

Table S1. O₂ exchange rates in the dark, under CO₂-saturated and CO₂-limited photosynthesis, and after addition of NaHCO₃, in four mutants of *S. 6803* (Δpgp , $\Delta glcD1/2$, $\Delta gcvT$, and $\Delta glyk$).

	Δpgp	$\Delta glcD1/2$	$\Delta gcvT$	$\Delta glyk$
Dark	-47 ± 19	-20 ± 6	-22 ± 12	-13 ± 4
CO ₂ -saturated photosynthesis (α)	110 ± 10	110 ± 17	122 ± 3	125 ± 16
CO ₂ -limited photosynthesis (β)	8.5 ± 2.3	5.9 ± 1.7	12 ± 7	8 ± 4
Addition of NaHCO ₃ (γ)	98 ± 23	94 ± 8	98 ± 9	115 ± 7

O₂ exchange rates were determined simultaneously with Chl fluorescence measurement under actinic light (AL; 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) at the times indicated by α , β , and γ in Supplemental Fig. S6 (see also Fig. 1 and 2). The reaction mixture contained cyanobacterial cells (10 $\mu\text{g Chl mL}^{-1}$) and 50 mM HEPES-KOH (pH 7.5). The O₂ exchange rates in the dark were determined before illumination with measuring light (ML). Negative values indicate O₂ uptake. Values ($\mu\text{mol O}_2 [\text{mg Chl}]^{-1} \text{h}^{-1}$) are means \pm standard deviation, $n = 4$.

Table S2. Oligonucleotides used for the disruption of the *pgp*, *glcD1/2*, *gcvT*, *glyk*, *flv2*, and *flv4* genes.

Primer name	Sequence (5'-3')	Primer name	Sequence (5'-3')
<i>Kan^r</i> f	TAGACTGGGCGGTTTTATGG	<i>sll0171</i> up f	ATTCTCTGAGCCGGAGAAATAGG
<i>Kan^r</i> r	ATTCCGAAGCCCAACCTTT	<i>sll0171</i> up r	CCATAAAACCGCCCAGTCTACTGATCCACTAAAGGCTGCAAAA
<i>Cm^r</i> f	GATCGGCACGTAAGAGGTTC	<i>sll0171</i> dn f	AAAGGTTGGGCTTCGGAATCAGAAGTGGGAAAACAACCTCTGG
<i>Cm^r</i> r	AGAAGCACACGGTCACACTG	<i>sll0171</i> dn r	CCCCTAAATCCCACCTAAAAAGG
<i>slr0458</i> up f	GGAACCTACATTCGAGCCTTAGC	<i>slr1840</i> up f	TTTTGATTGCACCAGACTCTTTT
<i>slr0458</i> up r	CCATAAAACCGCCCAGTCTAAGTTGGGGGAGATTTTCGATGTA	<i>slr1840</i> up r	CCATAAAACCGCCCAGTCTAAACCCAATGCCATTAGTAAACCT
<i>slr0458</i> dn f	AAAGGTTGGGCTTCGGAATATTACCGCATTCTAGCCTGCAC	<i>slr1840</i> dn f	AAAGGTTGGGCTTCGGAATTAGTACGGGAGTTTGTATGGCTTT
<i>slr0458</i> dn r	GGGAGACTGATGCAAAAACCTCAC	<i>slr1840</i> dn r	TGTTTCACTGAACCAATCTTTT
<i>sll0404</i> up f	GTGGCTACCTGTTCCGTTGT	<i>sll0219</i> up f	CATTCTGCTCATTCTGGGTGTT
<i>sll0404</i> up r	GAACCTCTTACGTGCCGATCGAAGCTAATCCCAGCACCAA	<i>sll0219</i> up r	CCATAAAACCGCCCAGTCTAGTAGGCAAGGTCATCAACTGGAG
<i>sll0404</i> dn f	CAGTGTGACCGTGTGCTTCTCATCAGTCTTGCCTGCTCA	<i>sll0219</i> dn f	AAAGGTTGGGCTTCGGAATAATCTCGATGCCCTAGATGTTT
<i>sll0404</i> dn r	TGCCCAATATGTTCAACGAA	<i>sll0219</i> dn r	ACAGAAACACCGTTTTGCTCAGT
<i>slr0806</i> up f	CTGGTTTGTCCCGCAATAAT	<i>sll0217</i> up f	GCCATAGAGCCCTCTCGATAGAT
<i>slr0806</i> up r	CCATAAAACCGCCCAGTCTAATTGCCTCTGGTTCTGATCG	<i>sll0217</i> up r	GAACCTCTTACGTGCCGATCGAAATCAGGTTTCGAGTCGTTCTG
<i>slr0806</i> dn f	AAAGGTTGGGCTTCGGAATGTGATCGCCTGGAAGAAATC	<i>sll0217</i> dn f	CAGTGTGACCGTGTGCTTCTCAAGTTATCGGCCTGTTTGAAG
<i>slr0806</i> dn r	AATACACCTCCGGCAAACCTG	<i>sll0217</i> dn r	GAATCCACATTGGTTGAGAGGAG

