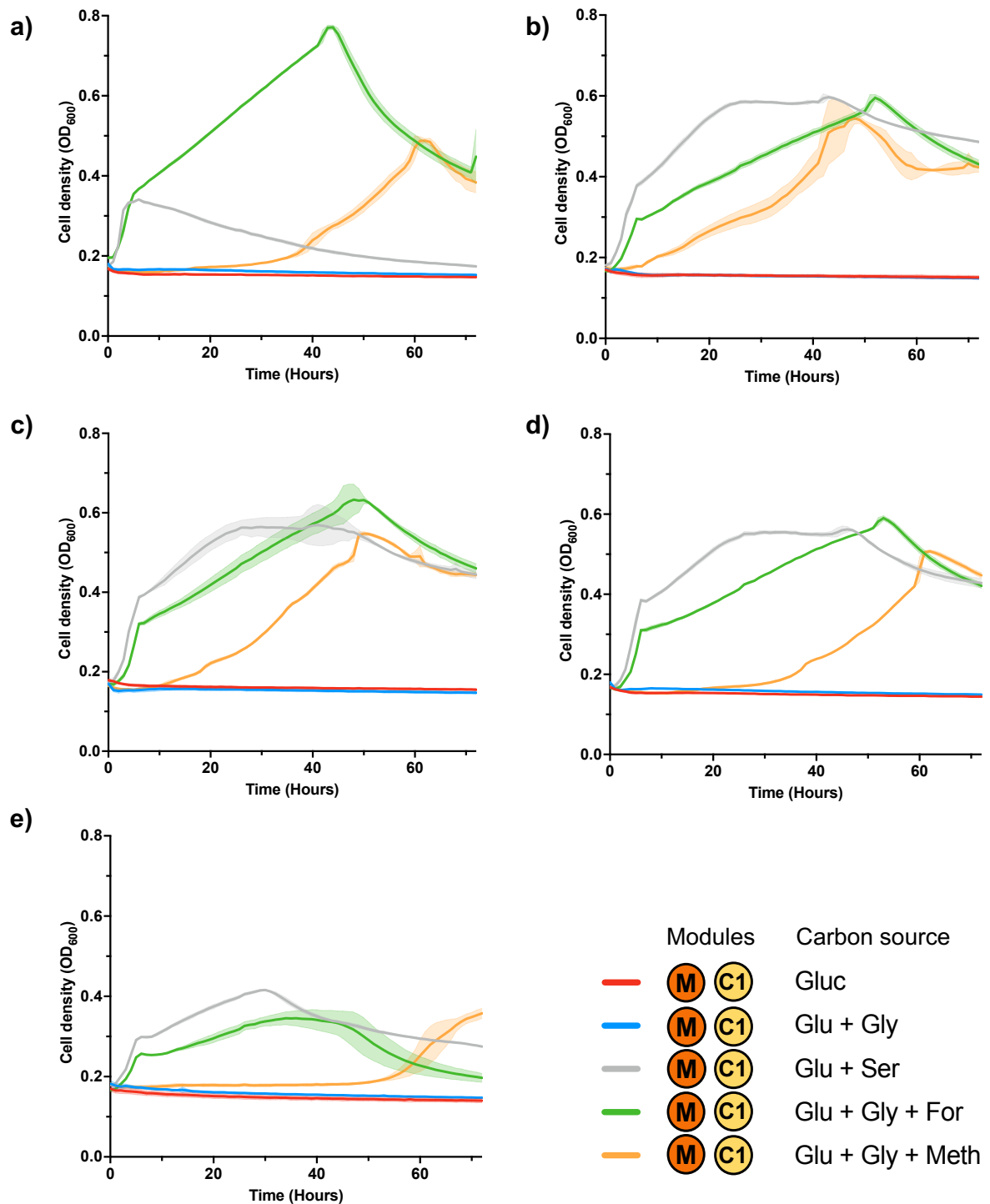


SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES



Figures S1 Methanol-dependent growth in C1-S-Aux with different methanol dehydrogenases a)

G. stearothermophilus, **b)** *B. methanolicus*, **c)** *C. glutamicum*, **d)** *adhP P. putida*, **e)** *pedE P. putida*.

Growth curves are averages from biological triplicates.

