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Supplemental Data for

Dark accumulation of downstream glycolytic intermediates initiates robust photosynthesis in cyanobacteria

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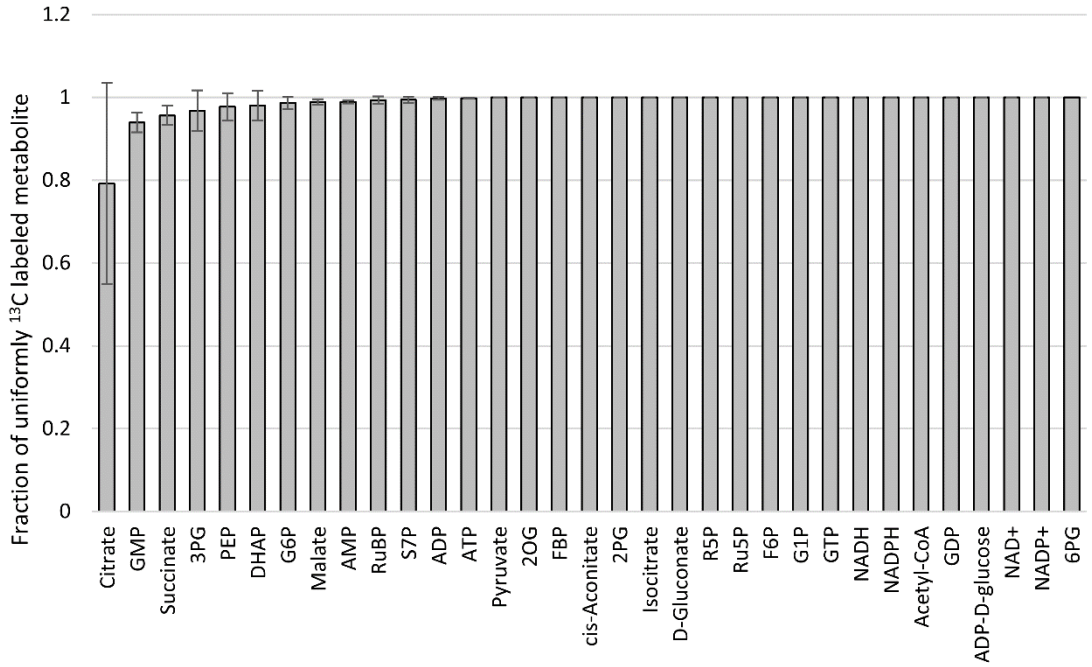
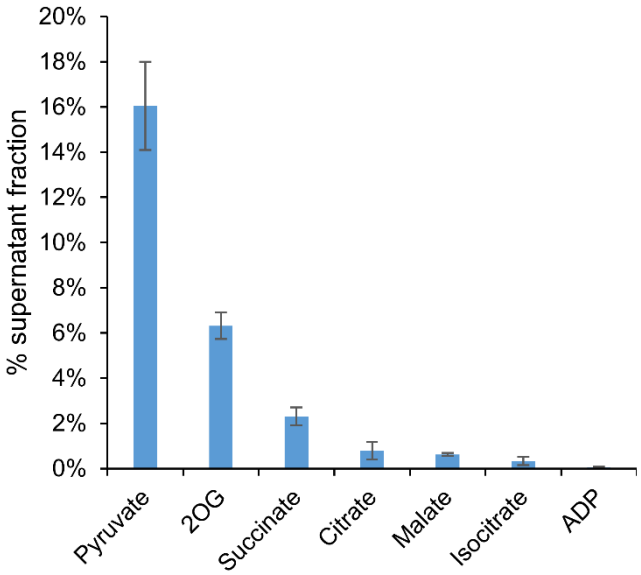
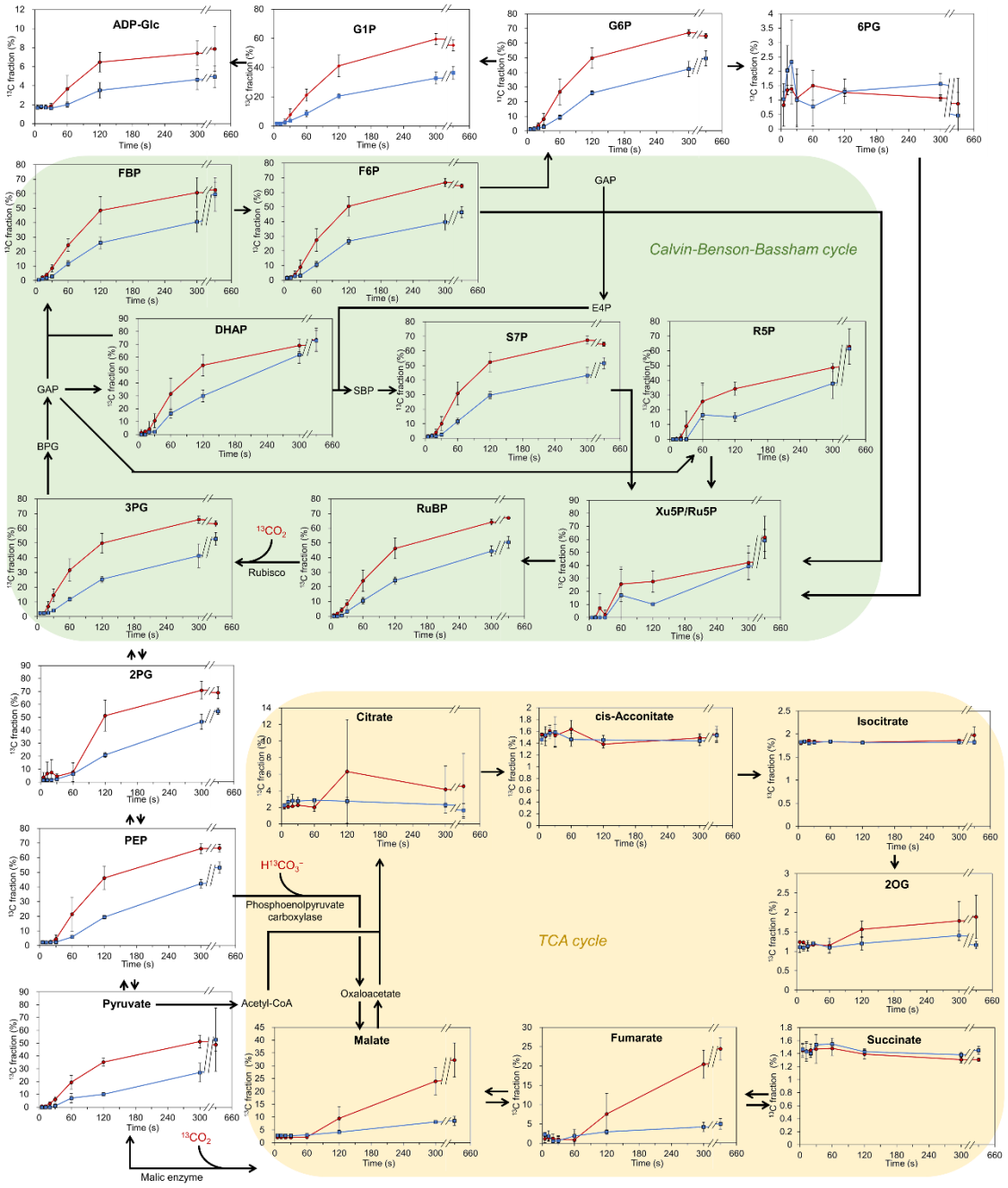