All sequences are displayed in the 5'-to-3' orientation.

Table S3. Oligonucleotides used for the construction of FLV2 and 4 expression vectors. All sequences are displayed in the 5'-to-3' orientation.

Primer name	Sequence (5'-3')
FLV2-Infusion f	GGTTCGCGTGGATCCATGATTTCTCCAATTGGTGGTCTT
FLV2-Infusion r	GGGAATTCGGGGATCCTAATATTGTCCCCCG
FLV4-Infusion f	GGTTCGCGTGGATCCATGGTTACCCTAATTGATTCT
FLV4-Infusion r	GGGAATTCGGGGATCTTAGTAGTGGTTGCCAGTTT

All sequences are displayed in 5'-3' orientation.

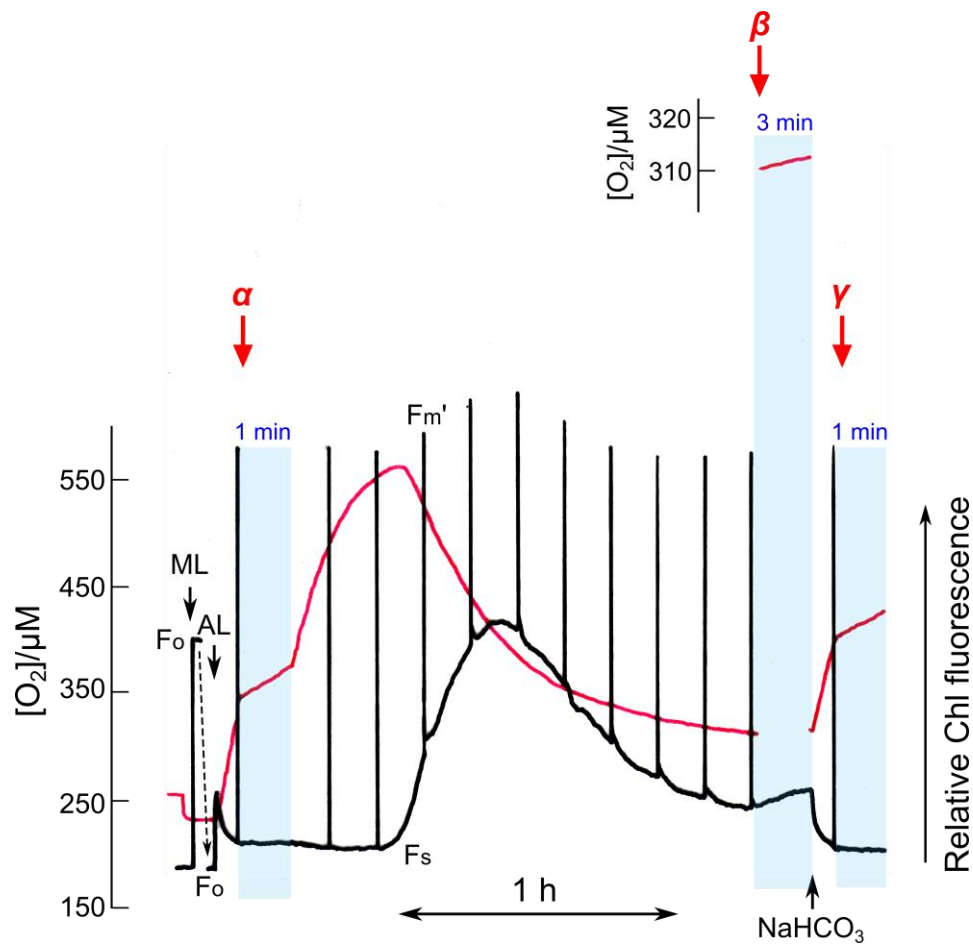


Fig. S1. Determination of O_2 evolution rates during the monitoring of medium $[O_2]$ (red trace) and relative Chl fluorescence (black trace) in the *S. 6803* wild type. A representative experiment is shown. At times α , β , and γ , the top of the O_2 electrode chamber was closed for 1–3 min (indicated by blue shading), and the O_2 evolution rates were determined. Thereafter, the chamber was opened again. For the measurement of the rate at β , the sensitivity of the recording was increased temporarily from 2 V to 500 mV. The medium containing *S. 6803* cells ($10 \mu\text{g Chl mL}^{-1}$) was illuminated with actinic light ($200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) from the indicated starting time (AL). The cells were added to the medium before illumination with measuring light commenced (ML). Further details as in Fig. 1.