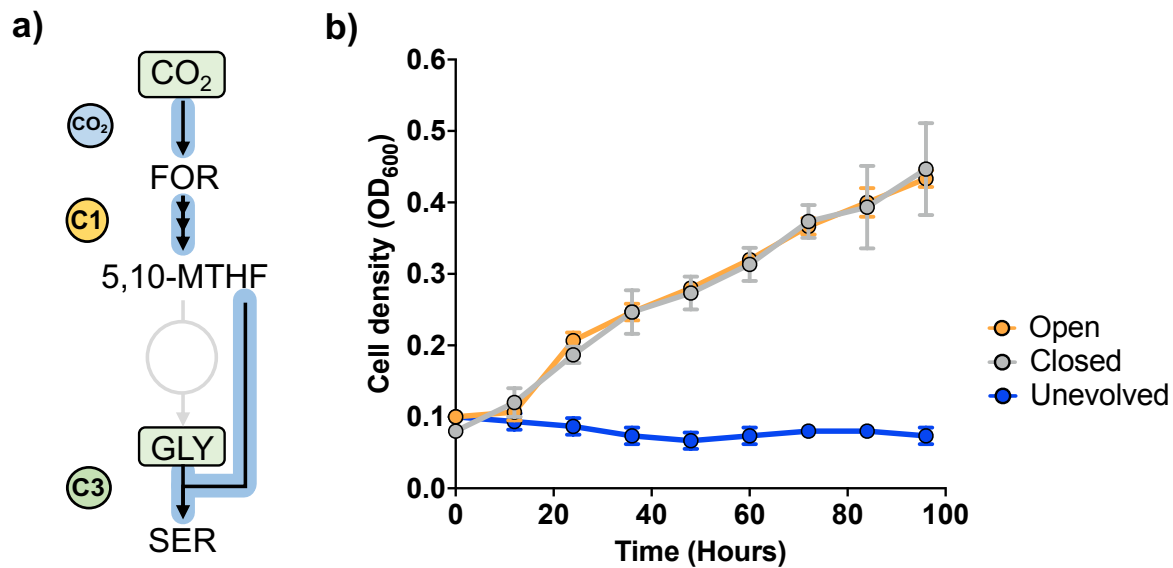


Figure S2 CO₂ - dependent growth at ambient CO₂ with different cultivation systems. a) Selection of the CO₂-dependent growth in C1- S-Evo. **b)** Growth patterns of C1-S-Evo cultivated in closed anaerobic bottles and open falcon tubes where the CO₂ can freely escape. The unevolved C1-S Aux strain was taken as a control. Growth curves are averages from biological triplicates.

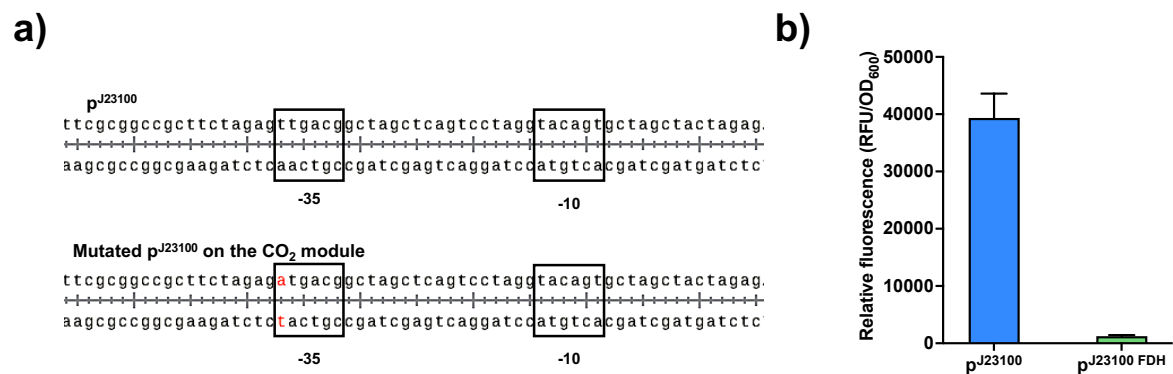


Figure S3 Characterization of the mutated CO₂ module during short-term evolution. a). Promoter sequences of the J23100 promoter and the mutated CO₂ module. **b).** Characterization of the mutated J23100 promoter of the CO₂ module using fluorescence as output. Relative fluorescence averages were taken from biological triplicates.