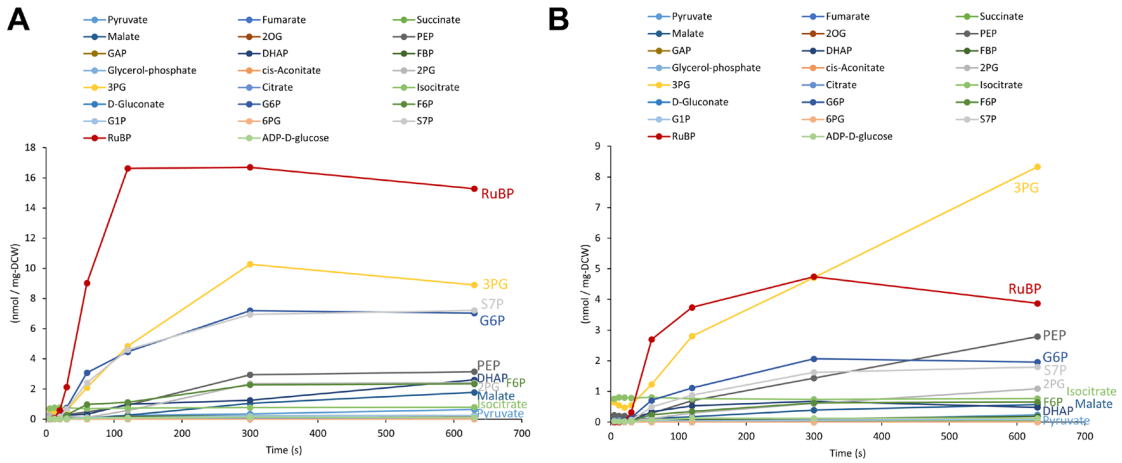
Supplemental Figure S1. Fraction of uniformly labeled metabolite in ^{13}C internal standards. Values are calculated as the ratio of uniformly labeled peak area to sum of peak areas of uniformly labeled and unlabeled signals. Values are the mean ± SD (bars) of six different ^{13}C-internal standards. Abbreviations are the same as in the Figure 2 legend.



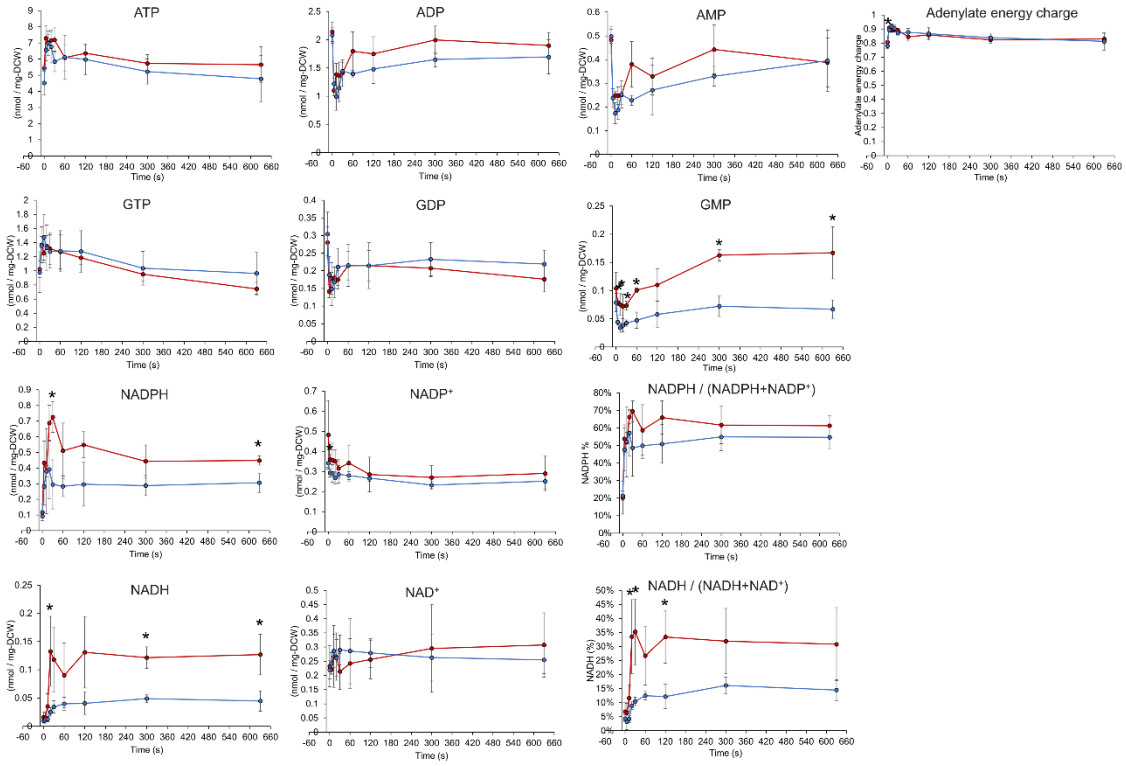
Supplemental Figure S2. Fractions of extracellular metabolites at 2 min after start of illumination with an intensity of 200 µmol m^{-2} s^{-1}. Cell suspensions in 1 mM $NaHCO_3$ are prepared in the same manner as used for absolute concentration measurement. After illumination for 2 min, cell suspensions are centrifuged, followed by harvesting supernatant as the extracellular fraction. After lyophilization, these extracellular metabolite samples are analyzed using capillary electrophoresis-mass spectrometry (CE-MS). The extracellular fractions are determined from the ratio of peak area of extracellular samples to that of the extracted samples. Values are the mean ± SD (bars) of three biological replicates. ADP, Adenosine diphosphate; 2OG, 2-oxoglutarate.