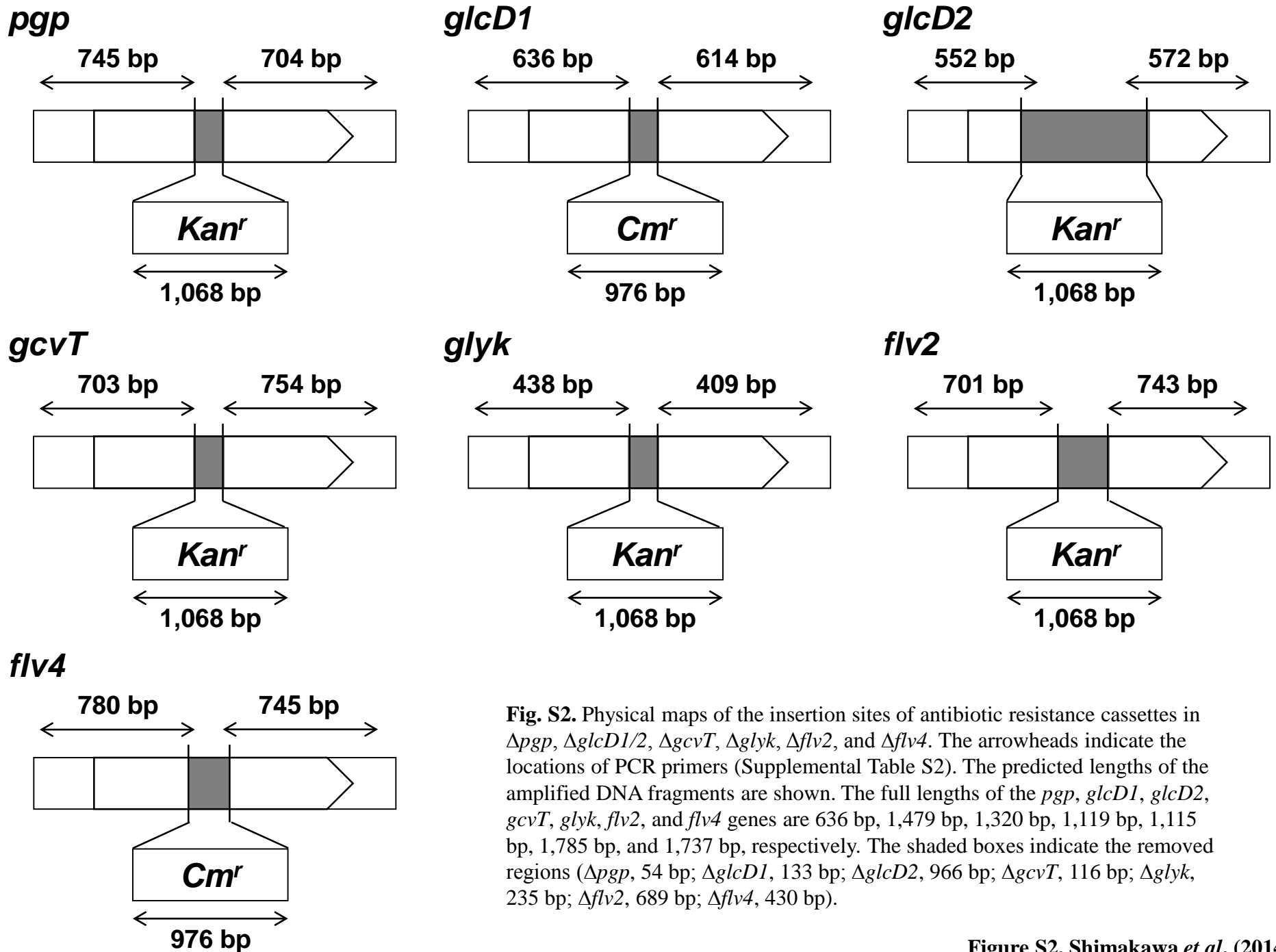


Fig. S2. Physical maps of the insertion sites of antibiotic resistance cassettes in Δpgp , $\Delta glcD1/2$, $\Delta gcvT$, $\Delta glyk$, $\Delta flv2$, and $\Delta flv4$. The arrowheads indicate the locations of PCR primers (Supplemental Table S2). The predicted lengths of the amplified DNA fragments are shown. The full lengths of the *pgp*, *glcD1*, *glcD2*, *gcvT*, *glyk*, *flv2*, and *flv4* genes are 636 bp, 1,479 bp, 1,320 bp, 1,119 bp, 1,115 bp, 1,785 bp, and 1,737 bp, respectively. The shaded boxes indicate the removed regions (Δpgp , 54 bp; $\Delta glcD1$, 133 bp; $\Delta glcD2$, 966 bp; $\Delta gcvT$, 116 bp; $\Delta glyk$, 235 bp; $\Delta flv2$, 689 bp; $\Delta flv4$, 430 bp).

