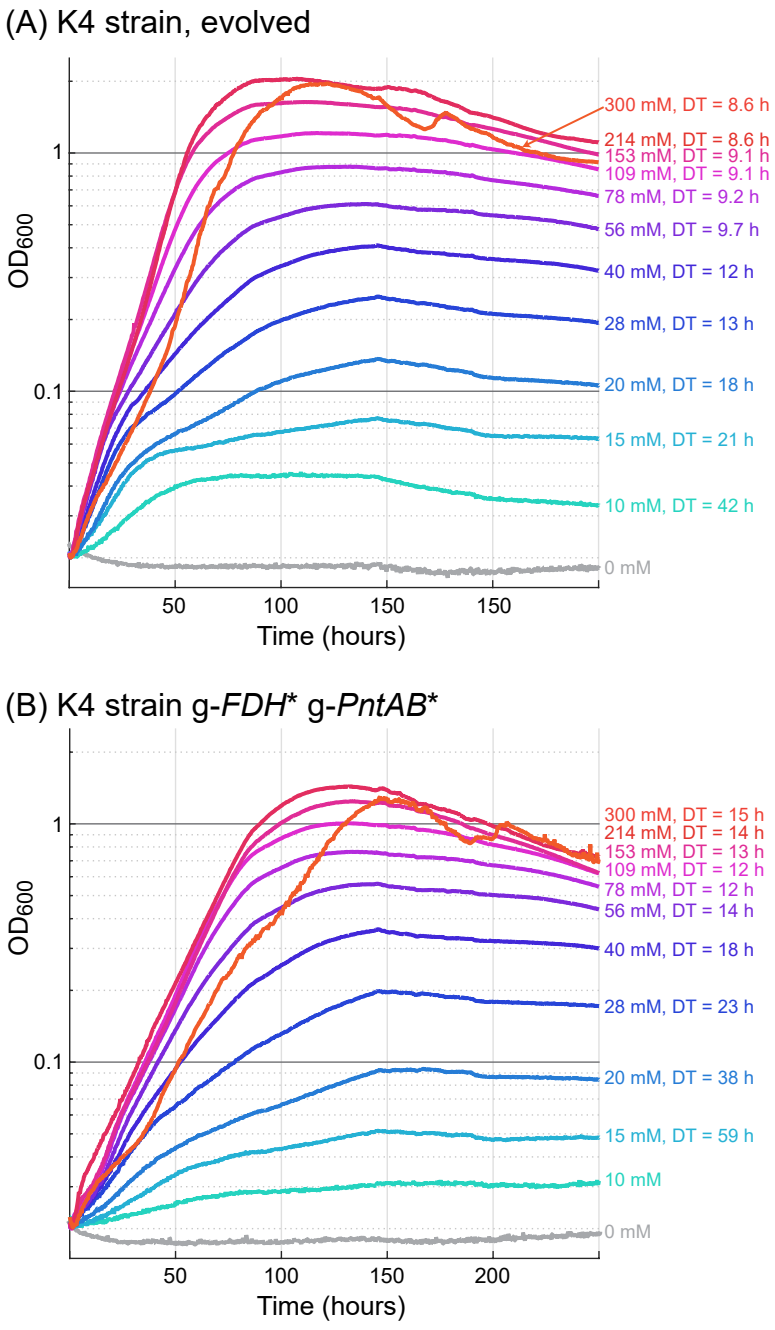
Supplementary Figure 8

Evolved strain displays 7.4-fold higher activity of FDH in cell extract. FDH activity was measured in 96-well plate by the addition of formate and NAD^+ and was followed by increase in absorbance at 340 nm by the accumulation of NADH. The results were normalized to mg of total cell protein. Strain K4 corresponds to a strain in which the four modules of the reductive glycine pathway were introduced into its genome, that is, gC_1M gC_2M gC_3M gEM, while strain K4e the same strain after short term evolution. Experiments were repeated twice, showing nearly identical curves.