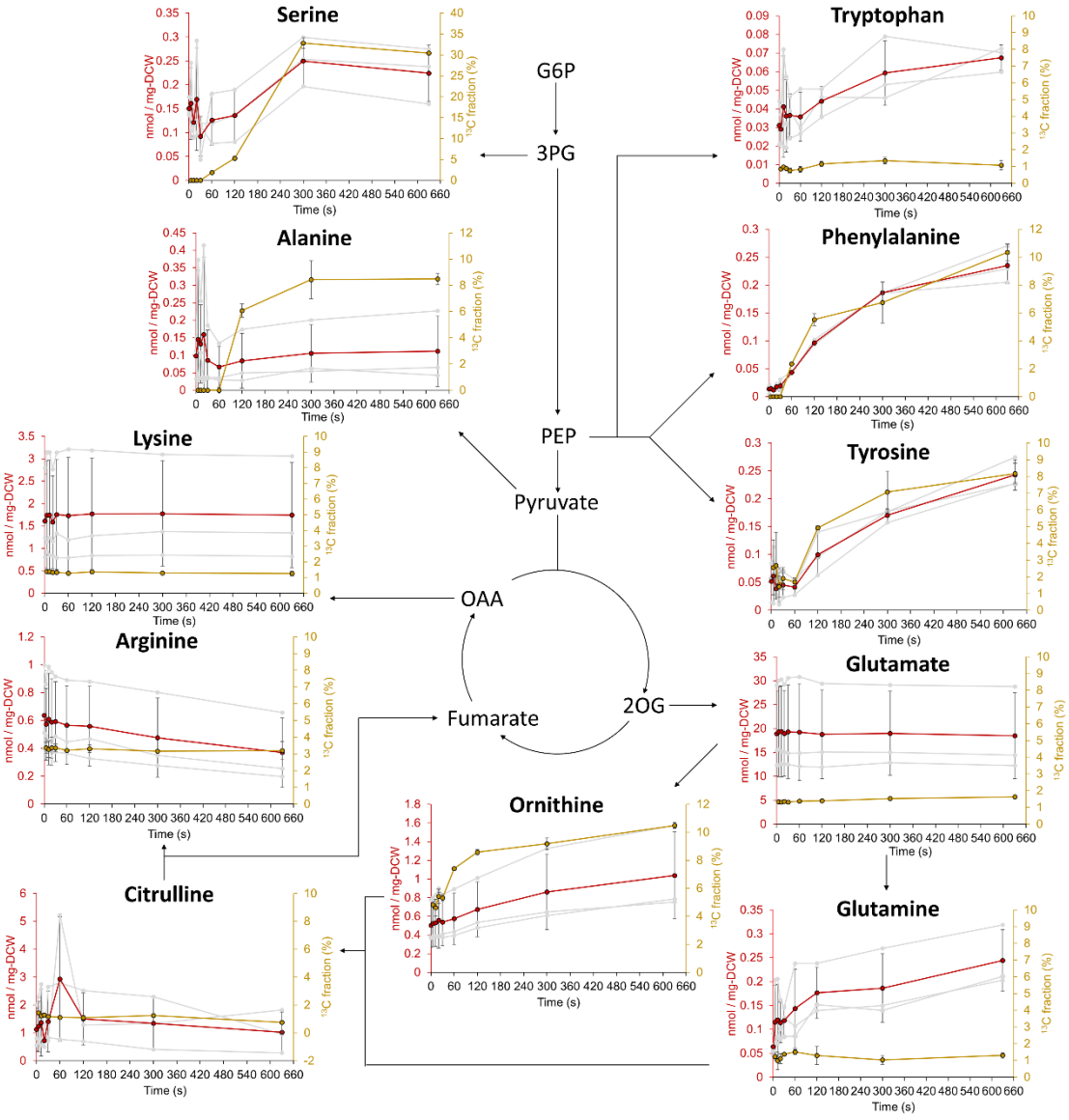
Supplemental Figure S3. Time course ^{13}C metabolite fractions following light irradiation at 200 µmol m^{-2} s^{-1} (red line) or 30 µmol m^{-2} s^{-1} (blue line). Values are the mean ± SD (bars) of three biological replicates. Black arrows indicate metabolic pathways. Abbreviations are the same as in the Figure 2 legend.



