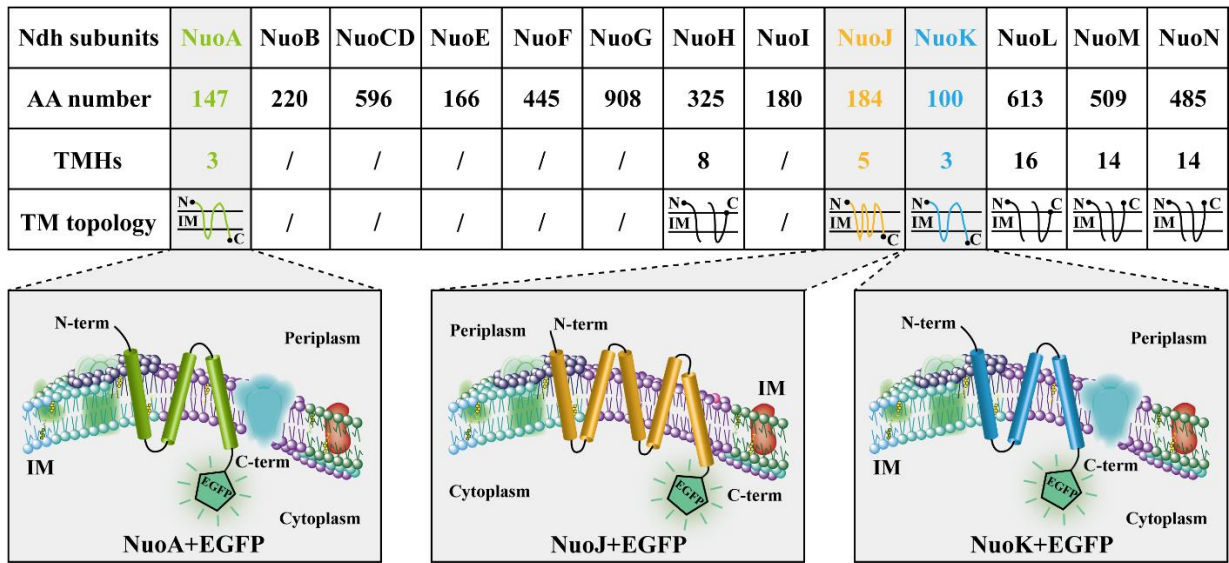
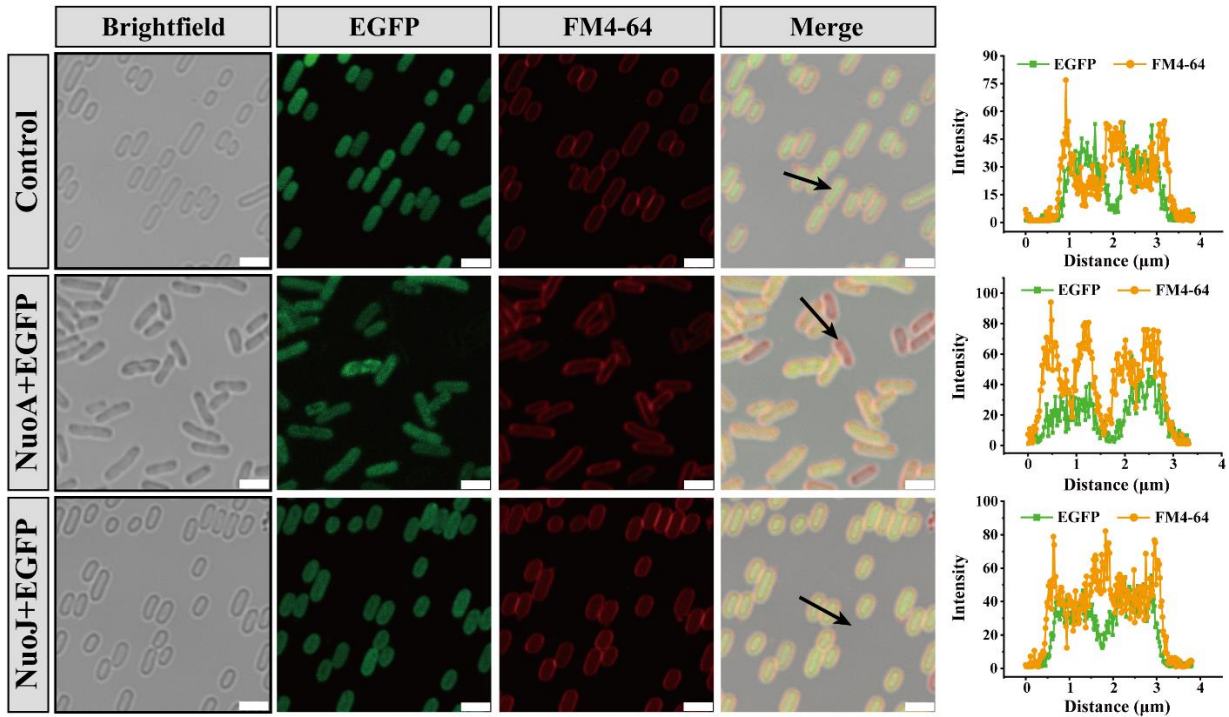


**A new-to-nature photosynthesis system enhances utilization of
one-carbon substrates in *Escherichia coli***

Tong *et al.*

a**b**

Supplementary Figure 1. Analysis and screening of anchor proteins. a, The number of transmembrane helical domains and transmembrane topology of 13 subunits (NuoA~N) in NADH dehydrogenase complex. Relevant information was obtained from online site: <http://www.cbs.dtu.dk/services/TMHMM/>. Schematic diagram of fusion expression of NuoA, NuoJ, and NuoK with EGFP, respectively. Abbreviation: Ndh, NADH dehydrogenase; AA, amino acid; TMHs, transmembrane helices; TM, transmembrane; N or N-term, N-terminal; C or C-term, C-terminal; IM, inner membrane; EGFP, enhanced green fluorescent protein. **b**, Fluorescence microscope images and pixel intensity analysis of EGFP (control), NuoA+EGFP, and NuoJ+EGFP fusion proteins in strains TL-1, TL-2, and TL-3, respectively. The white scale bar at the bottom right of microscope image represented 2.5 μ m. The pixel intensity data were collected by laser confocal microscopy software LAS AF Lite along the track of black solid arrow in microscope image. All experiments were repeated independently for three times with similar results (**b**). Source data are provided as a Source Data file.

a

Protein	Organism	AA number	TMHs	TM topology
crePsbA	<i>Chlamydomonas reinhardtii</i>	1-352	5	
PscA	<i>Chlorobaculum tepidum</i>	1-731	11	
PufL	<i>Rhodopseudomonas viridis</i>	1-274	5	
soePsbA	<i>Spinach</i>	1-353	5	

b

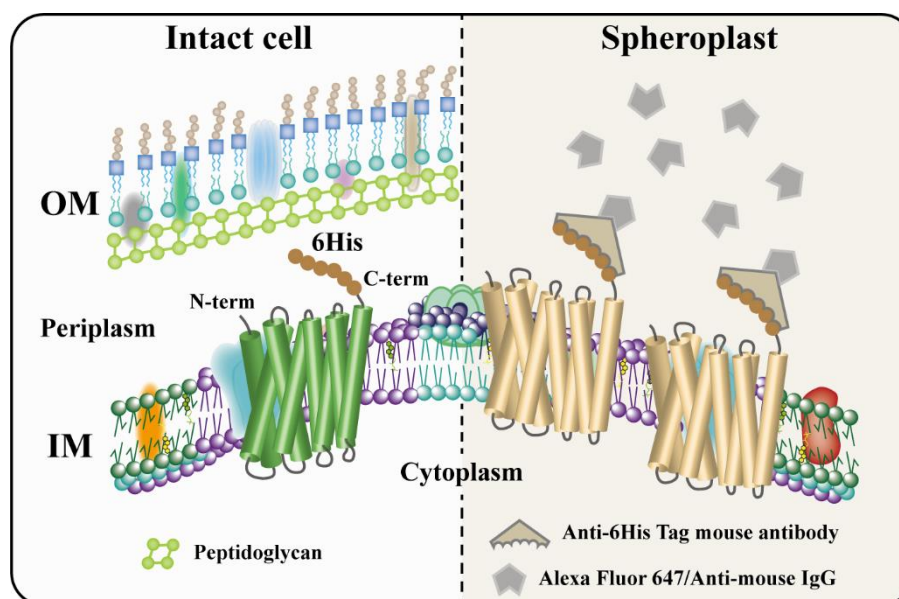
Protein	NuoK+crePsbA+6His	NuoK+PufL+6His	NuoK+soePsbA+6His
TMHs	3+5	3+5	3+5
TM topology			

NuoK+crePsbA+6His

NuoK+PufL+6His

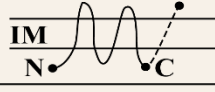
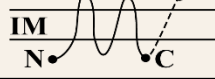
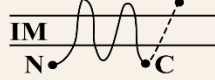
NuoK+soePsbA+6His

Supplementary Figure 2. Analysis of four core proteins. **a**, The number of transmembrane helical domains and transmembrane topology of four core proteins (crePsbA, PscA, PufL, and soePsbA) in photosynthesis reaction center. Relevant information was obtained from online site: <http://www.cbs.dtu.dk/services/TMHMM/>. **b**, Schematic diagram of adding His-tag to the C-terminus localized on periplasmic side of backbone proteins NuoK+crePsbA, NuoK+PufL, and NuoK+soePsbA, respectively. Abbreviation: AA, amino acid; TMHs, transmembrane helices; TM, transmembrane; N or N-term, N-terminal; C or C-term, C-terminal; IM, inner membrane; 6His, His-tag.

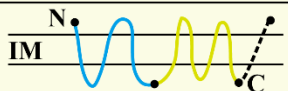
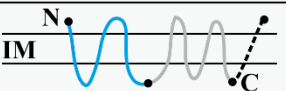
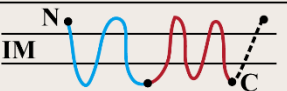


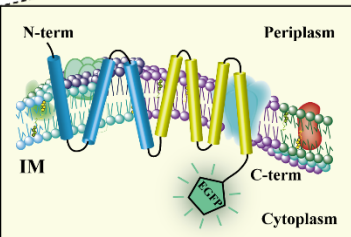
Supplementary Figure 3. The *E. coli* cells were treated through spheroplast¹ and stained with immunofluorescent probes Alexa Fluor 647. The Anti-6His Tag mouse antibody and Alexa Fluor 647/Anti-mouse IgG antibody were purchased from Sangon Biotech and diluted at 1:5000 and 1:1000, respectively. Abbreviation: OM, outer membrane; IM, inner membrane; N-term, N-terminal; C-term, C-terminal; 6His, His-tag.

a

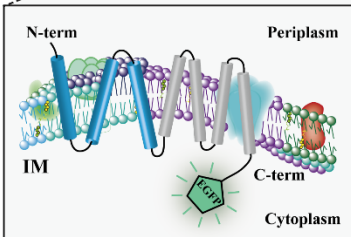
Protein	Organism	AA number	TMHs	TM topology
crePsbA*	<i>Chlamydomonas reinhardtii</i>	1-247	4	
PufL*	<i>Rhodospseudomonas viridis</i>	1-215	4	
soePsbA*	<i>Spinach</i>	1-247	4	

b

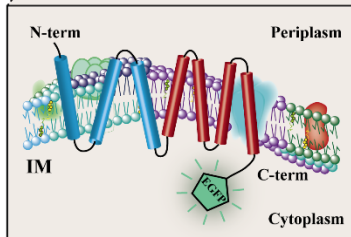
Protein	NuoK+crePsbA*+EGFP	NuoK+PufL*+EGFP	NuoK+soePsbA*+EGFP
TMHs	3+4	3+4	3+4
TM topology			