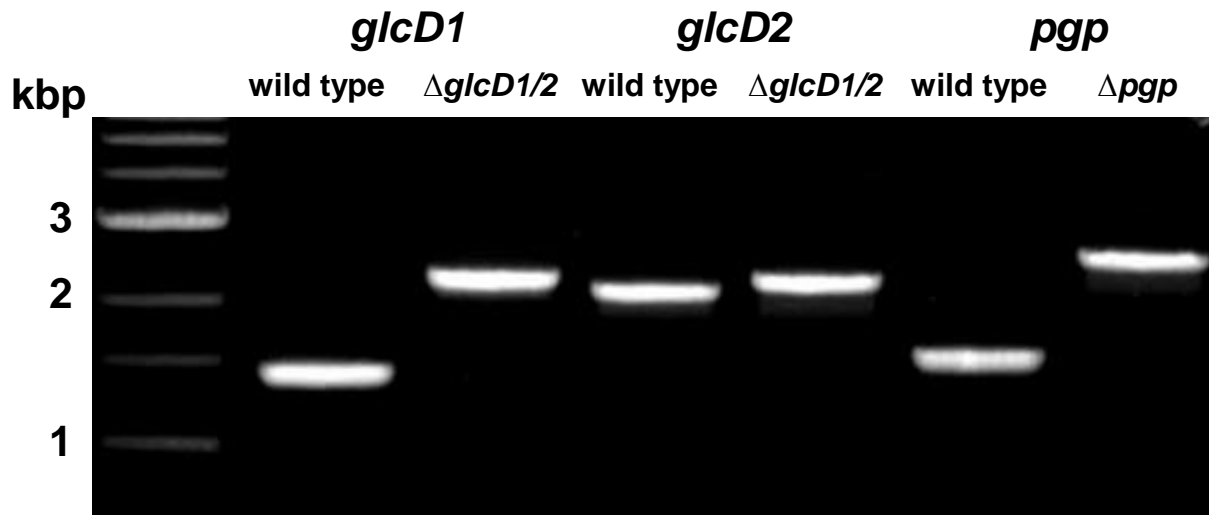
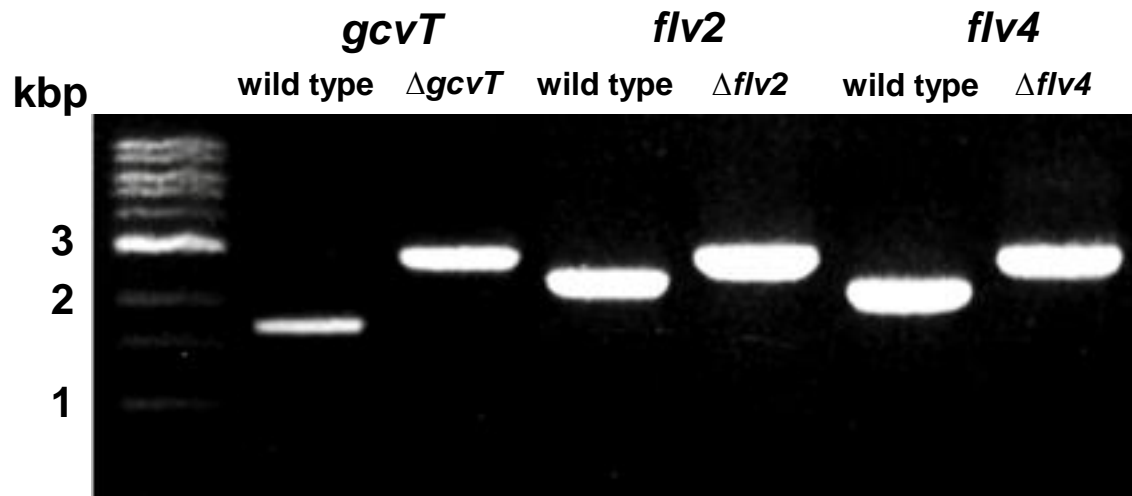