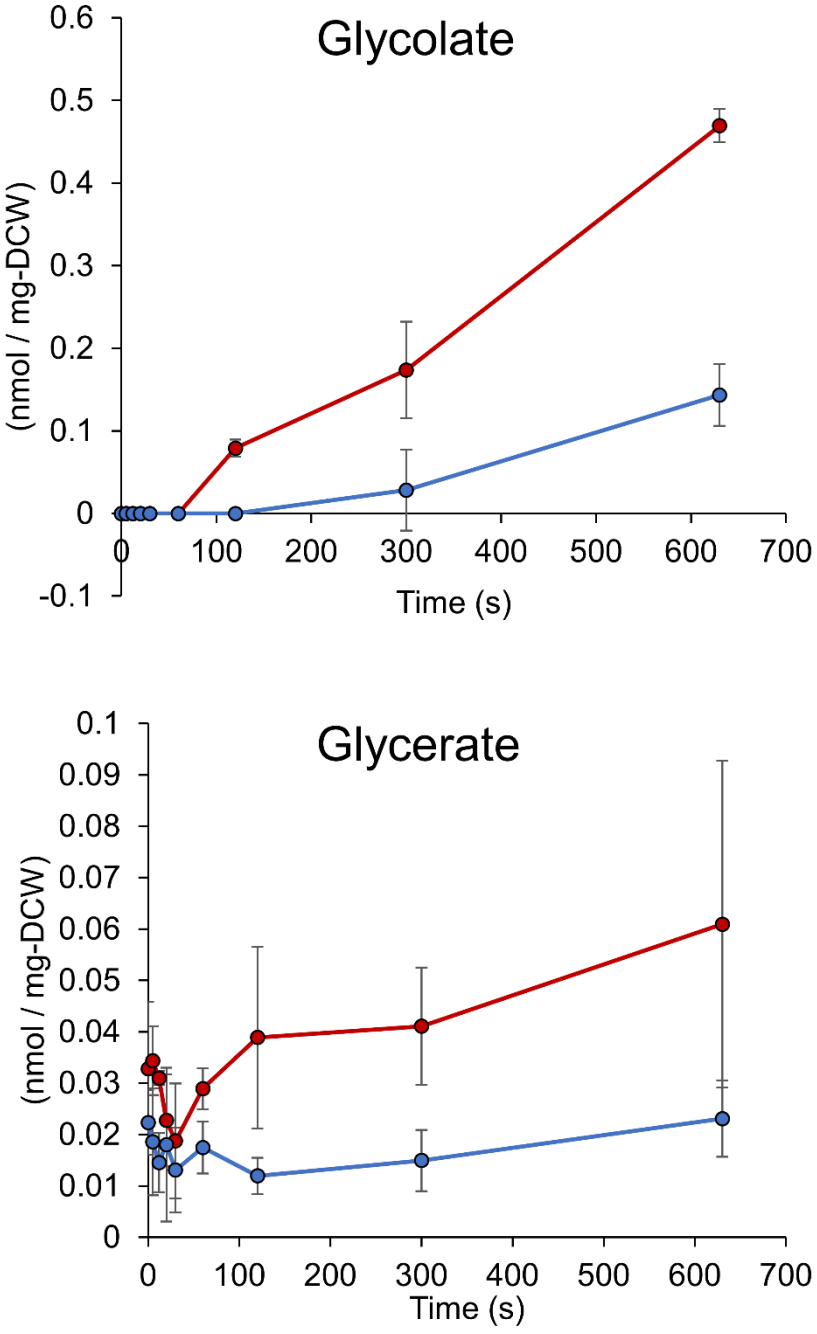
Supplemental Figure S4. Time course of ^{13}C level in each metabolite. Number of ^{13}C atoms is calculated as the mean of ^{13}C enrichment multiplied by mean metabolite concentration and the number of C atoms in the molecule for the two light intensities: (A) 200 µmol m^{-2} s^{-1}, (B) 30 µmol m^{-2} s^{-1}. Abbreviations are the same as in the Figure 2 legend.



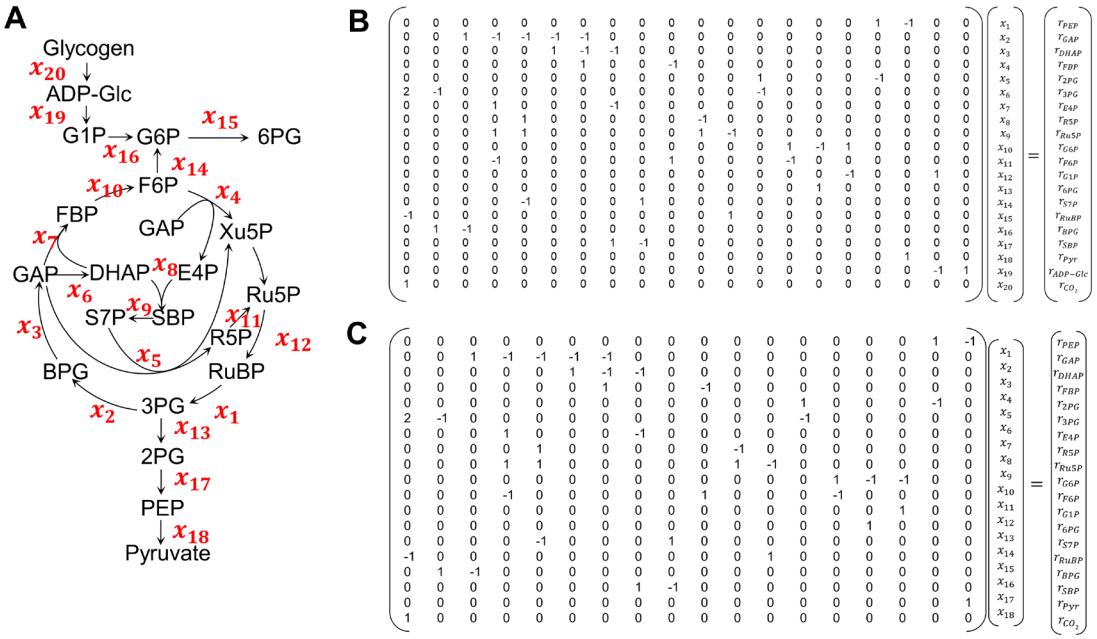
Supplemental Figure S5. Concentration transients of cofactors during photosynthetic induction. Intracellular metabolites are extracted at the indicated times after transition from darkness to light-irradiated conditions at the intensity of 200 μmol m^{-2} s^{-1} (red line) or 30 μmol m^{-2} s^{-1} (blue line). Values are the mean ± SD (bars) of three independent experiments. Significant differences between the two light intensities were evaluated by a two-tailed non-paired Student's t test (* $p < 0.05$). Abbreviations: ADP, Adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; GDP, Guanosine diphosphate; GMP, Guanosine monophosphate; GTP, Guanosine triphosphate; NAD(P), nicotinamide adenine dinucleotide (phosphate).