NuoK+crePsbA*+EGFP

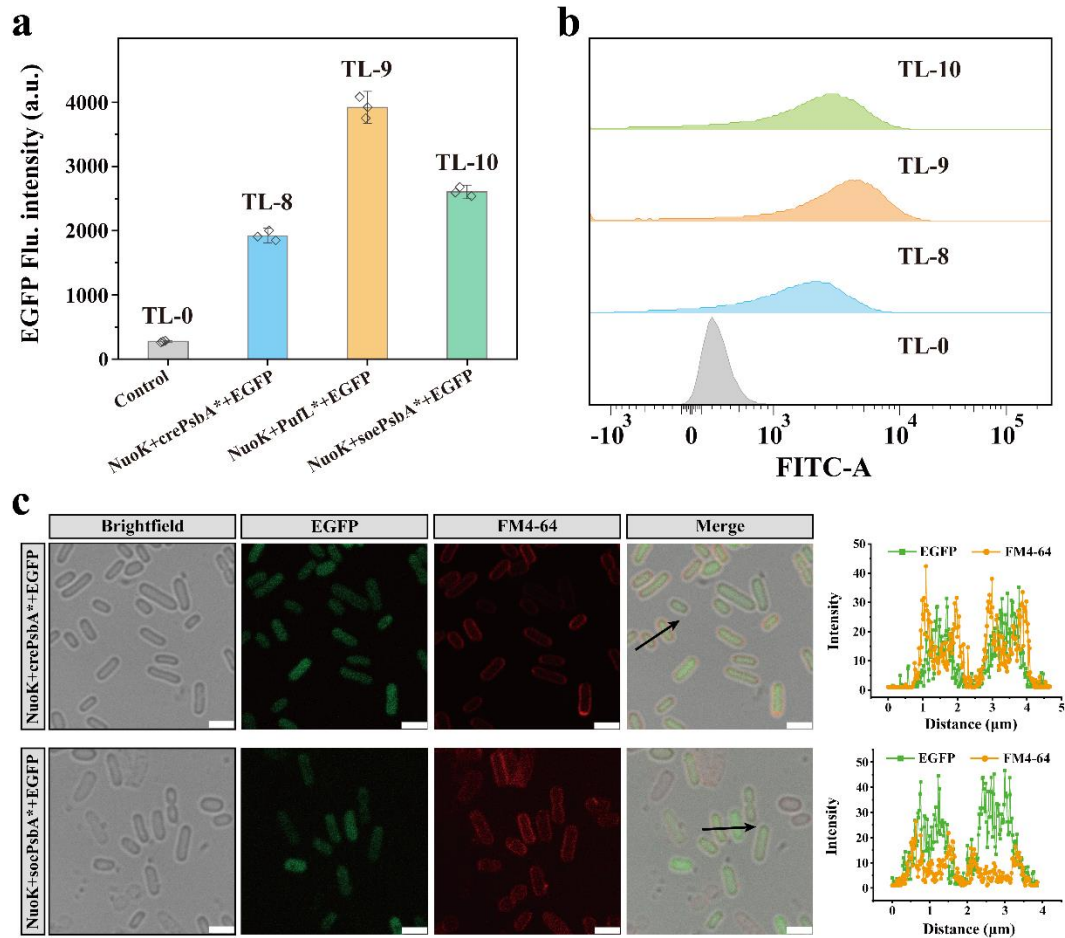


NuoK+PufL*+EGFP

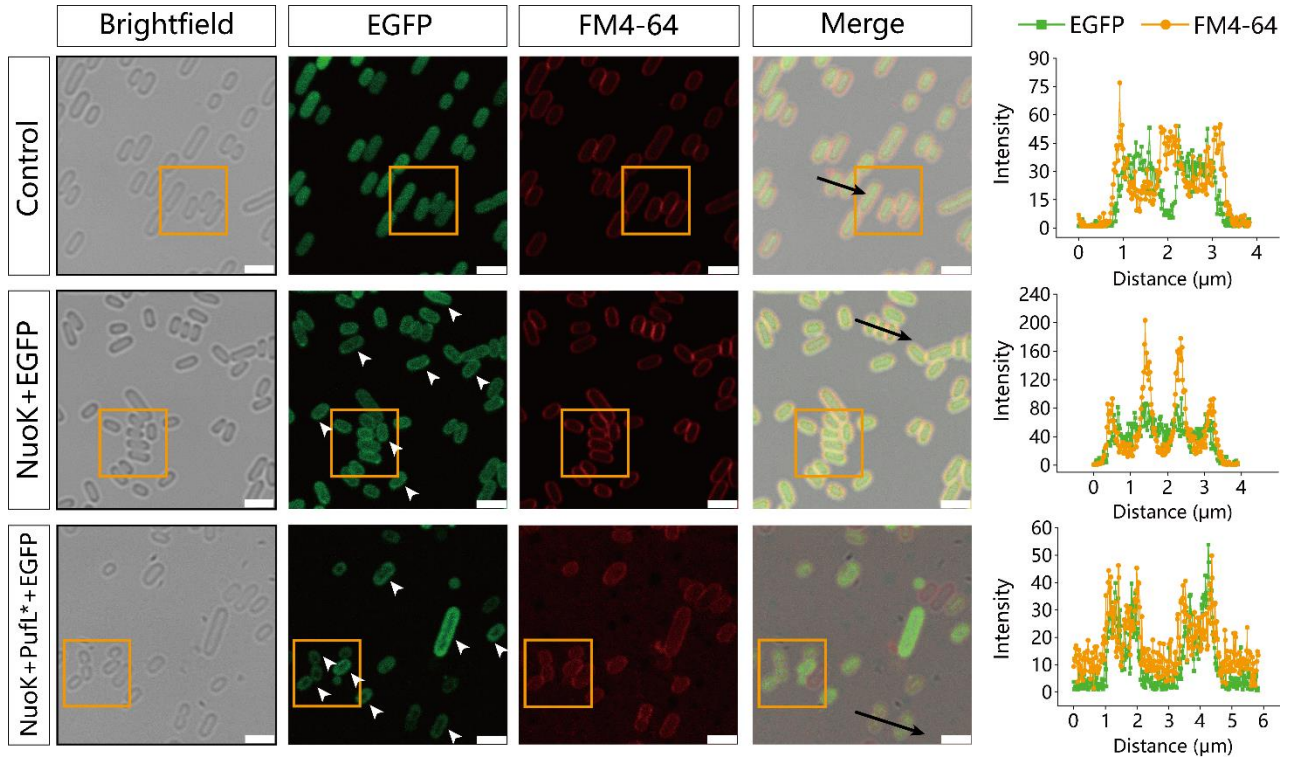


NuoK+soePsbA*+EGFP

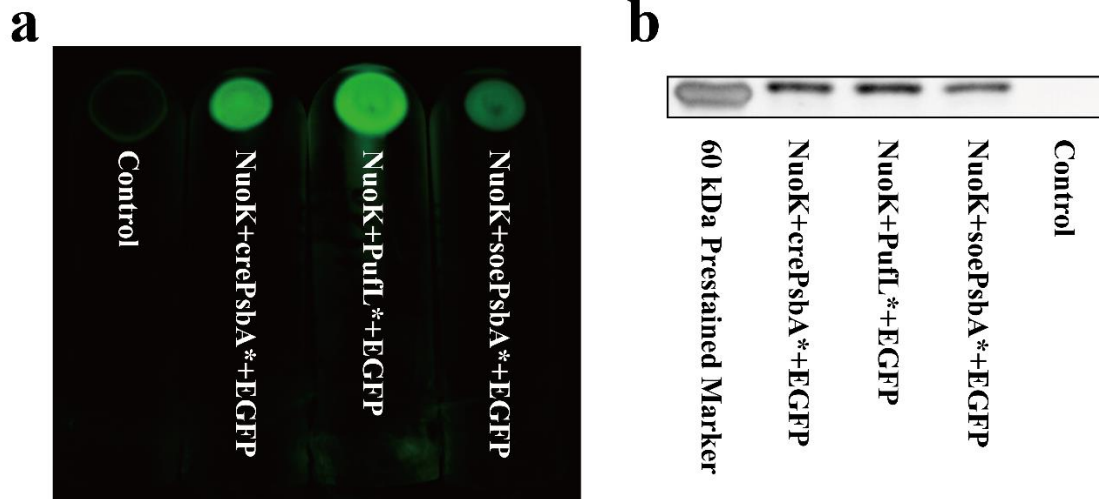
Supplementary Figure 4. Analysis of four core protein mutants. a, The number of transmembrane helical domains and transmembrane topology of three variant core proteins from photosynthesis reaction center. The fifth transmembrane α -helical domains of crePsbA, PufL, and soePsbA were truncated to obtain variant crePsbA*, PufL*, and soePsbA*, respectively. **b,** Schematic diagram of adding EGFP-tag to the C-terminus localized on the cytoplasmic side of backbone proteins NuoK+crePsbA*, NuoK+PufL*, and NuoK+soePsbA*, respectively. The black dashed lines in TM topology represented truncated transmembrane α -helical domains. Abbreviation: AA, amino acid; TMHs, transmembrane helices; TM, transmembrane; N or N-term, N-terminal; C or C-term, C-terminal; IM, inner membrane; EGFP, enhanced green fluorescent protein.



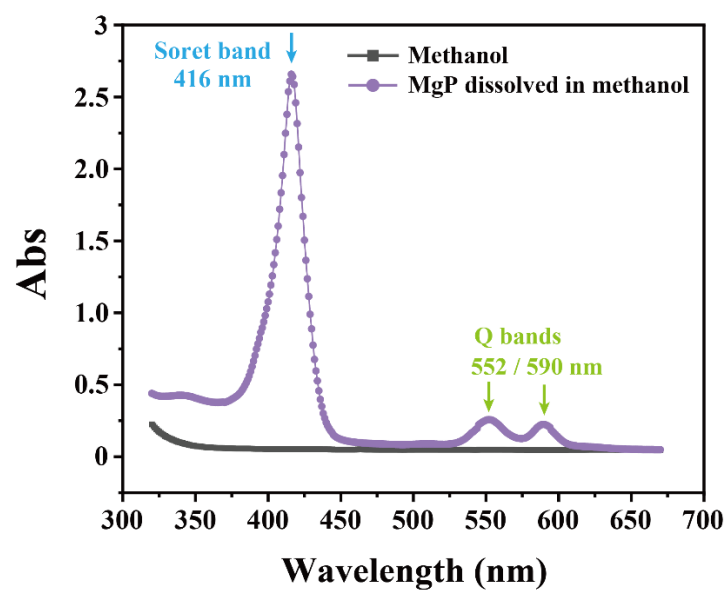
Supplementary Figure 5. Detection of EGFP fluorescence intensity for strains TL-0, TL-8, TL-9, and TL-10. **a**, The EGFP fluorescence intensity of strains TL-0 (control), TL-8 (NuoK+crePsbA*+EGFP), TL-9 (NuoK+PufL*+EGFP), and TL-10 (NuoK+soePsbA*+EGFP) were measured by flow cytometry, respectively. **b**, Half offset of histograms of flow cytometry for the EGFP fluorescence intensity of strains TL-0, TL-8, TL-9, and TL-10. **c**, Fluorescence images and pixel intensity analysis from laser confocal microscopy for strains TL-8 (NuoK+crePsbA*+EGFP) and TL-10 (NuoK+soePsbA*+EGFP). The white scale bar at the bottom right of microscope image represented 2.5 μ m. The pixel intensity data were collected by laser confocal microscopy software LAS AF Lite along the track of black solid arrow in microscope image. Values were shown as mean $\pm$ s.d. from three biological replicates (**a**). All experiments were repeated independently for three times with similar results (**b-c**). Source data are provided as a Source Data file.



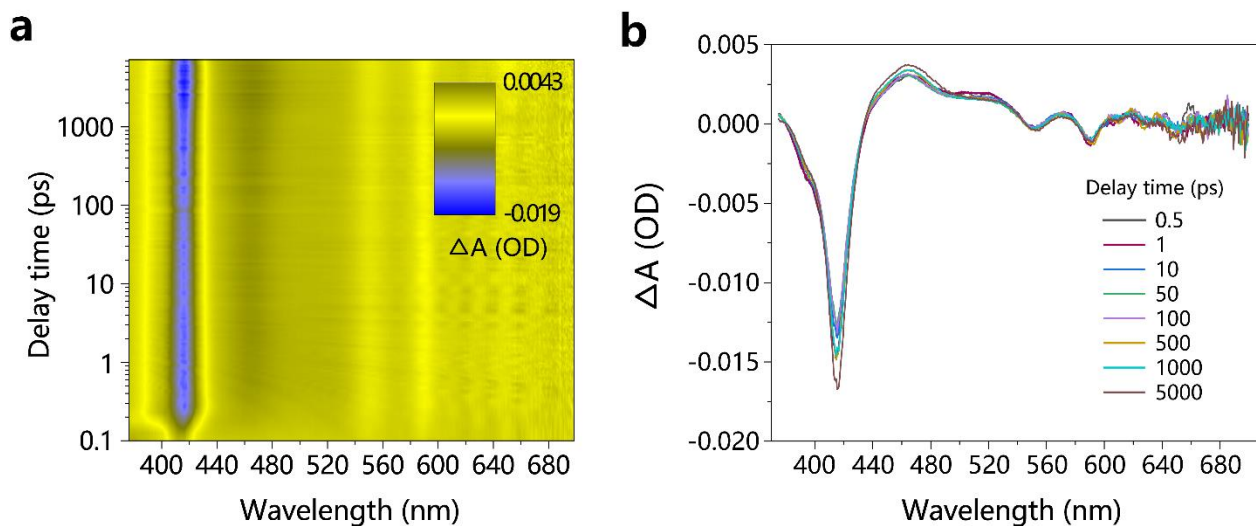
Supplementary Figure 6. Fluorescence microscope images and pixel intensity analysis from laser confocal microscopy for strains TL-1 (control), TL-4 (NuoK+EGFP), and TL-9 (NuoK+PufL*+EGFP). A zoomed-in detail of the area marked by the orange box is presented in Figure 2b. The pixel intensity data were collected by laser confocal microscopy software LAS AF Lite along the track of black solid arrow in microscope image. The white scale bar at the bottom right of microscope image represented 2.5 μm . Cells pointed by white triangular arrows had significant differences in microscope image. All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



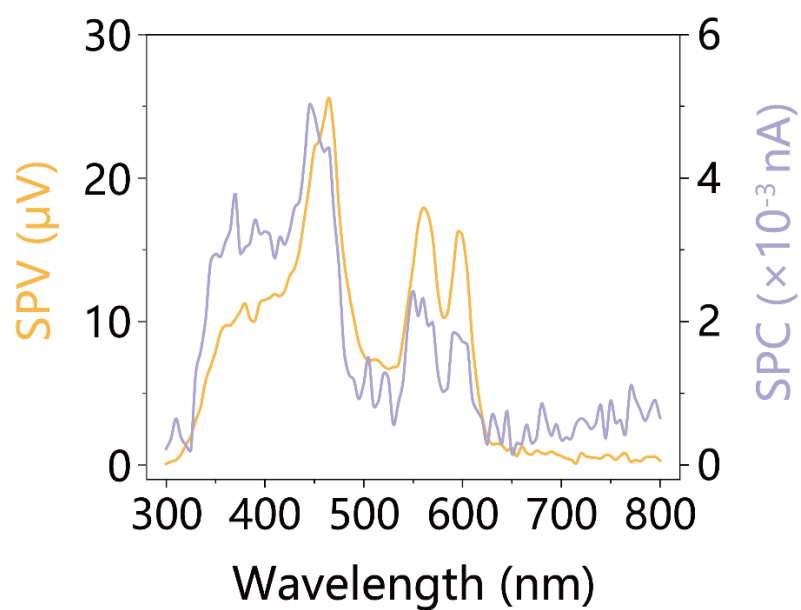
Supplementary Figure 7. Detection of EGFP fluorescence signal for membrane fractions. **a**, The EGFP fluorescence signals of membrane fractions extracted from strains TL-0 (control), TL-8 (NuoK+crePsbA*+EGFP), TL-9 (NuoK+PufL*+EGFP), and TL-10 (NuoK+soePsbA*+EGFP), respectively. **b**, Western blot analysis of fusion proteins NuoK+crePsbA*+EGFP (65.8 kDa), NuoK+PufL*+EGFP (62.3 kDa), and NuoK+soePsbA*+EGFP (65.7 kDa) in membrane fractions. The Anti-EGFP mouse antibody and HRP-conjugated rabbit anti-mouse IgG antibody were purchased from Sangon Biotech and diluted at 1:5000. Membrane fractions were prepared by ultracentrifugation. Fusion membrane protein samples were prepared by disorganizing the membrane fractions using 1.0% (wt/vol) DDM (*n*-dodecyl- β -D-maltopyranoside) for 2 h. The images of EGFP fluorescence signals and western blot were taken by iBright Imaging System FL1500. All experiments were repeated independently for three times with similar results (**a-b**). Source data are provided as a Source Data file.



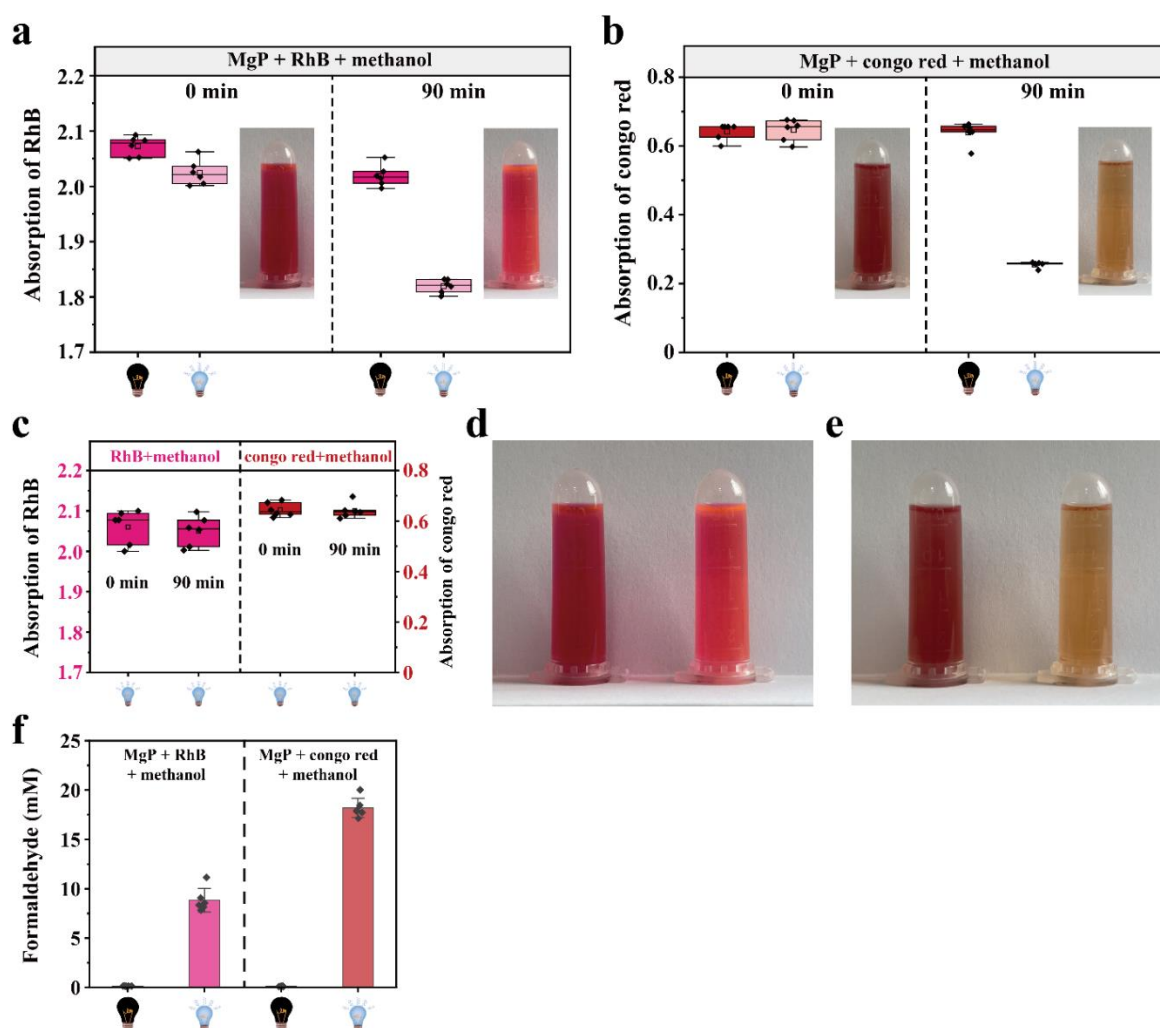
Supplementary Figure 8. UV-Vis spectrum scanning was performed on 2×10^{-5} mol/L MgP standard solution. MgP has obvious absorption peaks in the visible light range, with a strong absorption peak (Soret band) at 416 nm and two weak absorption peaks (Q band) at 522 nm and 590 nm, which is consistent with the spectroscopic characteristics of metalloporphyrin compounds. All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