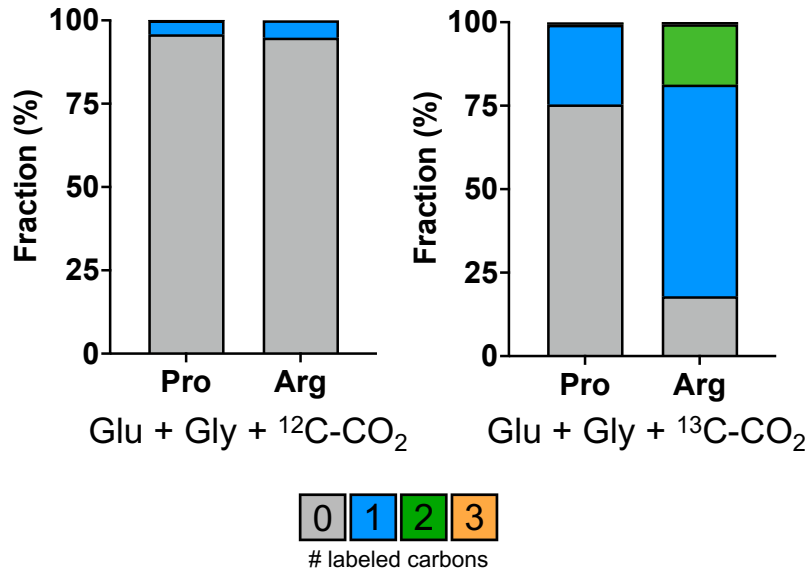


Figure S4 ^{13}C labeling of proline (Pro) and arginine (Arg) to determine intracellular CO_2 levels in C1-S-Evo. Labeling experiments are averages from biological triplicates.

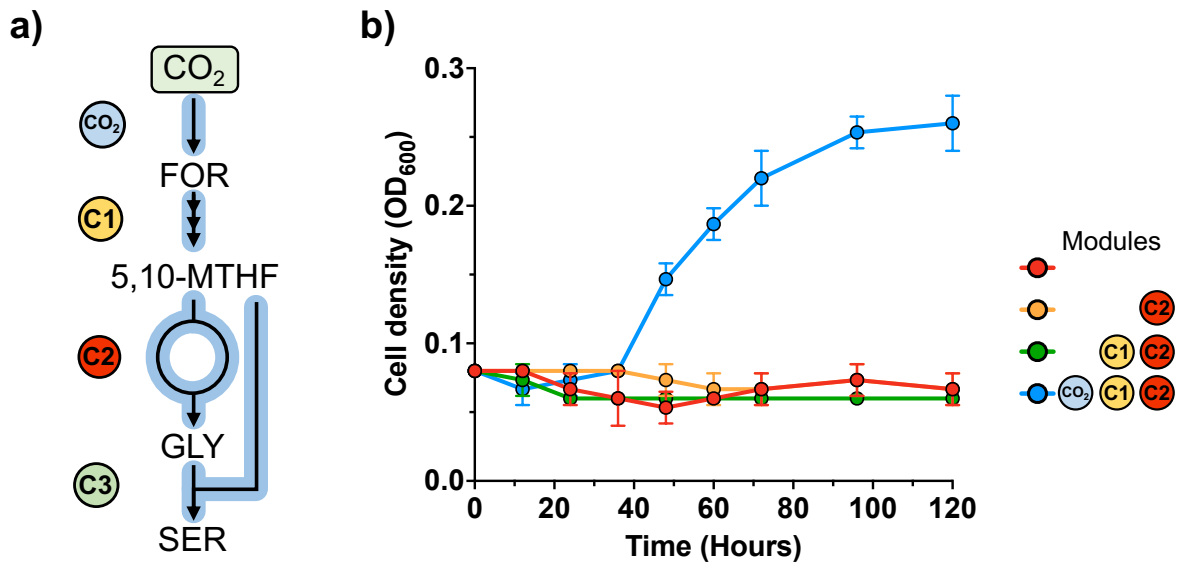


Figure S5 CO_2 - dependent growth in C1-G-S-Evo **a)** Selection of the combined effort of the CO_2 , C1, and C2 modules in C1-G-S- Evo. **b)** Growth was solely restored when the CO_2 , C1, and C2 modules are expressed. Growth curves are averages from biological triplicates.

SUPPLEMENTARY TABLES

Table S 1 Strains and plasmids used in the present study

External Excel file

Table S 2 Primers used in the present study.

External Excel file

Table S 3 Mutations identified in C1 -S –Evo after short-term evolution

Genome position	Gene name (Locus ID)	Mutation	Annotation	Gene annotation
9126	<i>rpmH</i> ← / → <i>dnaA</i>	+G	intergenic (-180/-416)	N.A.
278744	PP_0224 ← / ← <i>sctC</i>	+A	intergenic (-239/+75)	N.A.
2472058	PP_2166	Δ1 bp	coding (445/483 nt)	Anti-anti-sigma factor
4662562	<i>nuoG</i> (PP_4124)	C→T	Q476* (CAG→TAG)	NADH-quinone oxidoreductase subunit G

Table S 4 Codon optimized genes for the C1 module and M module

External Excel file