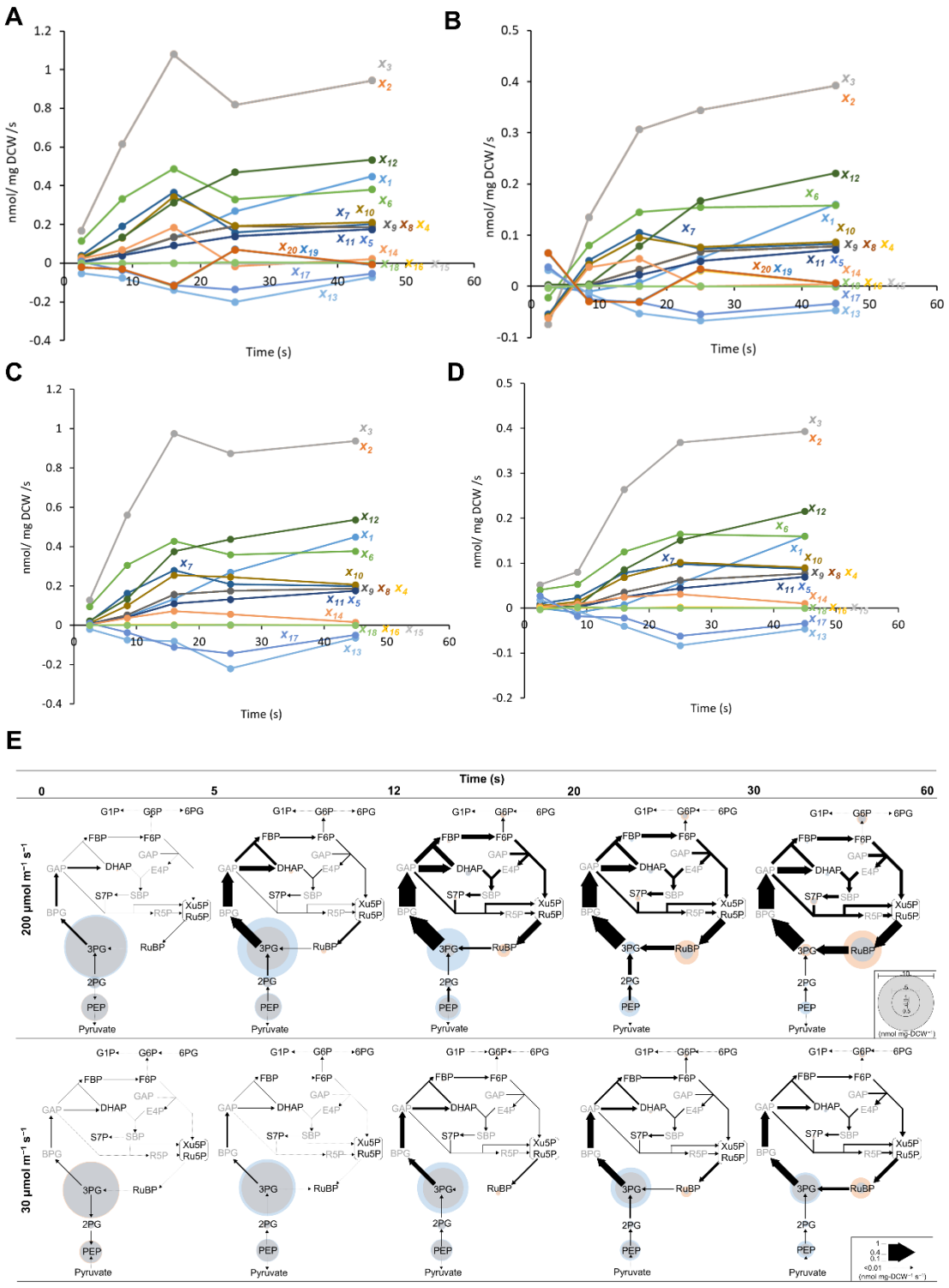
Supplemental Figure S6. Concentration and ^{13}C fraction transients of amino acids during photosynthetic induction of cells illuminated at an intensity of 200 µmol m^{-2} s^{-1}. Intracellular metabolites are extracted at the indicated times after transition from darkness to light-irradiated conditions at an intensity of 200 µmol m^{-2} s^{-1}. Red lines and yellow lines indicate metabolite concentration and ^{13}C fraction, respectively. Simplified central metabolic pathways are indicated by arrows. Values of concentrations and ^{13}C fractions are the mean ± SD (bars) of three and two independent experiments, respectively. Values of metabolite concentrations for individual experiments are indicated by gray lines. Abbreviations are the same as in the Figure 2 legend.



Supplemental Figure S7. Concentration transients of intermediates in photorespiration pathway during photosynthetic induction. Intracellular metabolites are extracted at the indicated time after transition from darkness to light-irradiated conditions at intensities of 200 µmol m^{-2} s^{-1} (red line) or 30 µmol m^{-2} s^{-1} (blue line). Values are the mean ± SD (bars) of three independent experiments. DCW, dry cell weight.