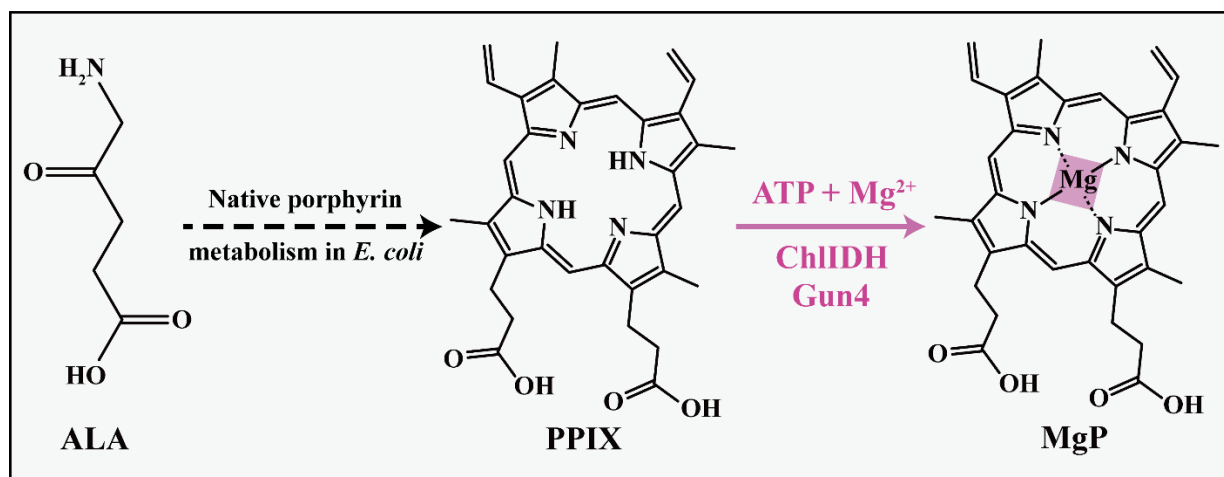
Supplementary Figure 9. Analysis of transient absorption spectroscopy of MgP in methanol solution. a, Two-dimensional isotropic transient absorption spectroscopy of MgP in methanol solution. **b**, Isotropic transient absorption spectroscopy of MgP in methanol solution at a few specific delay time. Source data are provided as a Source Data file.



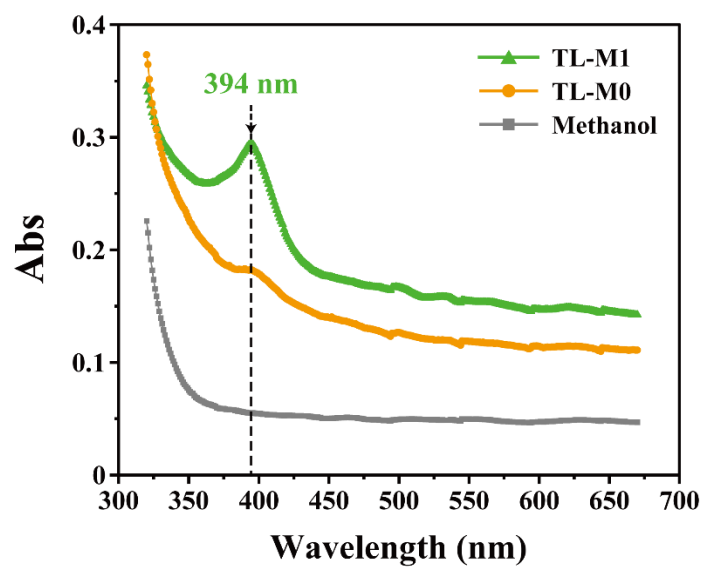
Supplementary Figure 10. The surface photovoltage (SPV) and photocurrent (SPC) curves of MgP standard powder. All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



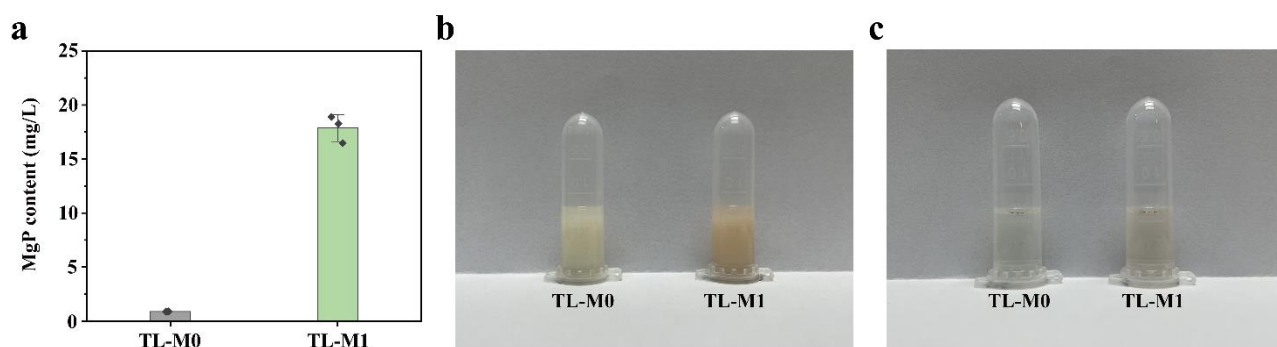
Supplementary Figure 11. The validation of the photoelectron-generating properties of MgP molecules in methanol (as an electron sacrificial agent for MgP). **a-b**, The degradation of dyes Rhodamine B (RhB) and congo red in the presence of MgP molecules was measured with and without blue light illumination. **c**, The degradation of dyes RhB and congo red in the absence of MgP molecules was measured with blue light illumination. **d-e**, Comparison of dyes RhB (**d**) and congo red (**e**) before (left) and after (right) photocatalytic degradation by MgP molecules. **f**, Formaldehyde content in dyes RhB and congo red degradation systems with and without blue light illumination. Formaldehyde content was determined by the acetylacetone method². Values were shown as mean $\pm$ s.d. from six biological replicates (**a-c** and **f**). All experiments were repeated independently for three times with similar results (**d-e**). Source data are provided as a Source Data file.



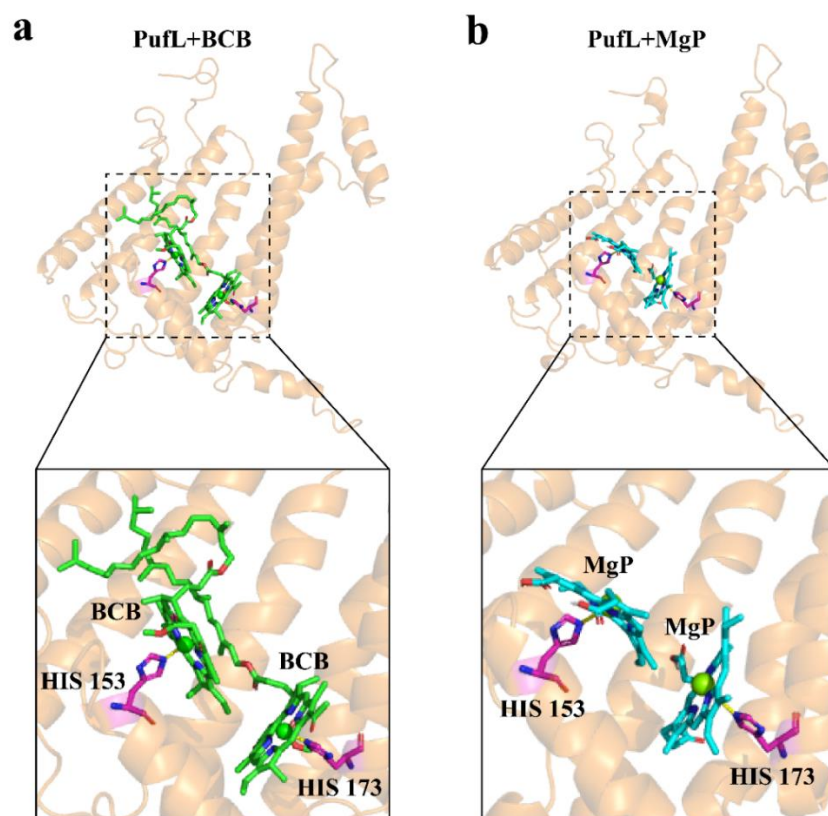
Supplementary Figure 12. Schematic diagram of heterologous MgP biosynthesis pathway. The heterologous MgP biosynthesis pathway³ was introduced into strain F0701 (generating strain TL-M1) to connect the native porphyrin metabolism for synthesizing MgP molecules in *E. coli*. Abbreviation: ALA, δ-aminolevulinic acid; PPIX, protoporphyrin IX; MgP, magnesium protoporphyrin IX; ATP, adenosine triphosphate; ChlIDH and Gun4, pathway enzyme subunits ChlI, ChlD, ChlH, and Gun4.



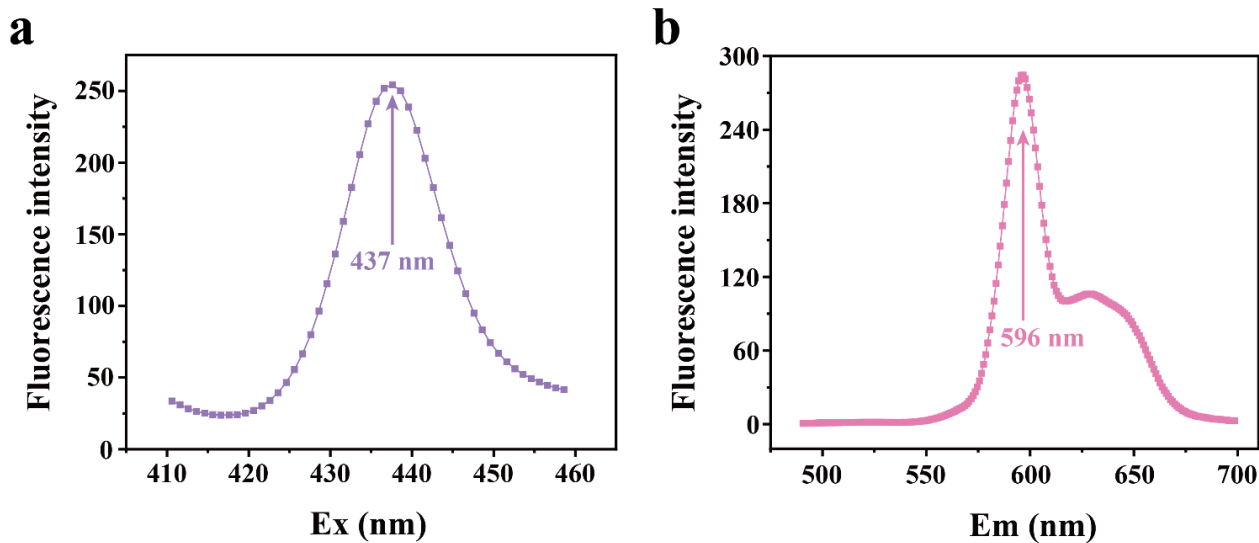
Supplementary Figure 13. UV-Vis spectrum scanning of the methanol extract of strains TL-M0 and TL-M1. All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



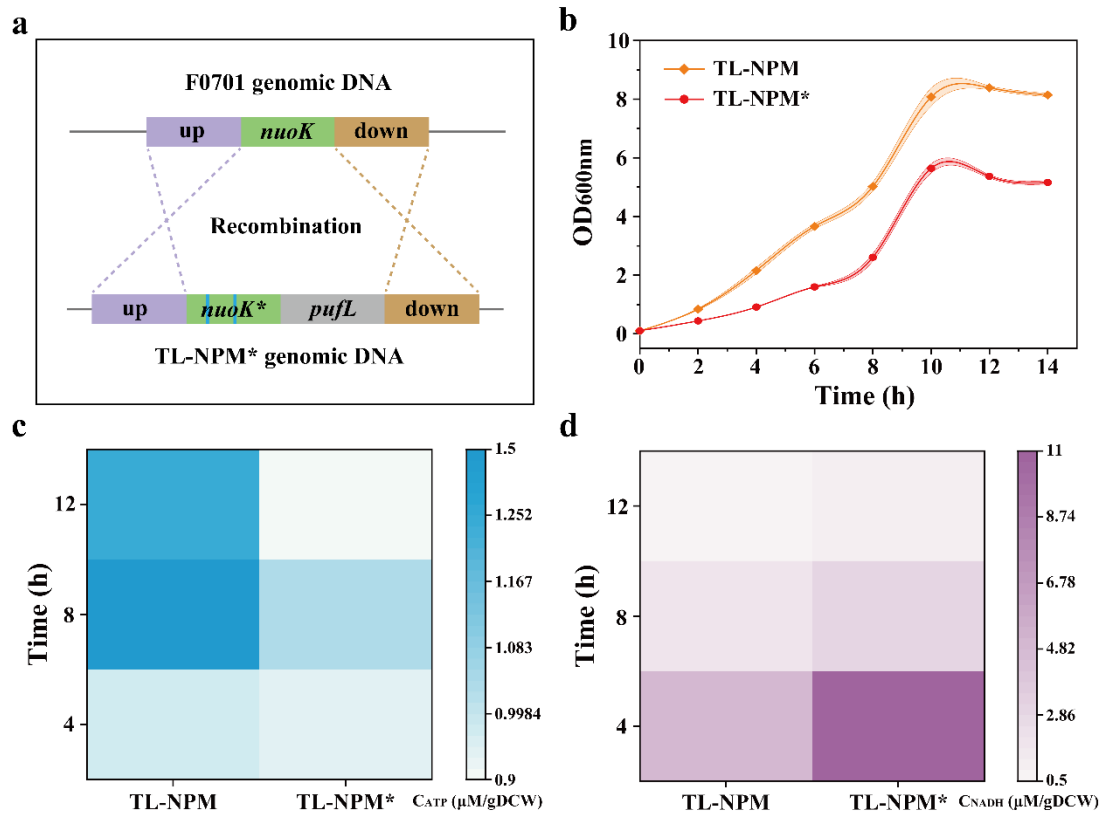
Supplementary Figure 14. Detection of MgP content in the methanol extract of strains TL-M0 and TL-M1. a, The MgP content in the methanol extract of strains TL-M0 and TL-M1. **b-c,** The pictures of cell disruption solution (**b**) and supernatants (**c**) extracted by methanol for strains TL-M0 and TL-M1, respectively. Solutions containing MgP appear lavender. Values were shown as mean $\pm$ s.d. from three biological replicates (**a**). All experiments were repeated independently for three times with similar results (**b-c**). Source data are provided as a Source Data file.