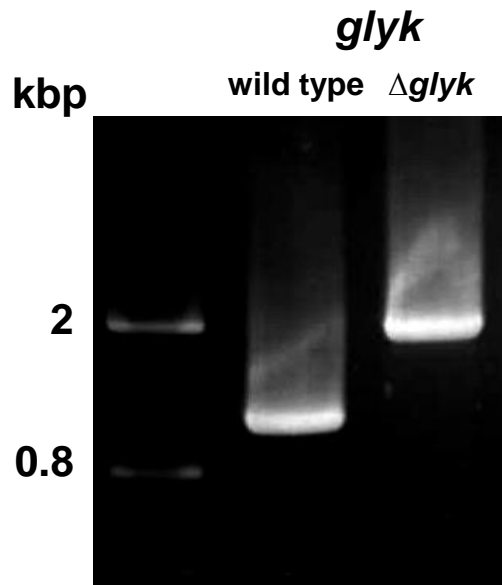
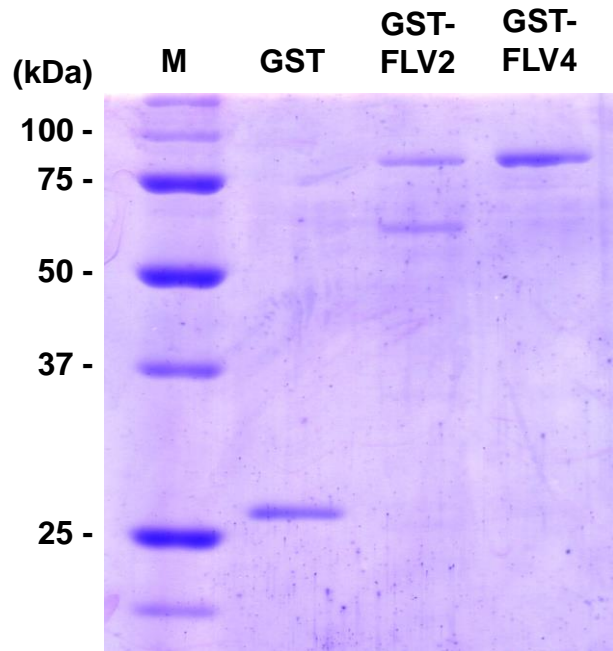