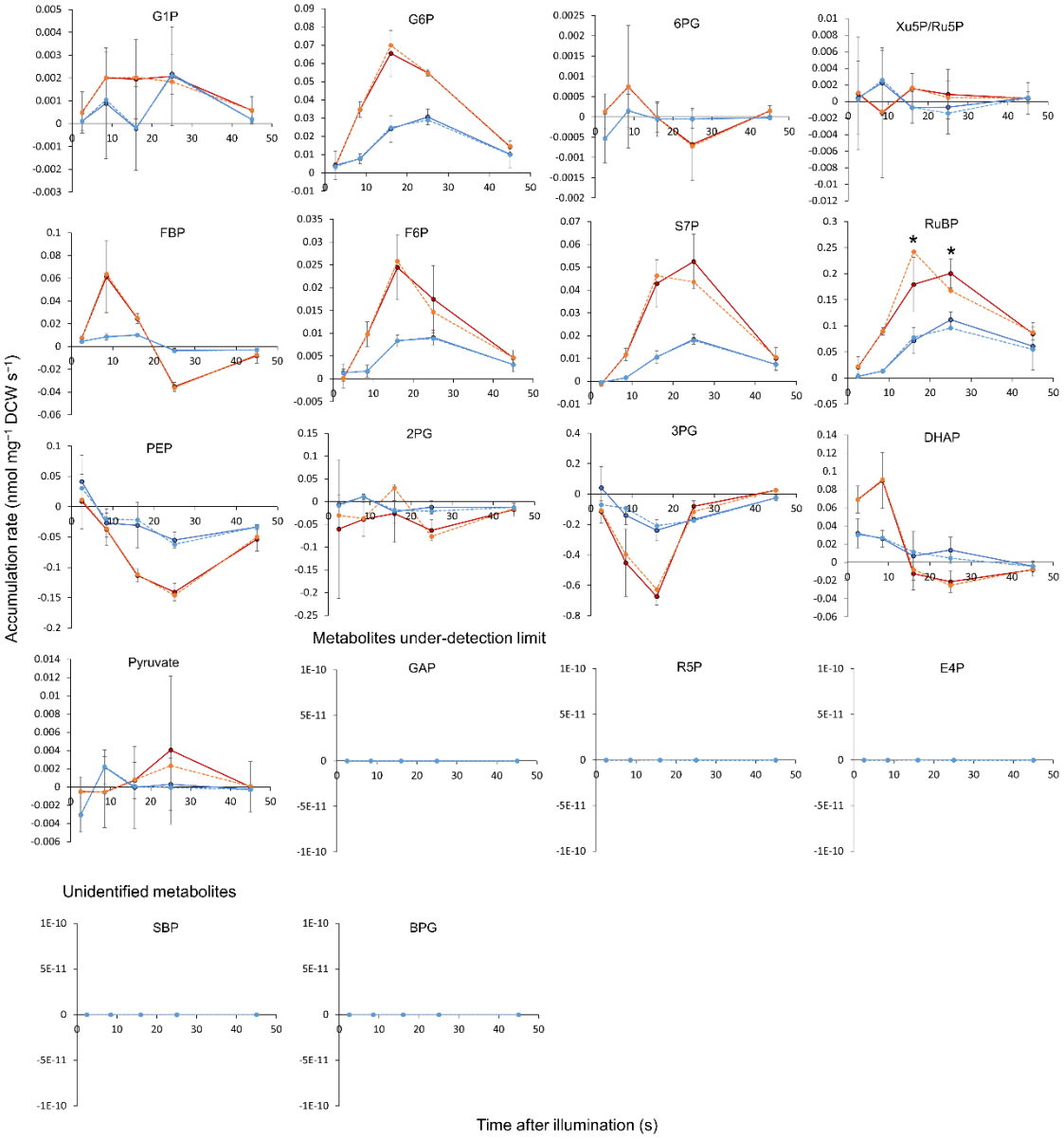


Supplemental Figure S8. Short-term dynamic metabolic flux analysis (STD-MFA). (A) Metabolic pathways included in STD-MFA calculations. The experimental data of absolute concentration and ^{13}C incorporation change allow us to simplify the metabolic model during photosynthetic induction. x_i represents net flux of *i*th metabolic pathway. (B, C) Detailed form of the mass balance equation used for STD-MFA (B) with and (C) without glycogen degradation pathways. For more details, see Methods section.