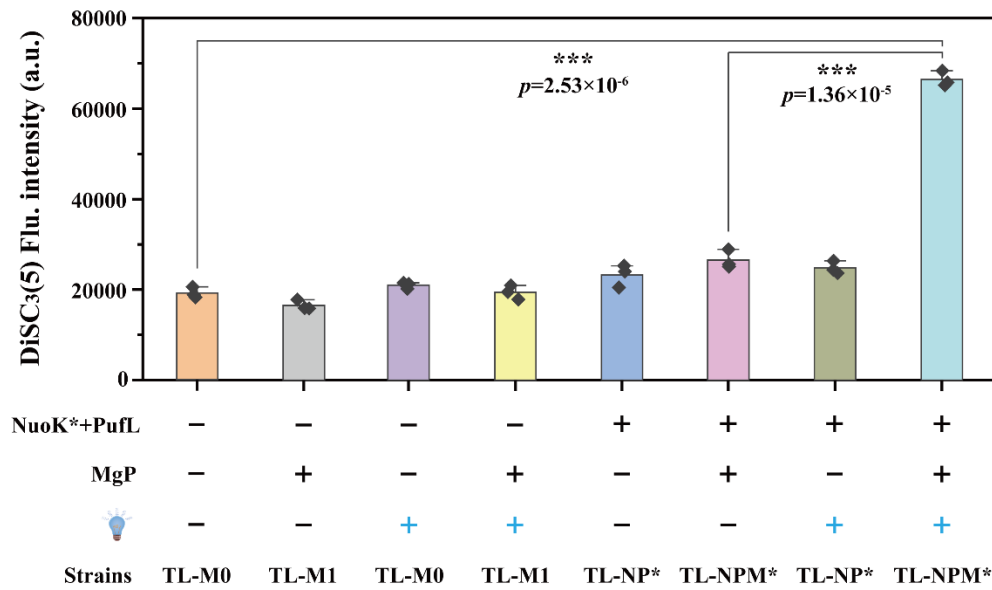
Supplementary Figure 15. Molecular docking analysis of MgP and PufL. **a**, Natural PufL+BCB model (PDB ID code 5PRC)⁴. **b**, PufL+MgP model in our study. The explanation of molecular docking was presented in Supplementary Note 4.



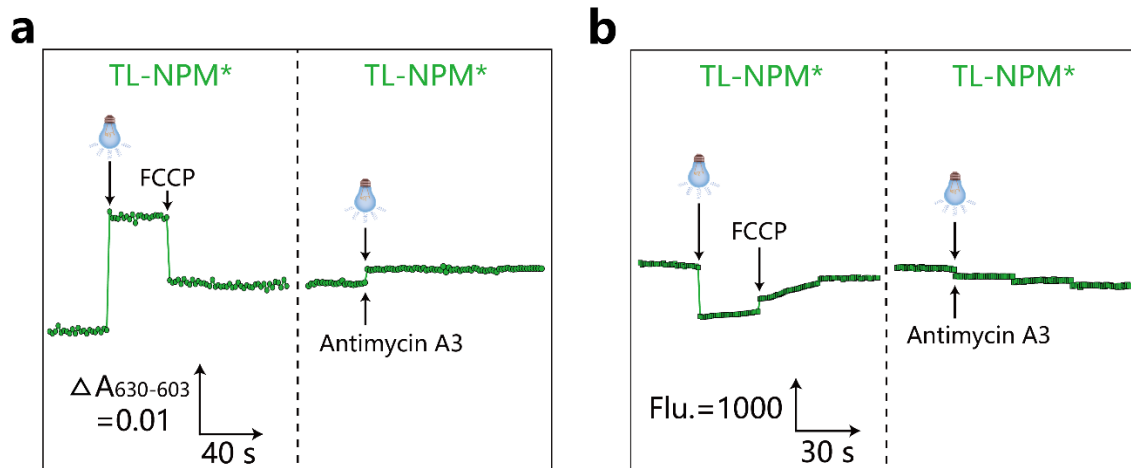
Supplementary Figure 16. Analysis of fluorescence spectrum scanning for MgP standard solution. Fluorescence spectrum scanning (excitation spectrum scanning (a) and emission spectrum scanning (b)) was performed on the MgP standard solution, and its excitation/emission wavelengths were 437 nm and 596 nm, respectively. Abbreviation: Ex, excitation wavelength; Em, emission wavelength. All experiments were repeated independently for three times with similar results (a-b). Source data are provided as a Source Data file.



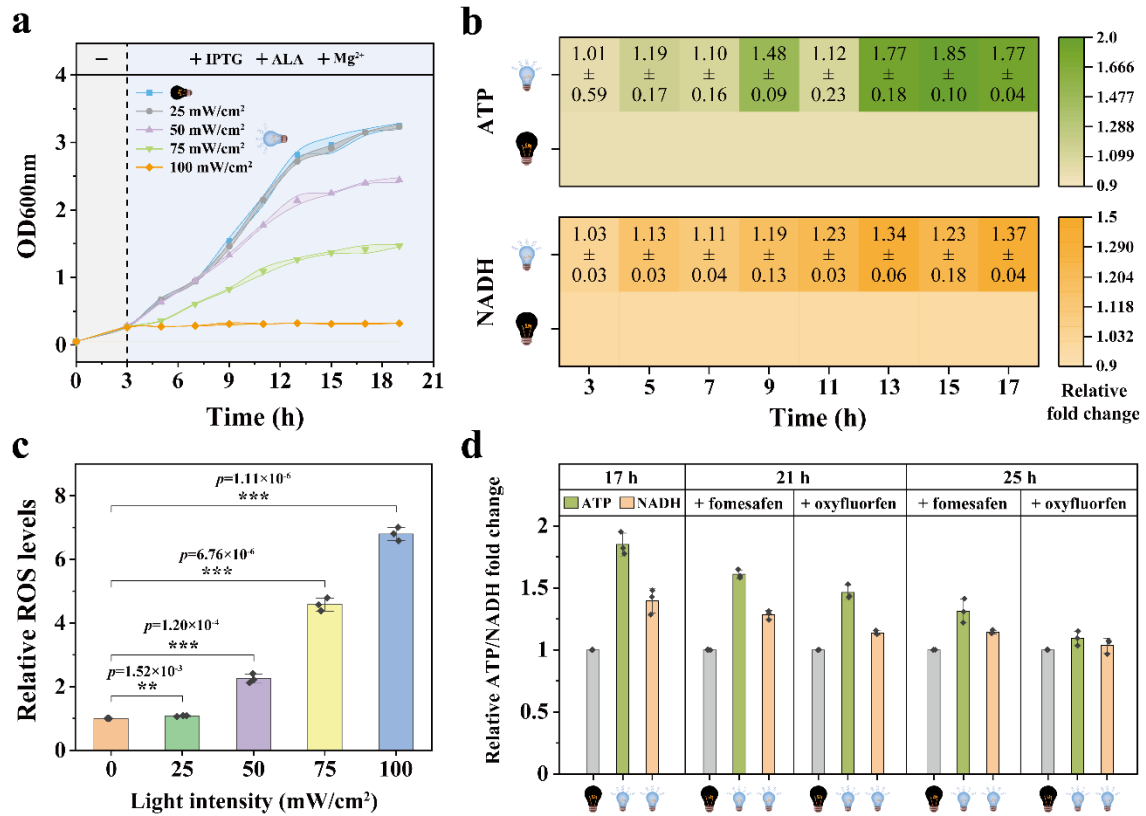
Supplementary Figure 17. Evaluation of growth performance and intracellular ATP and NADH contents for strains TL-NPM and TL-NPM*. **a**, Schematic representation of recombination to integrate backbone protein gene *nuoK**+*pufL*. **b**, Growth performance (OD_{600nm}) of strains TL-NPM and TL-NPM*. **c-d**, The intracellular ATP (**c**) and NADH (**d**) content of strains TL-NPM and TL-NPM*. Abbreviation: C_{ATP}, intracellular ATP content; C_{NADH}, intracellular NADH content. Values were shown as mean ± s.d. from three biological replicates (**b-d**). Source data are provided as a Source Data file.



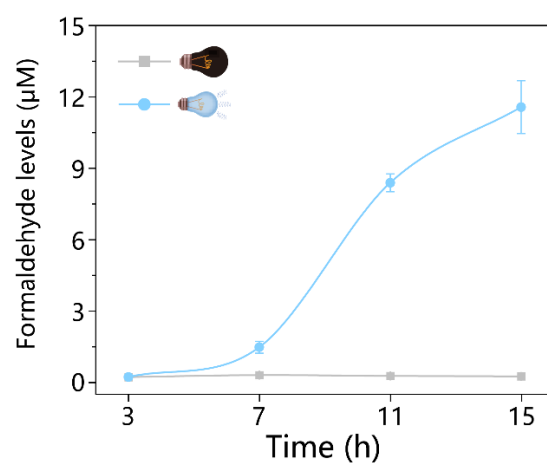
Supplementary Figure 18. The DiSC₃(5) fluorescence intensity of strains TL-M0, TL-M1, TL-NP*, and TL-NPM* with or without blue light illumination. Values were shown as mean $\pm$ s.d. from three biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided t -test. Source data are provided as a Source Data file.



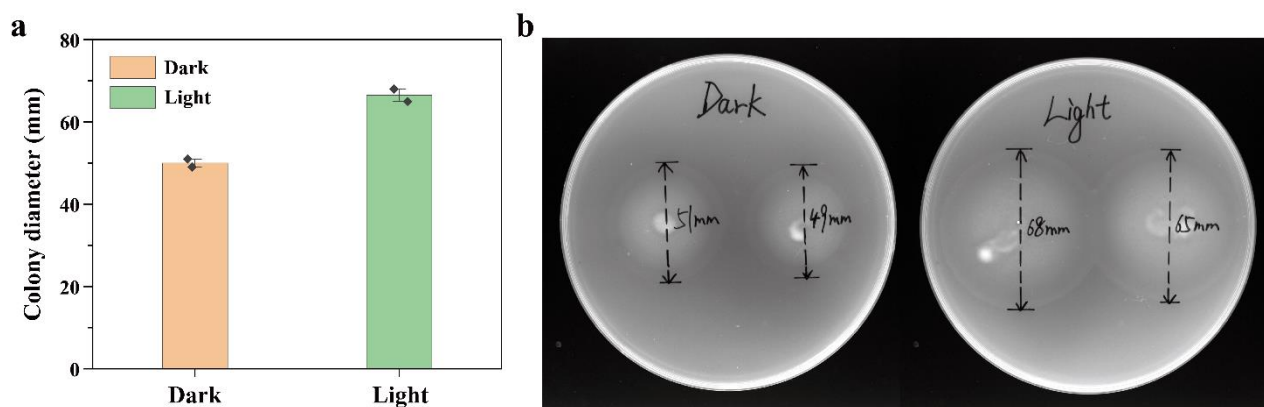
Supplementary Figure 19. The signal of membrane potential probe oxonol VI (a) and proton gradient probe ACMA (b) for the membrane fractions of strain TL-NPM*. Antimycin A3 is a potent inhibitor of complex III by inhibiting the oxidation of ubiquinol to ubiquinone^{5, 6}. Abbreviation: $\Delta A_{630-603}$, absorbance change at 630 nm minus 603 nm; FCCP, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, an uncoupler of oxidative phosphorylation. All experiments were repeated independently for three times with similar results (**a-b**). Source data are provided as a Source Data file.



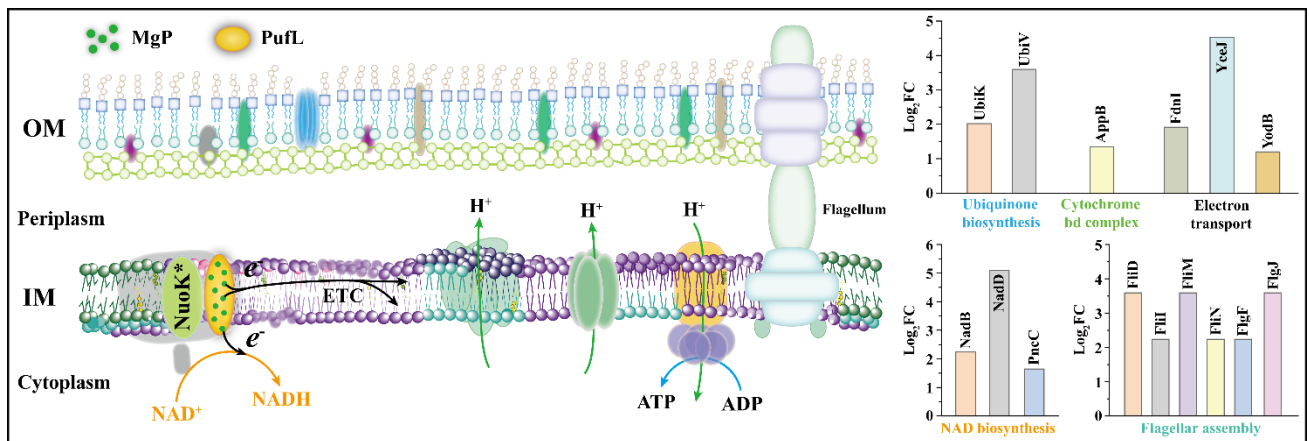
Supplementary Figure 20. Evaluation of growth performance, intracellular ATP and NADH content, and ROS levels for strain TL-NPM*. **a**, Growth performance (OD_{600nm}) of strain TL-NPM* under dark and different intensities of blue light illumination. Abbreviation: IPTG, isopropyl- β -D-thiogalactoside; ALA, δ -aminolevulinic acid. **b**, The relative fold change of intracellular ATP and NADH content for strain TL-NPM* under dark and blue light illumination. **c**, Relative ROS levels of strain TL-NPM* under different light intensities for 17 h. ROS levels were determined using ROS Assay Kit (Beyotime Biotechnology S0033S). **d**, Relative fold change of intracellular ATP and NADH contents for strain TL-NPM* after addition of 5 mM fomesafen and oxyfluorfen (as inhibitors of photosynthesis reaction center), respectively. Values were shown as mean $\pm$ s.d. from three biological replicates (**a-d**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided t -test. Source data are provided as a Source Data file.



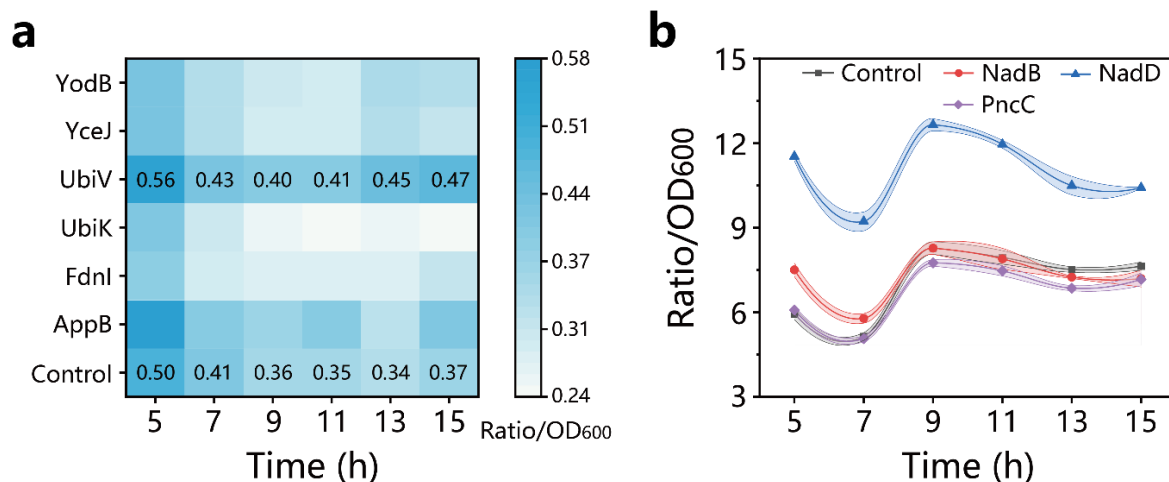
Supplementary Figure 21. Intracellular formaldehyde levels timecourse in strain TL-NPM* under dark and 25 mW/cm² blue light illumination. Values were shown as mean $\pm$ s.d. from three biological replicates. Source data are provided as a Source Data file.