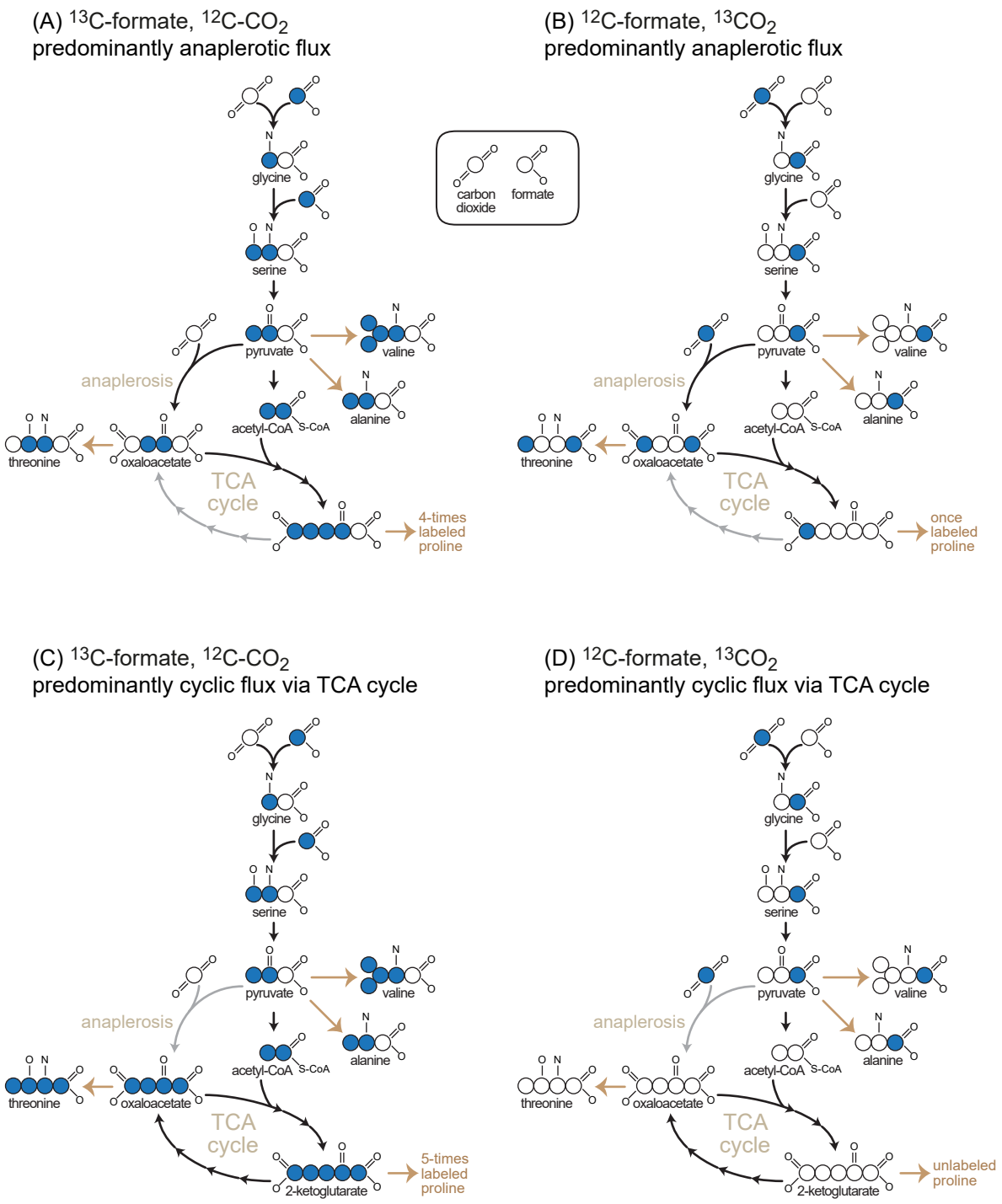
Supplementary Figure 9

Introduction of the two mutations found in genome sequencing of the evolved strain (5'UTR of *fdh* and promoter region of *pntAB*) improved growth on formate dramatically and resulted in a growth pattern very similar to that of the evolve strain (see Figure 3C). Cultivation of the evolved strain on formate as a sole carbon source within a 96-well plate. Experiments were conducted at 10% CO_2. Plate reader experiments were performed in triplicate, which displayed identical growth curves (±5%), and hence were averaged. Strain K4 corresponds to a strain in which the four modules of the reductive glycine pathway were introduced into its genome, that is, gC_1M gC_2M gC_3M gEM. All experiments (in triplicates) were repeated four times, which showed highly similar growth behavior.



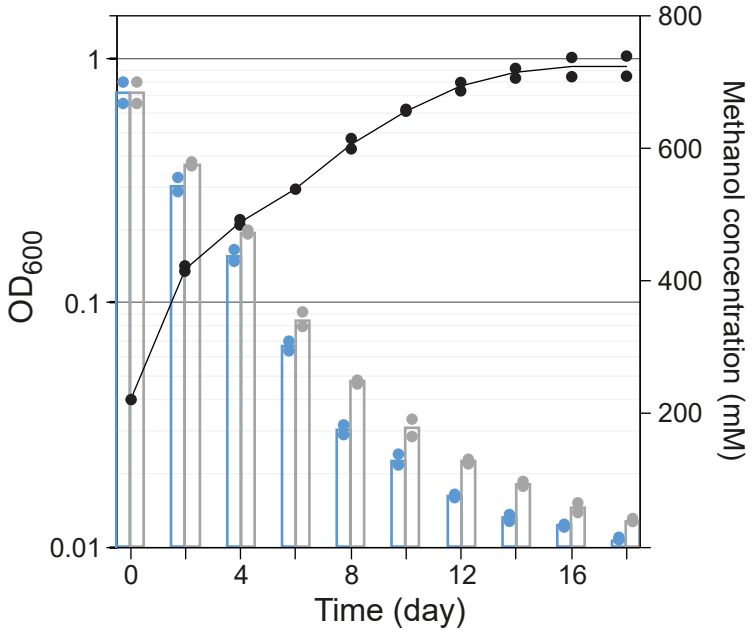
Supplementary Figure 10

Addition of 100 mM sodium bicarbonate enables growth on higher concentrations for formate, as demonstrated with the evolved K4 strain and a K4 strain to the genome of which the two mutations found in the evolved strain were introduced. Cultivation of the evolved strain on formate as a sole carbon source within a 96-well plate. Experiments were conducted at 10% CO_2. Plate reader experiments were performed in triplicate, which displayed identical growth curves (±5%), and hence were averaged. Strain K4 corresponds to a strain in which the four modules of the reductive glycine pathway were introduced into its genome, that is, gC_1M gC_2M gC_3M gEM. All experiments (in triplicates) were repeated four times, which showed highly similar growth behavior.



Supplementary Figure 11

Expected labeling of proteinogenic amino acids upon feeding with ^{13}C-formate/^{12}C-CO_2 or ^{12}C-formate/^{13}C-CO_2 and according to different metabolic scenarios.



Supplementary Figure 12

Addition of 100 mM sodium bicarbonate increases final OD_{600} on methanol, reaching 0.9 instead of 0.2 (Fig. 5C). Consumption of methanol is depicted by the bars: the grey bars correspond to methanol concentration in a test tube without cells (concentration decrease due to evaporation), while the blue bars represent the concentration of methanol in a test tube in which cells are growing on methanol. Dots corresponds to measured values (biologically independent duplicates) and the line and bars to their average.