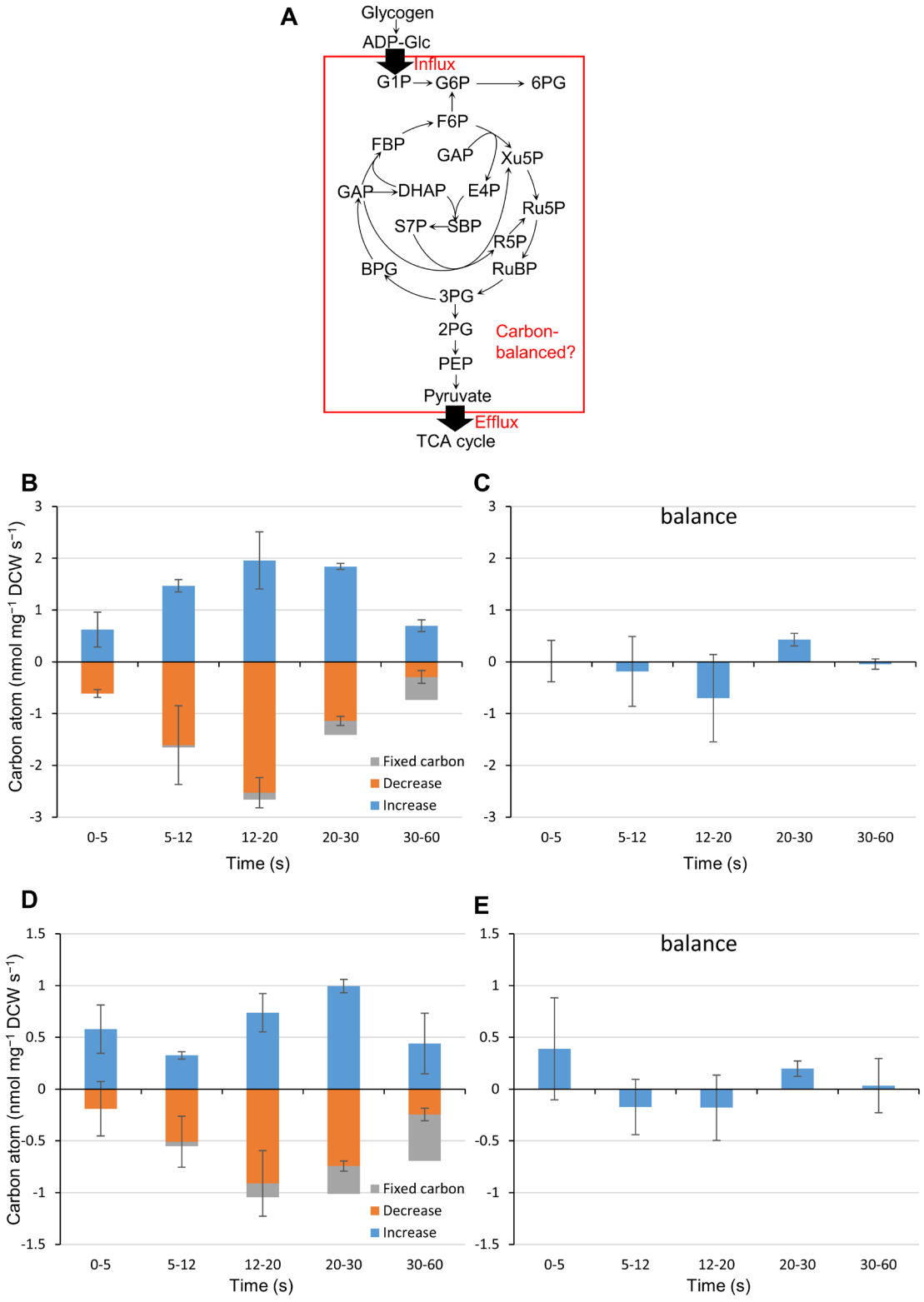


Supplemental Figure S9. Estimated metabolic flux changes during the initiation of photosynthesis. Transients of estimated metabolic fluxes during photosynthetic induction in illuminated conditions at (A, C) 200 µmol m^{-2} s^{-1} or (B, D) 30 µmol m^{-2} s^{-1}. Metabolic fluxes are

estimated using metabolic system (A, B) with or (C, D) without glycogen degradation pathways. The subscript numbers of x correspond to metabolic flux shown in Supplementary Figure 8A. Since the fluxes are estimated using metabolite accumulation rates derived from two consecutive concentration data points, flux values are plotted at a time point corresponding to the average (mean) of the two time points. (E) Metabolic fluxes estimated using a metabolic system without glycogen degradation pathways (Supplementary Figure 8C) are indicated by thickness of arrows. Metabolite concentrations are shown in the same way as Figure 4. Abbreviations are the same as in the Figure 2 legend.

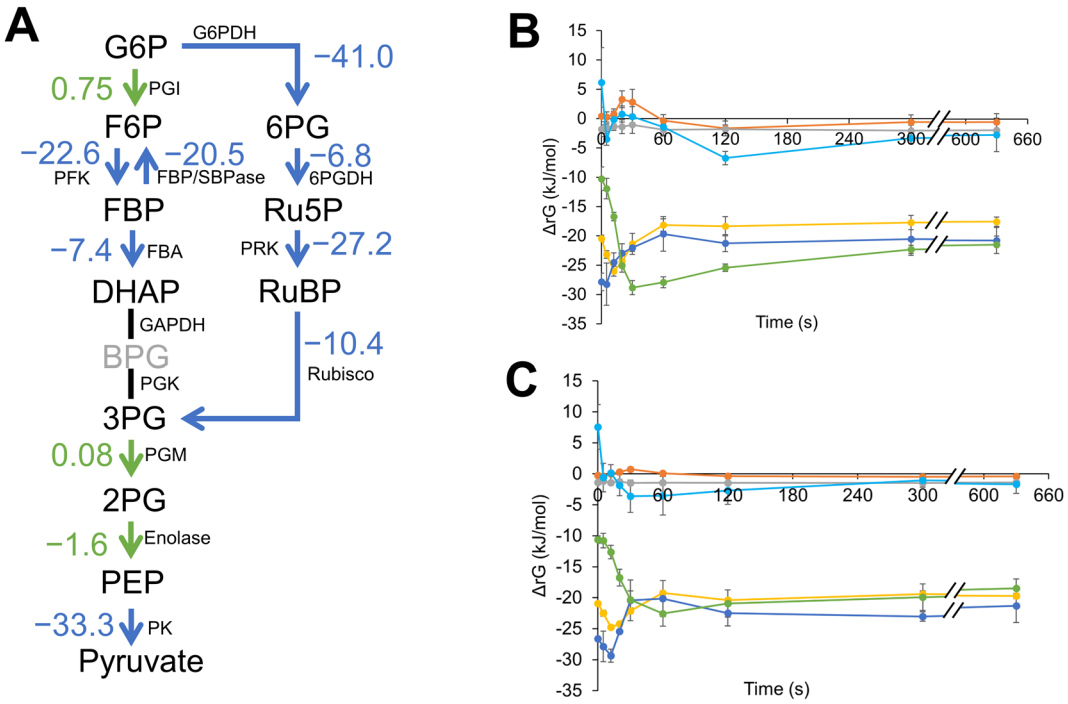


Supplemental Figure S10. Measured and estimated metabolic accumulation rates. Measured accumulation rates at light intensities of 200 µmol m^{-2} s^{-1} (red solid line) or 30 µmol m^{-2} s^{-1} (blue solid line) are derived from slope between two consecutive concentration data points shown in Fig. 2. Estimated accumulation rates at light intensities of 200 µmol m^{-2} s^{-1} (orange dotted line) or 30 µmol m^{-2} s^{-1} (blue dotted line) are shown for validation of the short-term dynamic metabolic flux analysis (STD-MFA) results in Supplemental Figure 9E. Abbreviations are the same as in the Figure 2 legend. Values are plotted at the average (mean) time point of the two time points used for the concentration data points. Values of measured accumulation rates are the mean ± SD (bars) of three independent experiments. Asterisks indicate that the estimated value is out of range of the measured mean ± SD.