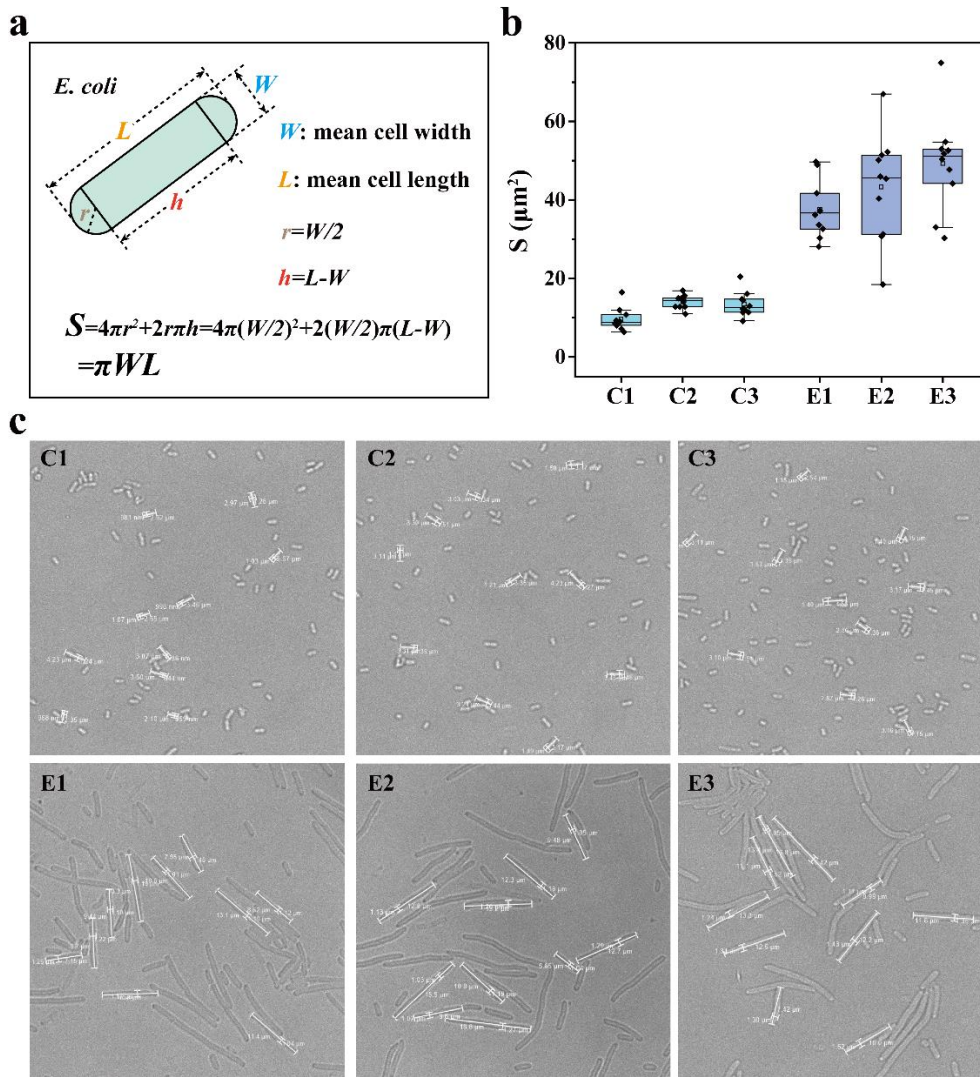
Supplementary Figure 22. Evaluation of flagella motility for strain TL-NPM*. **a**, The colony diameter of strain TL-NPM* under dark and blue light illumination. **b**, Colonies of strain TL-NPM* on solid medium under dark and blue light illumination. The image of colonies was taken by iBright Imaging System FL1500. All experiments were repeated independently for twice with similar results (**a-b**). Source data are provided as a Source Data file.



Supplementary Figure 23. Transcriptomic analysis for strain TL-NPM* under dark condition and blue light illumination. The significantly upregulated transcriptional levels of genes associated with ubiquinone biosynthesis, cytochrome bd complex, electron transport, NAD⁺ biosynthesis, and flagellar assembly were presented. Abbreviation: OM, outer membrane; IM, inner membrane; ETC, electron transport chain; ATP/ADP, adenosine triphosphate/diphosphate; NADH/NAD⁺, nicotinamide adenine dinucleotide reduced/oxidized. Source data are provided as a Source Data file.

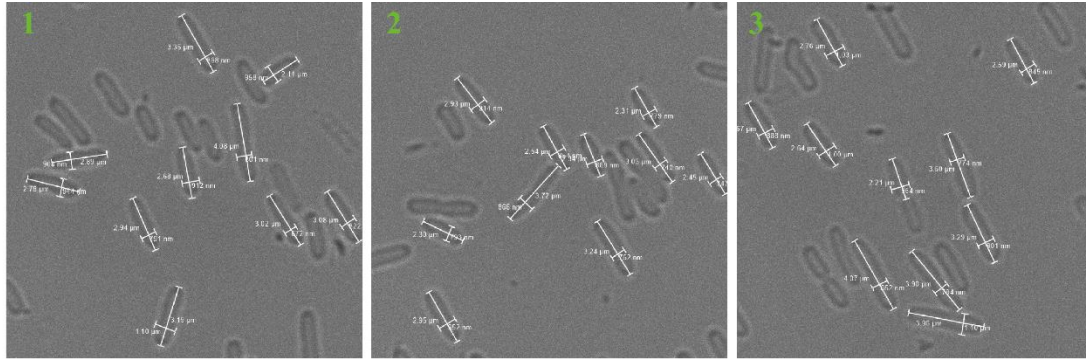


Supplementary Figure 24. Screening of target genes that regulate ATP and NADH generation. **a**, The QUEEN2m fluorescence signal of strains TL-A0 (control) and TL-A1~TL-A6 (the genes *appB*, *fdnI*, *ubiK*, *ubiV*, *yceJ*, and *yodB* were overexpressed, respectively) under blue light illumination. **b**, The Frex fluorescence signal of strains TL-N0 (control) and TL-N1~TL-N3 (the genes *nadB*, *nadD*, and *pncC* were overexpressed, respectively) under blue light illumination. Values were shown as mean $\pm$ s.d. from three biological replicates (**a-b**). Source data are provided as a Source Data file.

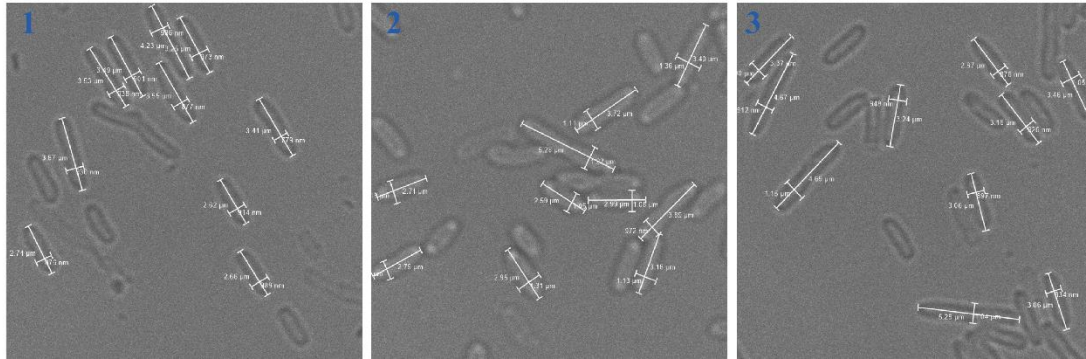


Supplementary Figure 25. The expression of *sula* gene on plasmid scale (pEtac-*sula*) increased *E. coli* cell mean surface area. **a**, The model of *E. coli* was used to calculate the mean surface area⁷. **b**, Mean surface area (S) of *E. coli* with and without plasmid pEtac-*sula*. The introduction of plasmid pEtac-*sula* into *E. coli* (experimental group) resulted in a 250.5% increase in the mean surface area compared with that of the control group. **c**, Microscope images of *E. coli* from the control group and experimental group. C1~C3 and E1~E3 were from three different microscope fields, respectively. White line segment represented cell width and length measured by laser confocal microscopy software LAS AF Lite in microscope images. Values were shown as mean $\pm$ s.d. from ten biological replicates (**b**). Source data are provided as a Source Data file.

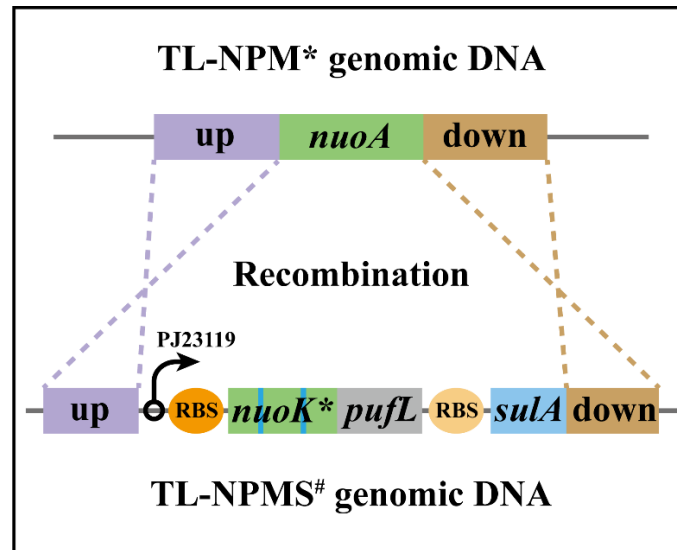
TL-NPM*



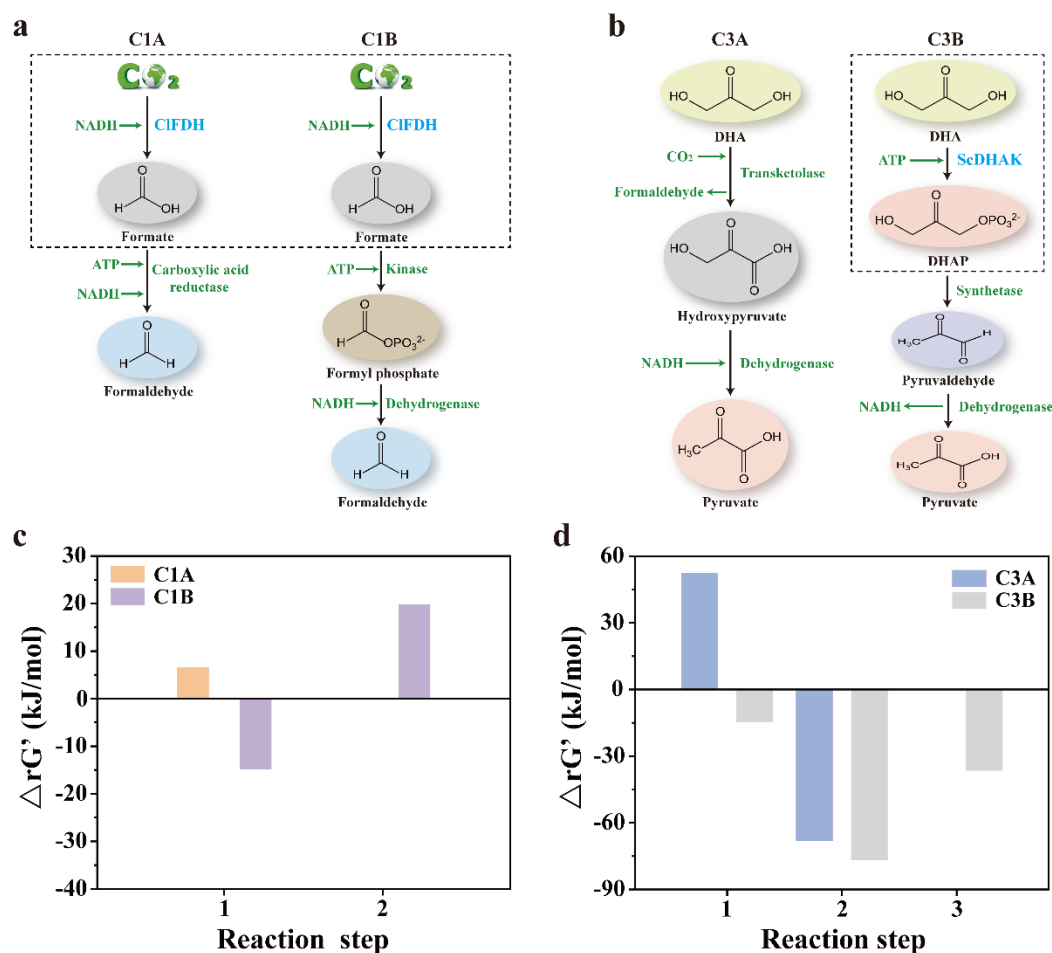
TL-NPMS*



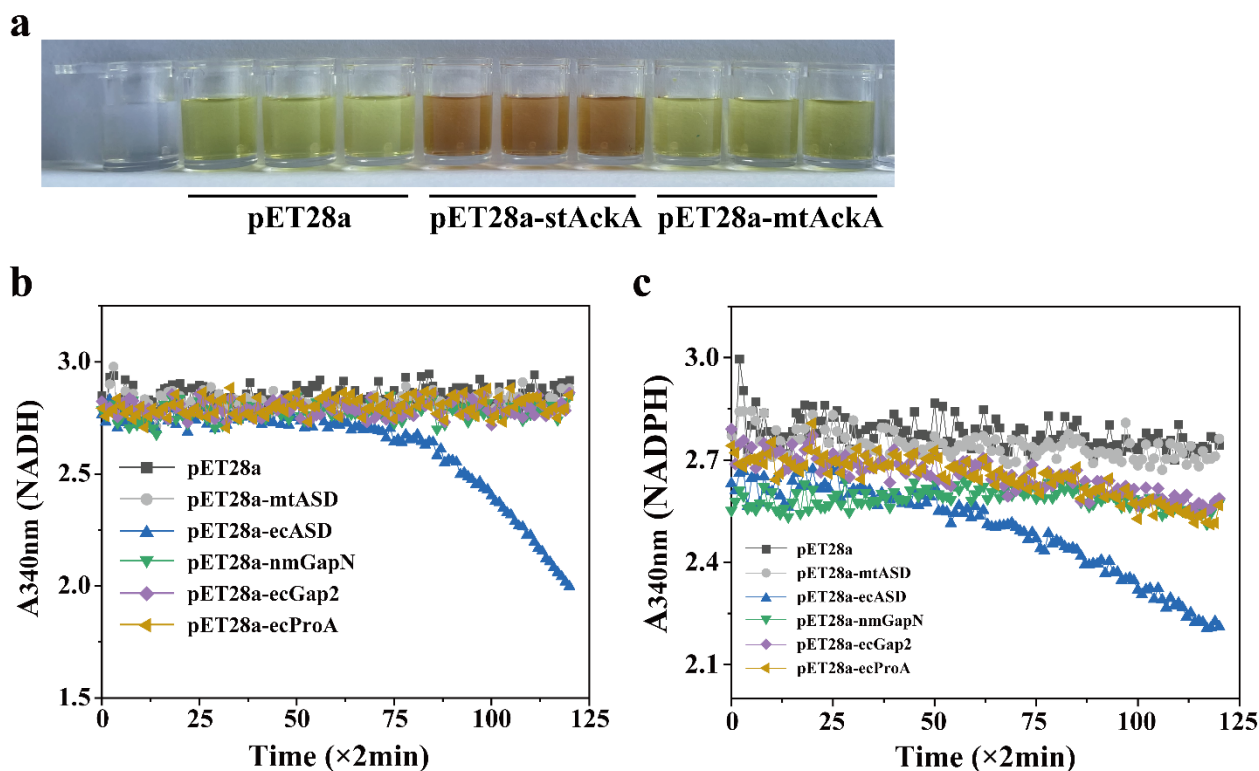
Supplementary Figure 26. Microscope images of strains TL-NPM* and TL-NPMS*. Images were taken from three different microscope fields to determine the mean cell width and length, respectively. White line segment represented cell width and length measured by laser confocal microscopy software LAS AF Lite in microscope images.