Fig. S3. DNA fragments amplified by PCR showing complete segregation of the inactivated genes, *pgp* (*slr0458*), *glcD1* (*sll0404*), *glcD2* (*slr0806*), *gcvT* (*sll0171*), *glyk* (*slr1840*), *flv2* (*sll0219*), and *flv4* (*sll0217*).

SDS-PAGE



Western blot

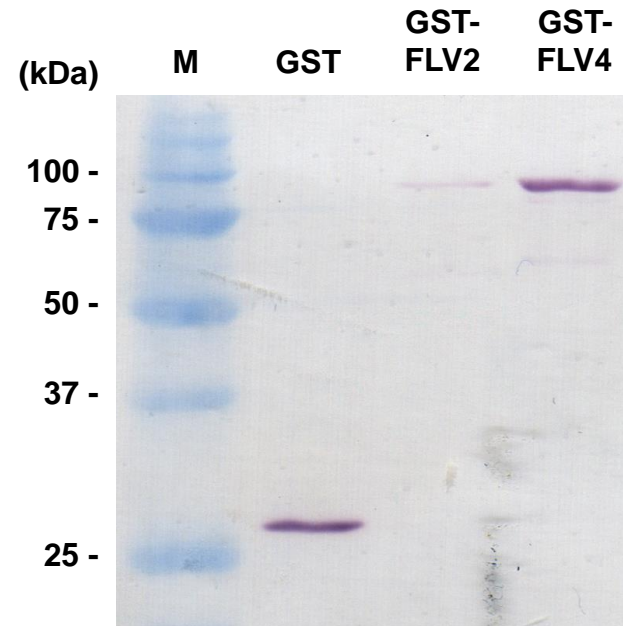


Fig. S4. SDS-PAGE and Western blot of GST, GST-FLV2, and GST-FLV4 proteins. Purified recombinant proteins (0.1 μ g each) were analyzed by SDS-PAGE; the gel was stained with CBB. GST, GST-FLV2, and GST-FLV4 proteins were detected by the GST-antibody in Western-blots. Expected sizes of recombinant proteins are 26 kDa (GST), 92 (= 26 + 66) kDa (GST-FLV2), and 90 (= 26 + 64) kDa (GST-FLV4).

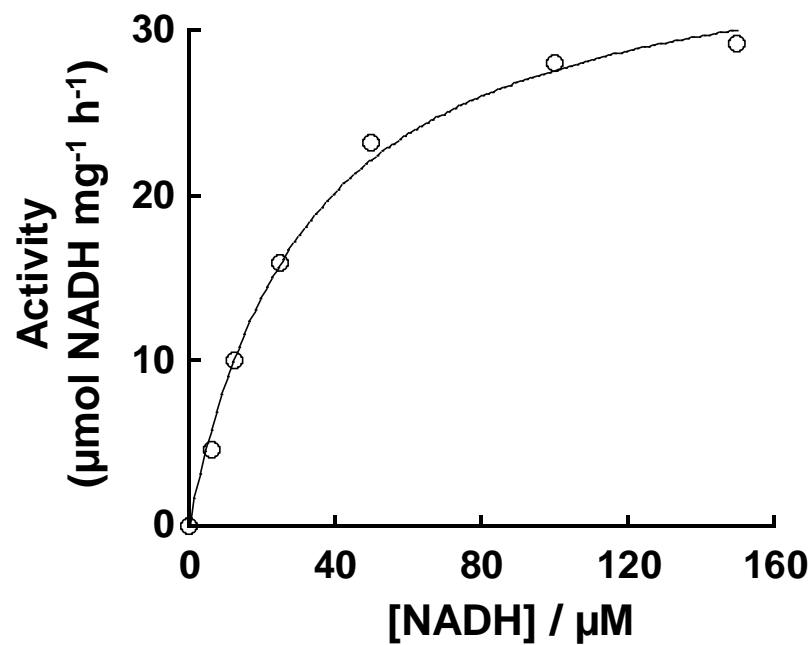


Fig. S5. Dependence of the NADH-oxidation activity on the concentration of NADH in the reaction catalyzed by the recombinant GST-FLV4 protein.