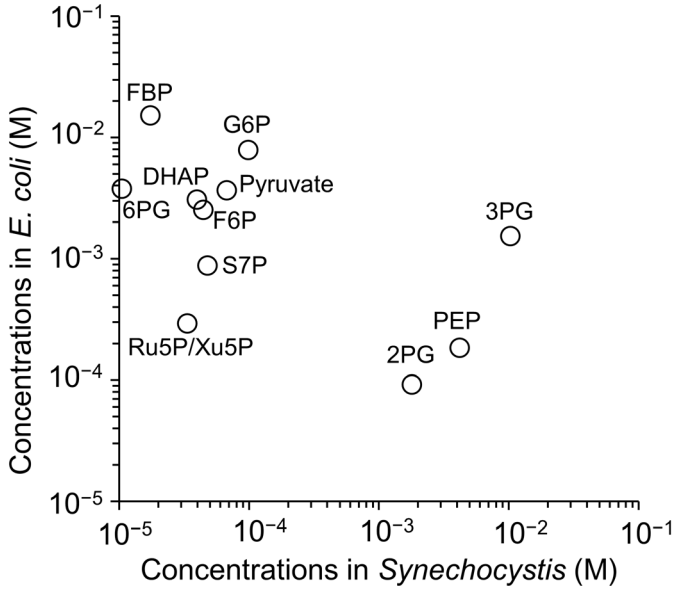


Supplemental Figure S11. Carbon mass balance among the metabolites shown in the red frame of (A) are calculated (B-D). Rates of carbon atomic changes at light intensities of 200 μmol m^{-2} s^{-1} (B)

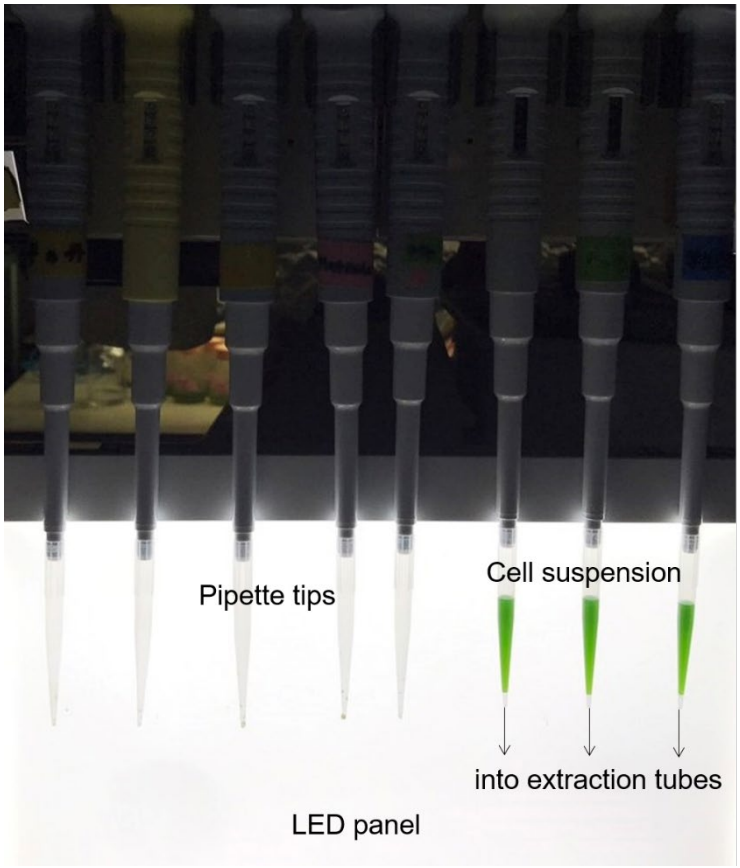
and 30 μmol m^{-2} s^{-1} (D) are summed up, and shown in (C) and (D), respectively. Values are the mean ± SD (bars) of three independent experiments.



Supplemental Figure S12. Free energy analysis. (A) Reaction Gibbs free energy change ($\Delta_r G$) values of glycolysis and pentose phosphate pathway in dark conditions are calculated from the absolute concentrations, and shown in the metabolic map. Blue and green arrows indicate $\Delta_r G$ is below −5, between −5 and 5, respectively. (B,C) Time course of $\Delta_r G$ at the illuminated light intensities of 200 µmol m^{-2} s^{-1} (B) and 30 µmol m^{-2} s^{-1} (C). $\Delta_r G$ values of reactions (3PG to 2PG: orange line, FBP to F6P: yellow line, Ru5P to RuBP: blue line, RuBP to 3PG: green line, 2PG to PEP: gray line, DHAP to FBP: light blue) were derived based on equation (2). Values are the mean ± SD (bars) derived from three independent experiments.



Supplemental Figure S13. Comparison of absolute metabolite concentrations between *Escherichia coli* and *Synechocystis*. Metabolite concentrations of *E. coli* are derived from Park et al., (2016). Metabolite concentrations of *Synechocystis* are the mean of six dark samples (Fig. 2, t = 0).



Supplemental Figure S14. Rapid sampling system used in this study. Setting the cell suspensions in pipette tips prior to light irradiation permits rapid sampling during photosynthetic induction into extraction tubes containing phenol-chloroform-isoamyl alcohol (PCI). LED, Light emitting diode.

Supplemental Table S1. Standard reaction Gibbs energy change from eQuilibrator database.

From	To	$\Delta_r G^{\circ}$ (pH 7.5)
FBP -->	F6P+Pi	-11.8 ± 1.4
2 DHAP-->	FBP	-17.5 ± 0.6
3PG+NADPH+ATP-->	DHAP+NADP+ADP+Pi	12.8 ± 1.3
3PG -->	2PG	4.5 ± 0.7
2PG -->	PEP	-3.8 ± 0.6
ATP+Ru5P-->	ADP+RuBP	-28.6 ± 4.5
H_2O+CO_2+RuBP-->	2 3PG	-26.4 ± 7.4 (pH 7)