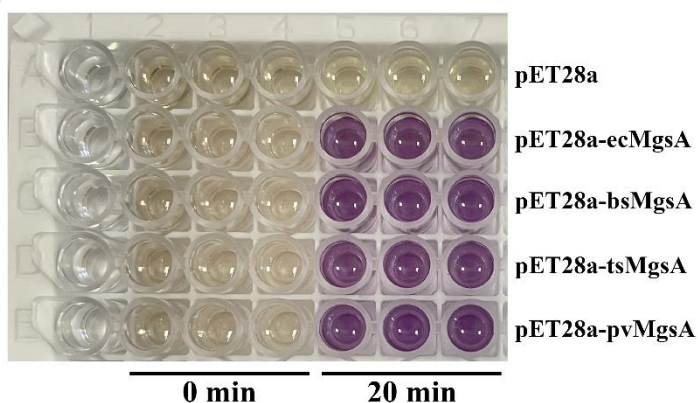
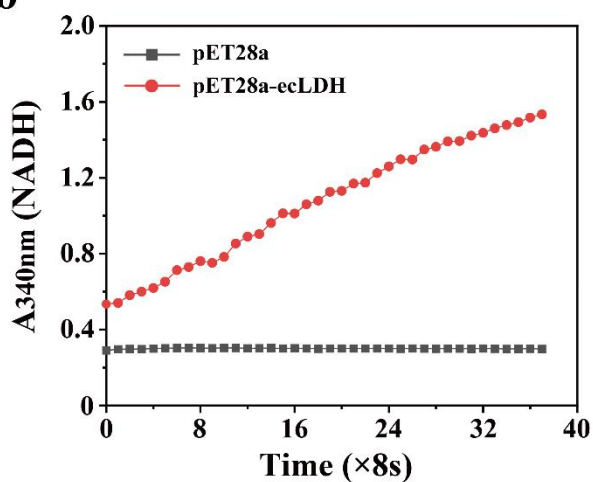
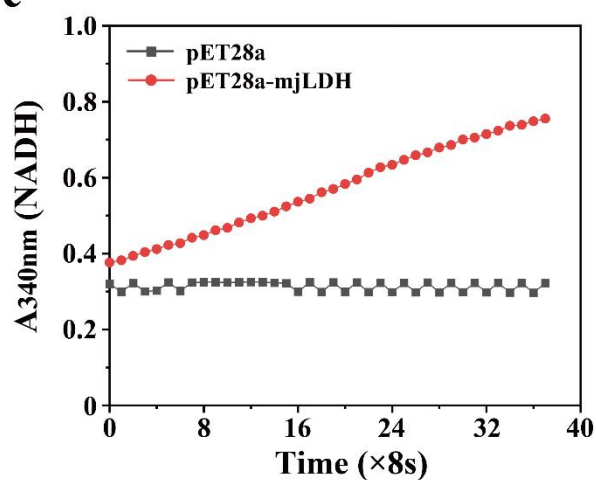
Supplementary Figure 27. Schematic representation of recombination to integrate the expression cassette containing the promoter P_{J23119}, backbone protein gene *nuoK**+*pufL*, and gene *sulA*.



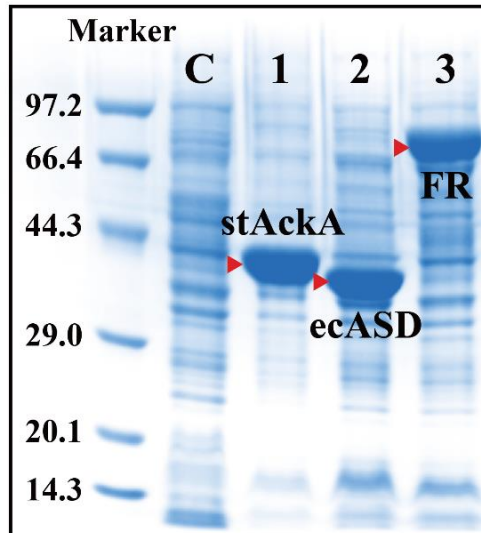
Supplementary Figure 28. Design and thermodynamic evaluation of the C1 module and C3 module. **a**, Two C1 module schemes for converting CO_2 to formaldehyde. **b**, Two C3 module schemes for converting dihydroxyacetone to pyruvate. Abbreviation: ATP, adenosine triphosphate; NADH, Nicotinamide adenine dinucleotide reduced; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate. **c-d**, Thermodynamic evaluation of biochemical reactions in C1 module and C3 module using eQuilibrator (<https://equilibrator.weizmann.ac.il/>). Specially parameter was listed in following: pH=7.5, pMg=3.0, ionic strength 0.2 M, NADH 2.0 mM, NAD^+ 2.0 mM, ATP 2.0 mM, ADP 2.0 mM, AMP 2.0 mM, substrates 1.0 mM, products 1.0 mM. Source data are provided as a Source Data file.



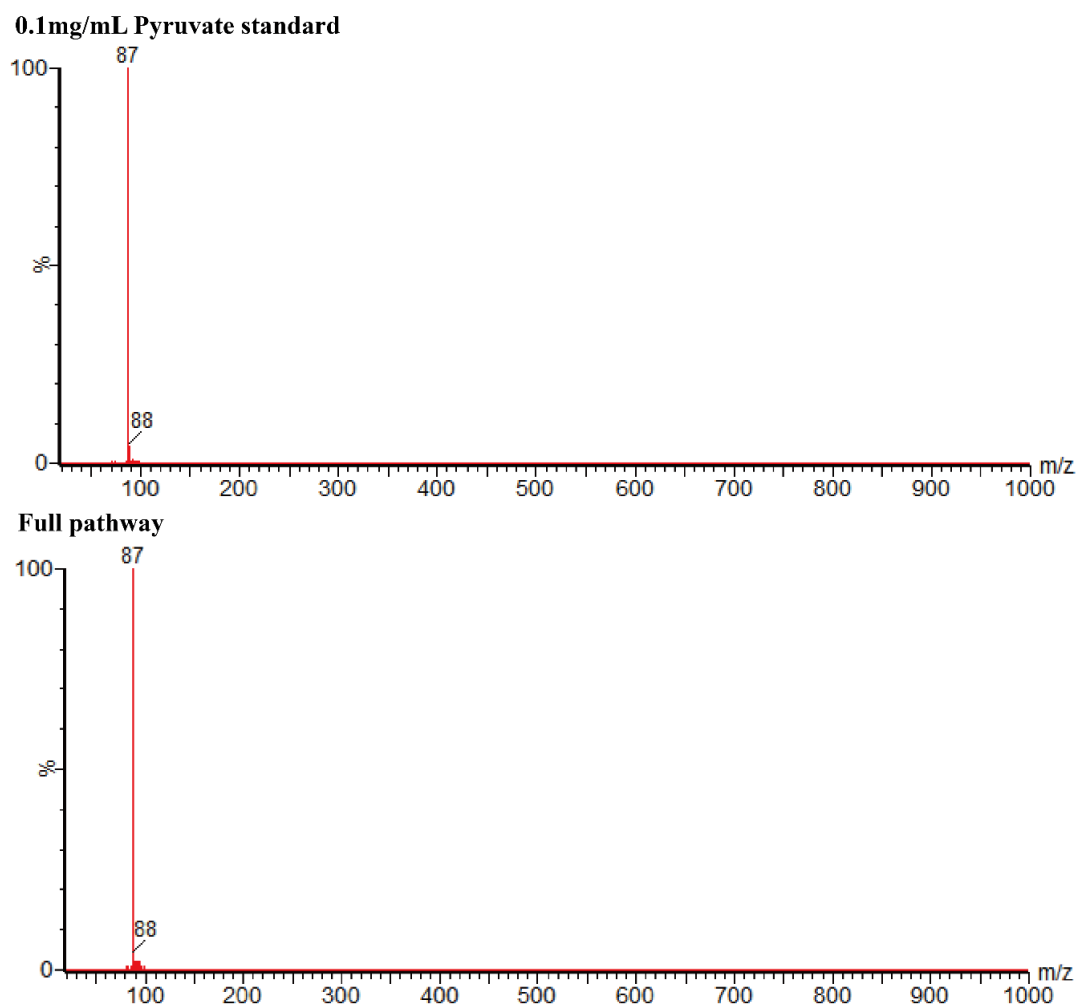
Supplementary Figure 29. Evaluation of candidate pathway enzymes for C1 module. a, The generation of formyl phosphate was determined by colorimetry to calculate the specific enzyme activities of stAckA and mtAckA. **b-c,** Time curve of NADH and NADPH consumption in the stAckA and five dehydrogenases cascade reaction. Reduction of the absorbance at 340 nm (absorbance of NADH or NADPH) indicated the conversion of formyl phosphate into formaldehyde. Source data are provided as a Source Data file.

a**b****c**

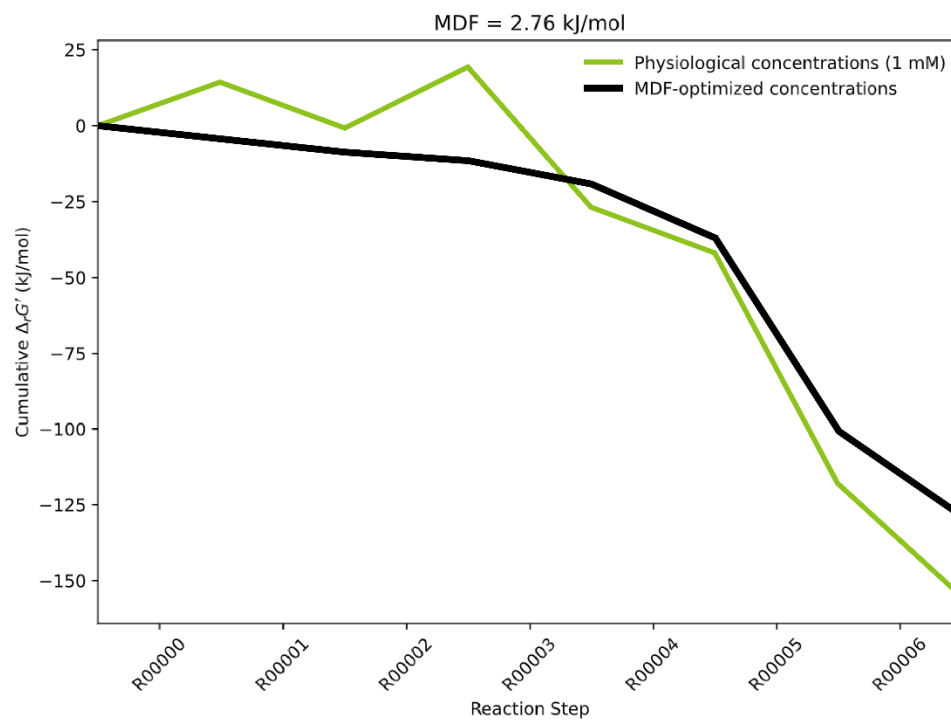
Supplementary Figure 30. Evaluation of candidate pathway enzymes for C3 module. **a**, The generation of methylglyoxal was determined by colorimetry to calculate the specific enzyme activities of ecMgsA, bsMgsA, tsMgsA, and pvMgsA. **b-c**, Time curve of NADH generation in the two dehydrogenases ecLDH and mjLDH reaction. The increase of absorbance at 340 nm (absorbance of NADH) indicated the conversion of methylglyoxal into pyruvate. Source data are provided as a Source Data file.



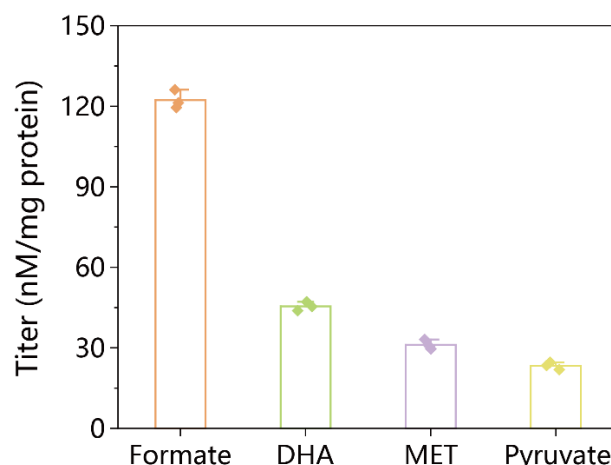
Supplementary Figure 31. SDS-PAGE analysis of pathway enzymes stAckA, ecASD, and FR (constructed by fusing stAckA and ecASD). All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



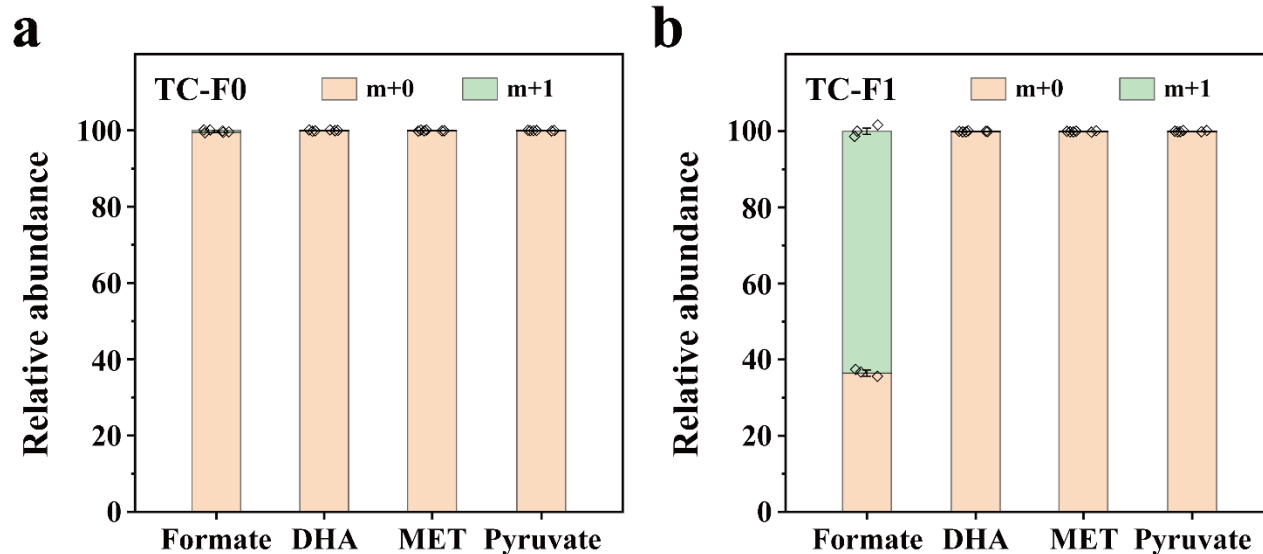
Supplementary Figure 32. LC-MS analysis of pyruvate standard and supernatant from one-pot catalytic reactions.