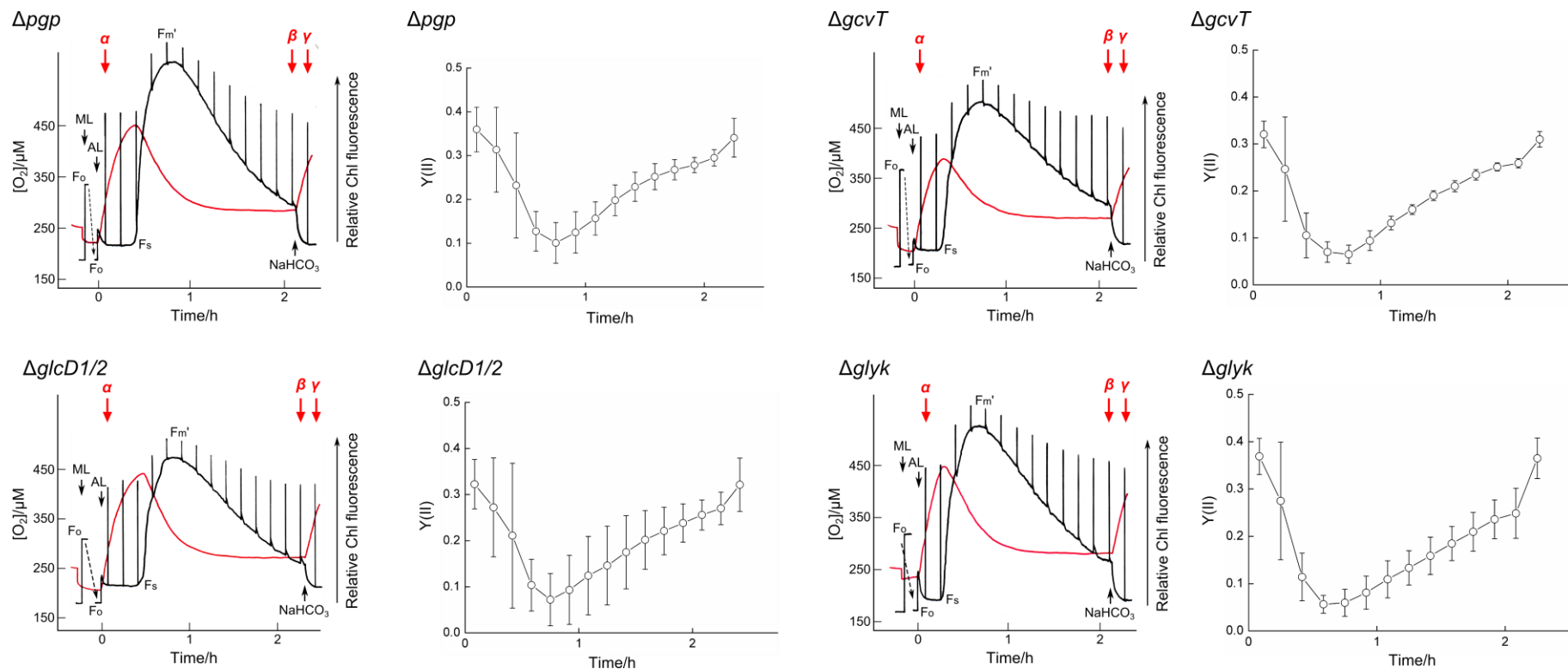


Fig. S6. Development of photosynthetic parameters in media containing four high- $[\text{CO}_2]$ -grown deletion mutants of *S. 6803*, Δpgp (top left), $\Delta glcD1/2$ (bottom left), $\Delta gcvT$ (top right), and $\Delta glyk$ (bottom right). Other details as in Fig. 1.