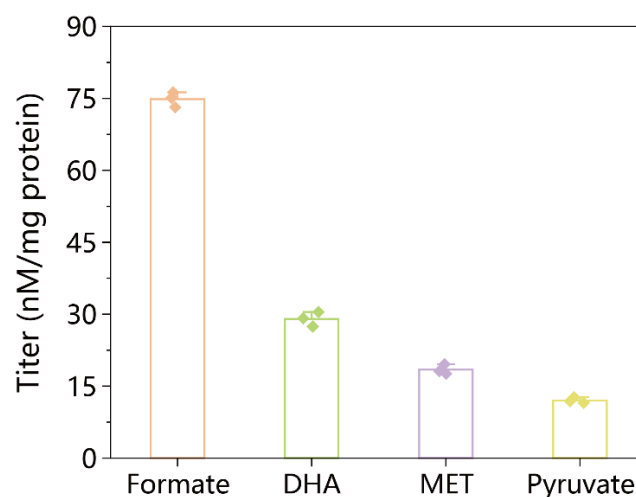
Supplementary Figure 33. The max-min driving force (MDF) of ADRP was analyzed through eQuilibrator pathway analysis (<https://equilibrator.weizmann.ac.il/pathway/>).



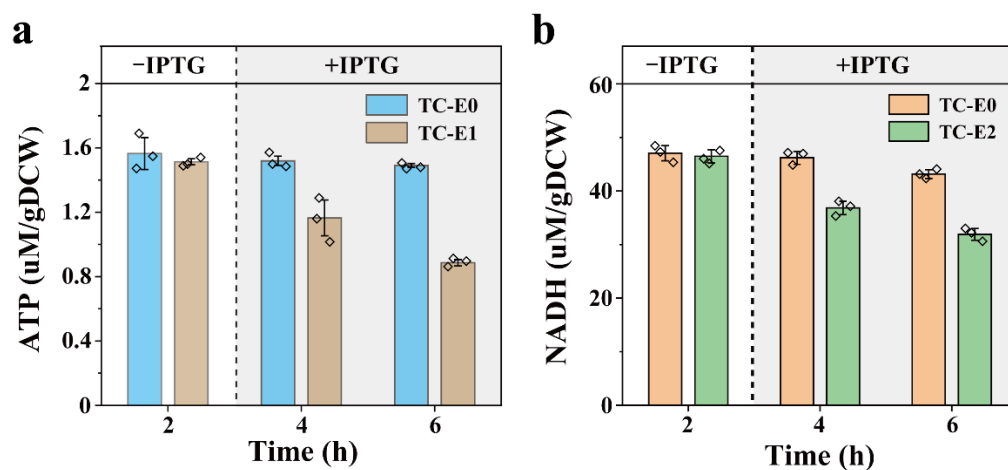
Supplementary Figure 34. The titers of intracellular formate (122.3 nM/mg protein), DHA (45.5 nM/mg protein), MET (31.1 nM/mg protein), and pyruvate (23.3 nM/mg protein) in strain TD-F1. As shown in Fig. 4g, the relative abundance of mass isotopomers for central metabolite pyruvate were 69.1% (m+0), 6.7% (m+1), 9.3% (m+2), and 14.9% (m+3). Based on these results, the proportion of C atom in central metabolite pyruvate derived from glucose and CO₂ was $(23.3 \times 69.1\% \times 3 + 23.3 \times 6.7\% \times 2 + 23.3 \times 9.3\% \times 1) / (23.3 \times 14.9\% \times 3 + 23.3 \times 9.3\% \times 2 + 23.3 \times 6.7\% \times 1) = 3.29/1$. In addition, the proportion of C atom in formate, DHA, and MET derived from glucose and CO₂ were 1/3.33, 2.17/1, and 2.62/1, respectively. Abbreviation: DHA, dihydroxyacetone; MET, methylglyoxal. Values were shown as mean $\pm$ s.d. from three biological replicates. Source data are provided as a Source Data file.



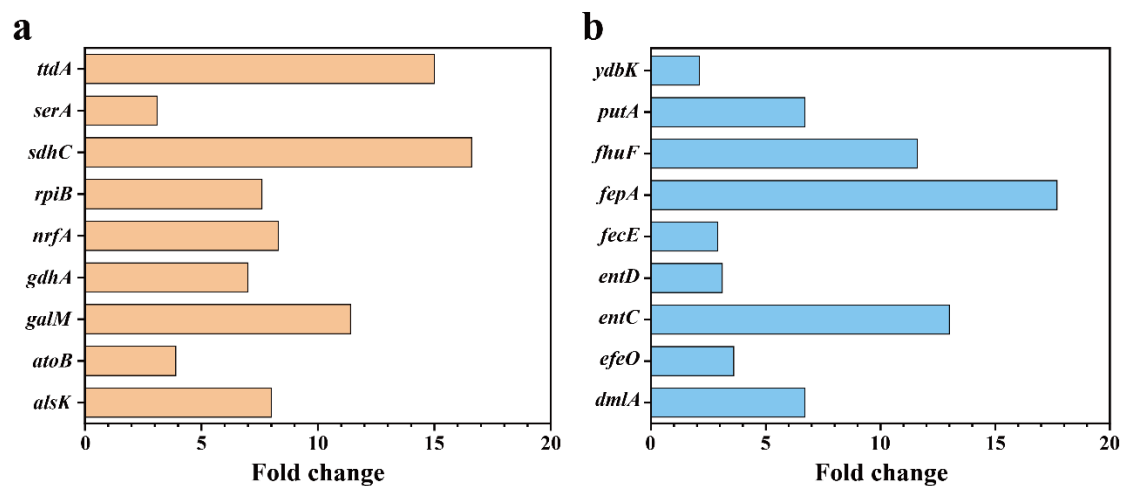
Supplementary Figure 35. Relative abundance of mass isotopomers for intracellular formate, DHA, MET, and pyruvate in strains TC-F0 (a) and TC-F1 (b) in the absence of blue light illumination, respectively. Abbreviation: DHA, dihydroxyacetone; MET, methylglyoxal. m+0 represented metabolites without ^{13}C atom and m+1 represented metabolites with one ^{13}C atom. Values were shown as mean $\pm$ s.d. from three biological replicates (**a-b**). Source data are provided as a Source Data file.



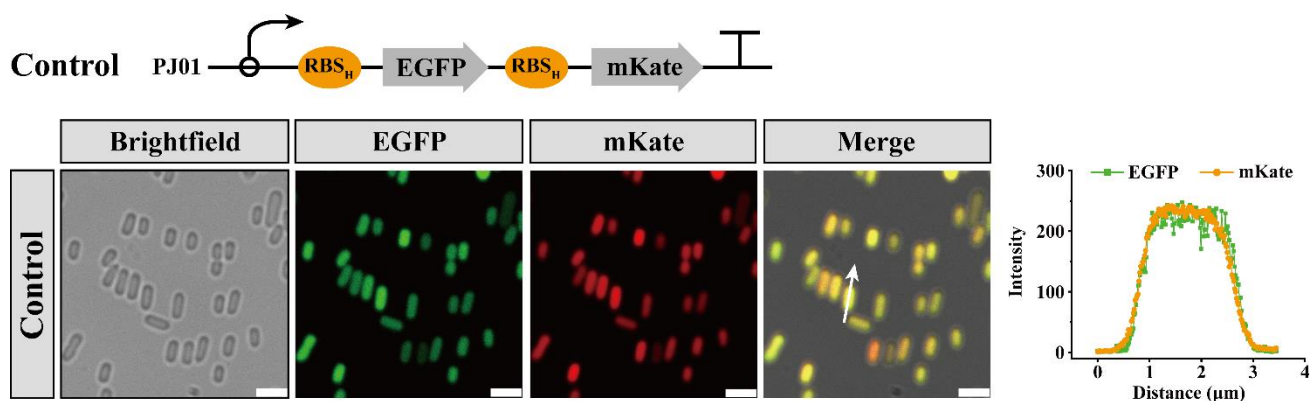
Supplementary Figure 36. The titers of intracellular formate (74.8 nM/mg protein), DHA (29.0 nM/mg protein), MET (18.4 nM/mg protein), and pyruvate (12.0 nM/mg protein) in strain TC-F1 under blue light illumination. Abbreviation: DHA, dihydroxyacetone; MET, methylglyoxal. Values were shown as mean $\pm$ s.d. from three biological replicates. Source data are provided as a Source Data file.



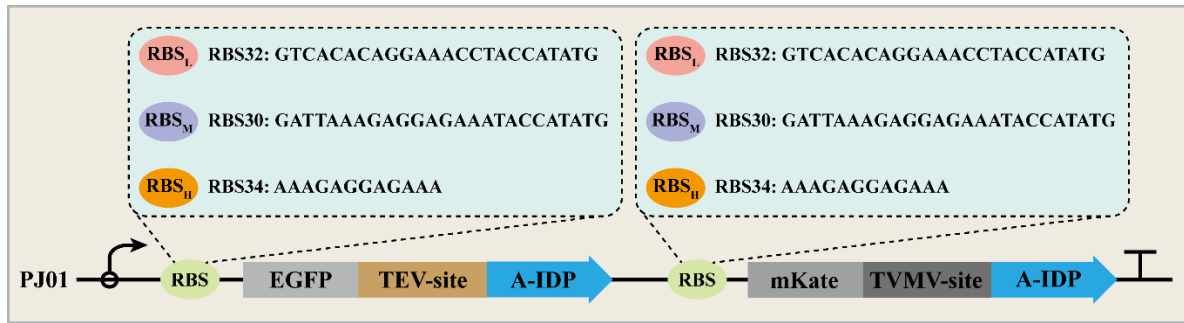
Supplementary Figure 37. Evaluation of intracellular ATP content for strain TC-E1 and intracellular NADH content for strain TC-E2. a, The intracellular ATP content of strains TC-E0 and TC-E1 before and after adding IPTG. **b,** The intracellular NADH content of strains TC-E0 and TC-E2 before and after adding IPTG. Values were shown as mean $\pm$ s.d. from three biological replicates (**a-b**). Source data are provided as a Source Data file.



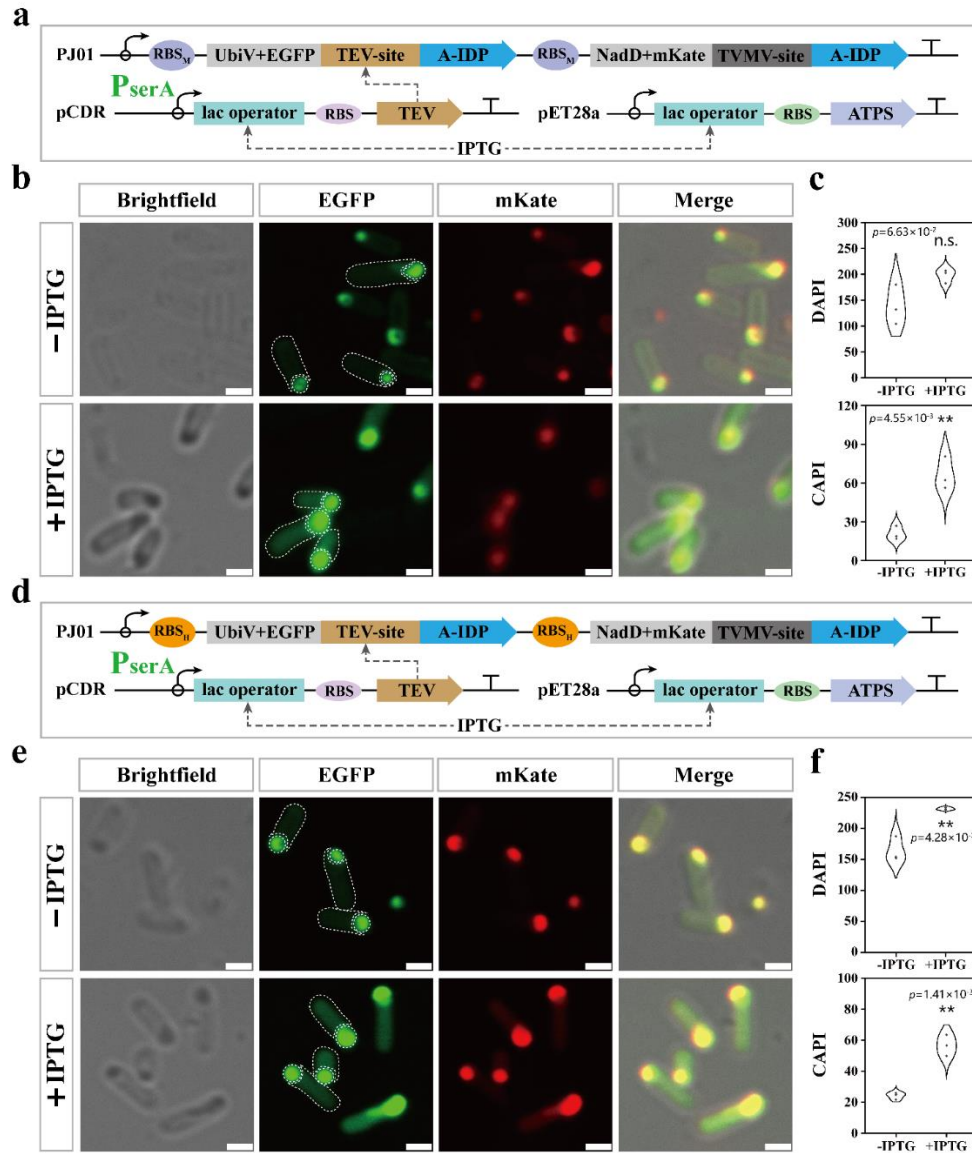
Supplementary Figure 38. The fold change in transcript levels of nine candidate genes in strains TC-E1 (a) and TC-E2 (b), respectively. Source data are provided as a Source Data file.



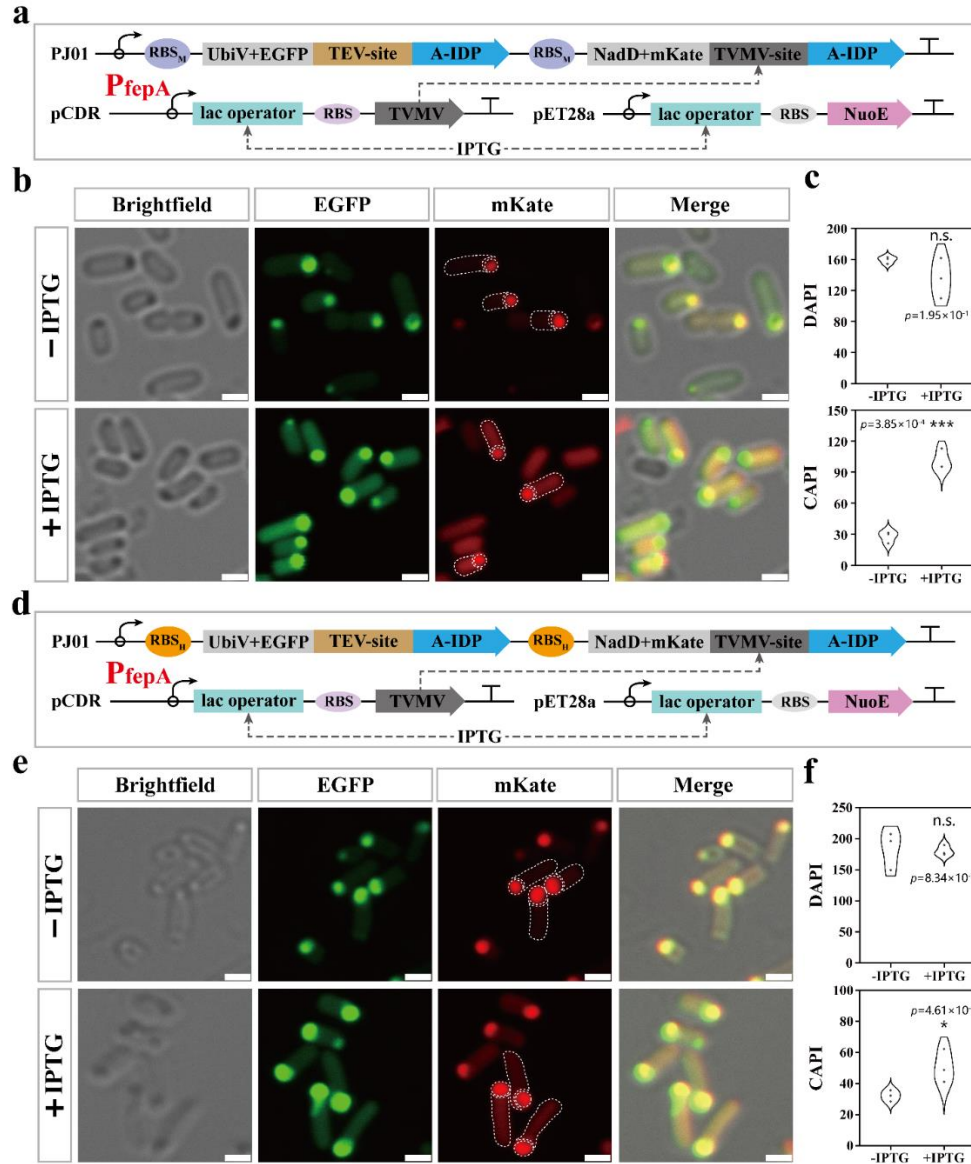
Supplementary Figure 39. Schematic diagram, laser confocal microscopy images, and pixel intensity analysis of the control group without protein capacitor. The white scale bar at the bottom right of microscope image represented 2.5 μm. The pixel intensity data were collected by laser confocal microscopy software LAS AF Lite along the track of white solid arrow in microscope image. Abbreviation: RBS_H, high strength ribosome binding site; EGFP, enhanced green fluorescent protein; mKate, a basic red fluorescent protein. All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



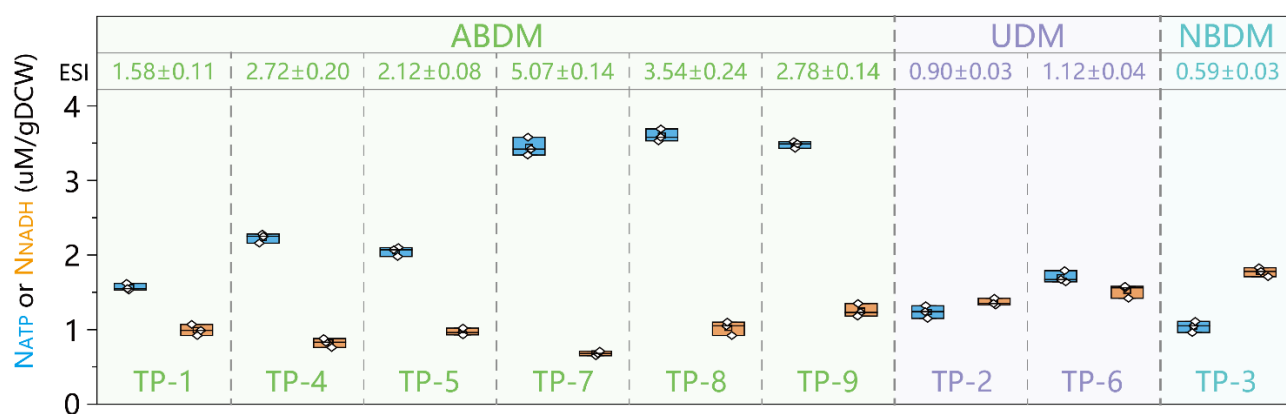
Supplementary Figure 40. Three degrees (low, medium, and high degree) of charging in protein capacitor was achieved through three strengths of RBS (RBS32, RBS30, and RBS34). Abbreviation: RBS_H, high strength ribosome binding site; RBS_M, medium strength ribosome binding site; RBS_L, low strength ribosome binding site; RBS, ribosome binding site; EGFP, enhanced green fluorescent protein; mKate, a basic red fluorescent protein; TEV-site, TEV recognition site; TVMV-site, TVMV recognition site; A-IDP, artificial intrinsically disordered protein.



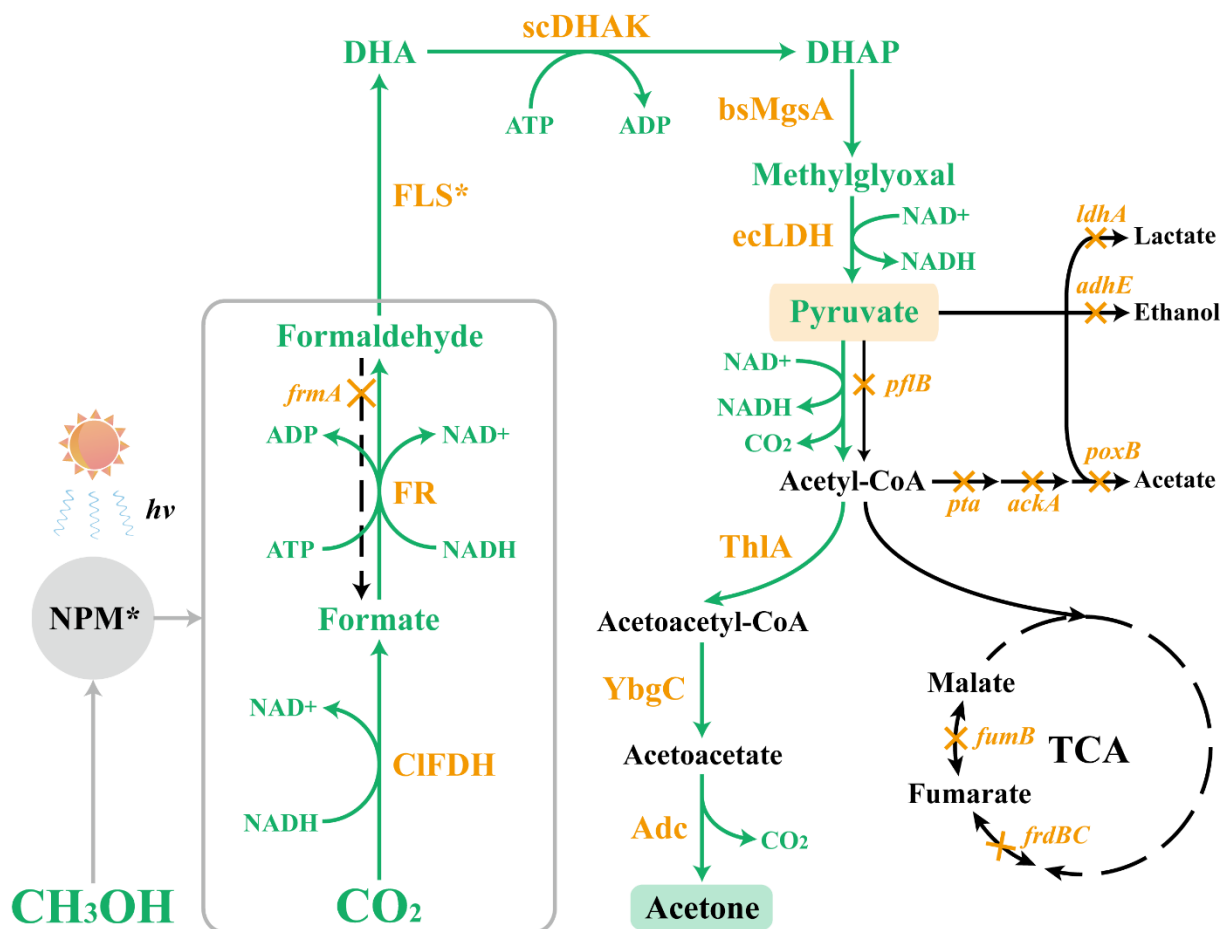
Supplementary Figure 41. Construction and evaluation of energy adapter in *E. coli*. **a, d**, Schematic diagram of constructing the energy adapter by assembling the P_{serA} promoter-based energy responder and the regulatory protein (UbiV and NadD)-based protein capacitor with medium (**a**) and high (**d**) degree of charging. Abbreviation: RBS_M, medium strength ribosome binding site; RBS_H, high strength ribosome binding site; EGFP, enhanced green fluorescent protein; mKate, a basic red fluorescent protein; TEV-site, TEV recognition site; TVMV-site, TVMV recognition site; A-IDP, artificial intrinsically disordered protein; P_{serA}, promoter serA; RBS, ribosome binding site; TEV, tobacco etch virus protease; ATPS, ATP sulfurylase; IPTG, isopropyl- β -D-thiogalactopyranoside. **b-c, e-f**, Laser confocal microscopy images and pixel intensity analysis to verify the effectiveness of energy adapter that senses and regulates the intracellular ATP levels in standby and running modes. The white scale bar at the bottom right of microscope image represented 1.0 μ m. The average pixel intensity data within the white dashed circle were collected by software Image J in microscope image. The white dashed circles represented the outlines of cytoplasmic region and droplet region in microscope image, respectively. Values were shown as mean $\pm$ s.d. from three biological replicates (**c, f**). All experiments were repeated independently for three times with similar results (**b, e**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided t -test. Source data are provided as a Source Data file.



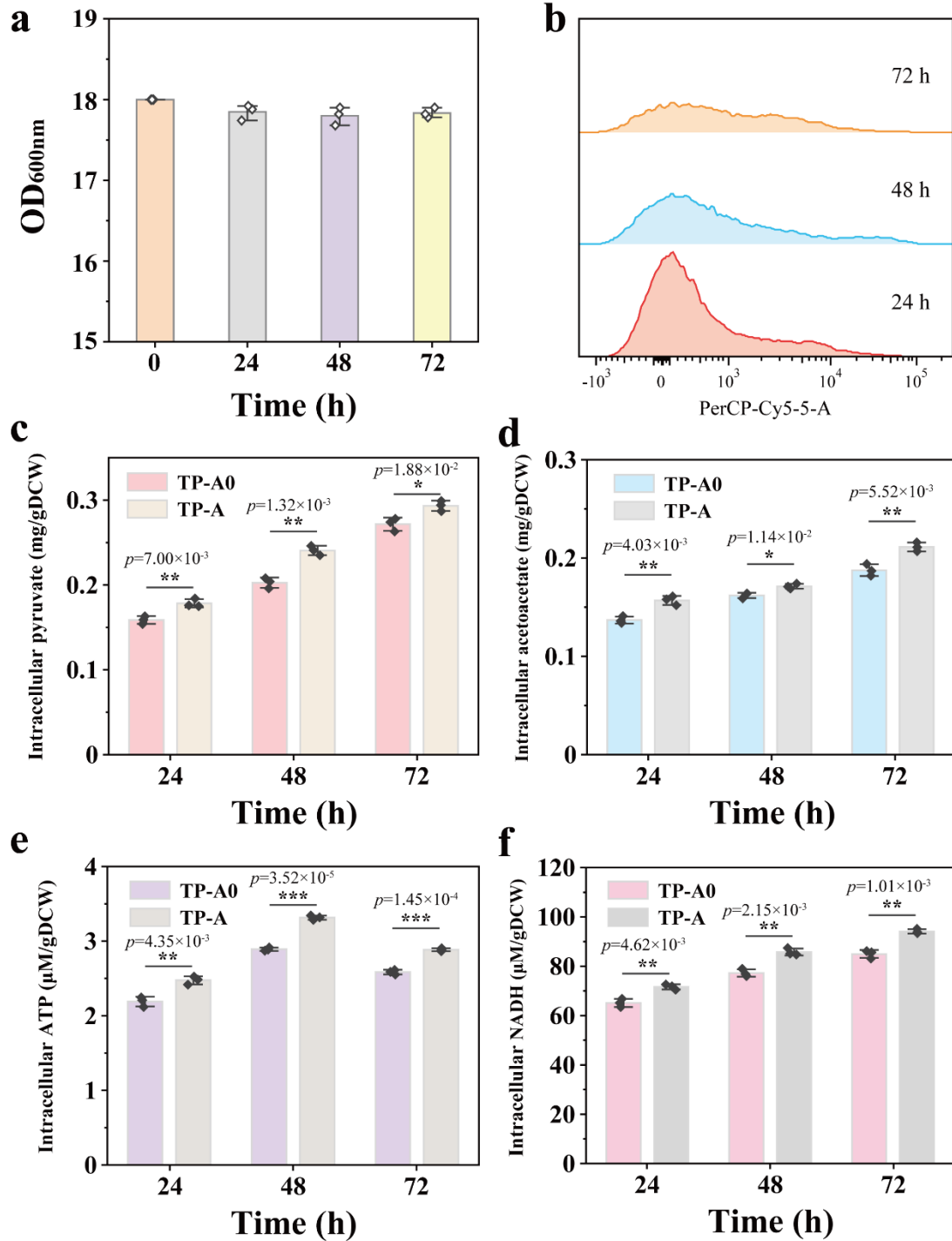
Supplementary Figure 42. Construction and evaluation of energy adapter in *E. coli*. **a, d**, Schematic diagram of constructing the energy adapter by assembling the P_{fepA} promoter-based energy responder and the regulatory protein (UbiV and NadD)-based protein capacitor with medium (**a**) and high (**d**) degree of charging. Abbreviation: RBS_M , medium strength ribosome binding site; RBS_H , high strength ribosome binding site; EGFP, enhanced green fluorescent protein; mKate, a basic red fluorescent protein; TEV-site, TEV recognition site; TVMV-site, TVMV recognition site; A-IDP, artificial intrinsically disordered protein; P_{fepA} , promoter *fepA*; RBS, ribosome binding site; TVMV, tobacco vein mottling virus protease; NuoE, NADH dehydrogenase complex subunit E; IPTG, isopropyl- β -D-thiogalactopyranoside. **b-c, e-f**, Laser confocal microscopy images and pixel intensity analysis to verify the effectiveness of energy adapter that senses and regulates the intracellular NADH levels in standby and running modes. The white scale bar at the bottom right of microscope image represented 1.0 μm . The average pixel intensity data within the white dashed circle were collected by software Image J in microscope image. The white dashed circles represented the outlines of cytoplasmic region and droplet region in microscope image, respectively. Values were shown as mean $\pm$ s.d. from three biological replicates (**c, f**). All experiments were repeated independently for three times with similar results (**b, e**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided *t*-test. Source data are provided as a Source Data file.



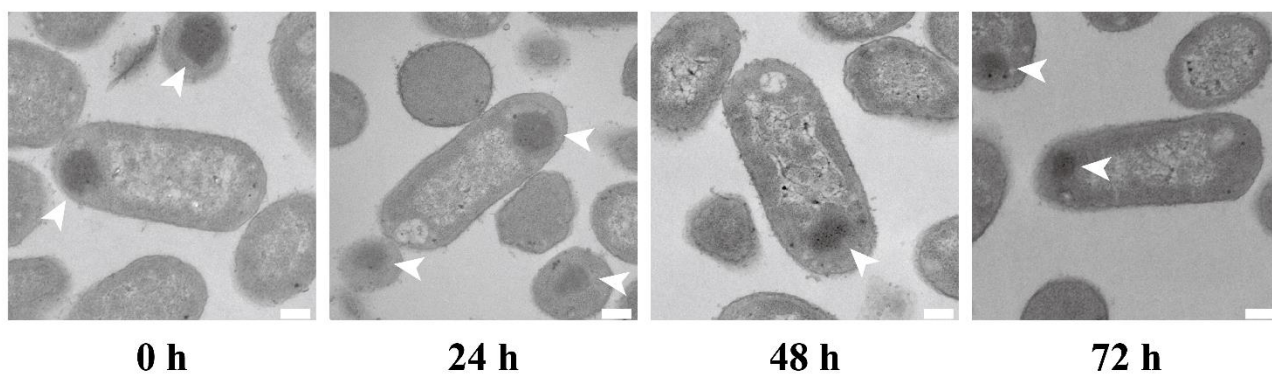
Supplementary Figure 43. The ESI value of strains TP-1~TP-9 was estimated by measuring the C_{ATP} and C_{NADH} before and after the discharging of protein capacitor. Abbreviation: ESI, energy supply index; ABDM, ATP-biased distribution mode; UDM, ATP and NADH-unbiased distribution mode; NBDM, NADH-biased distribution mode. Values were shown as mean $\pm$ s.d. from three biological replicates. Source data are provided as a Source Data file.



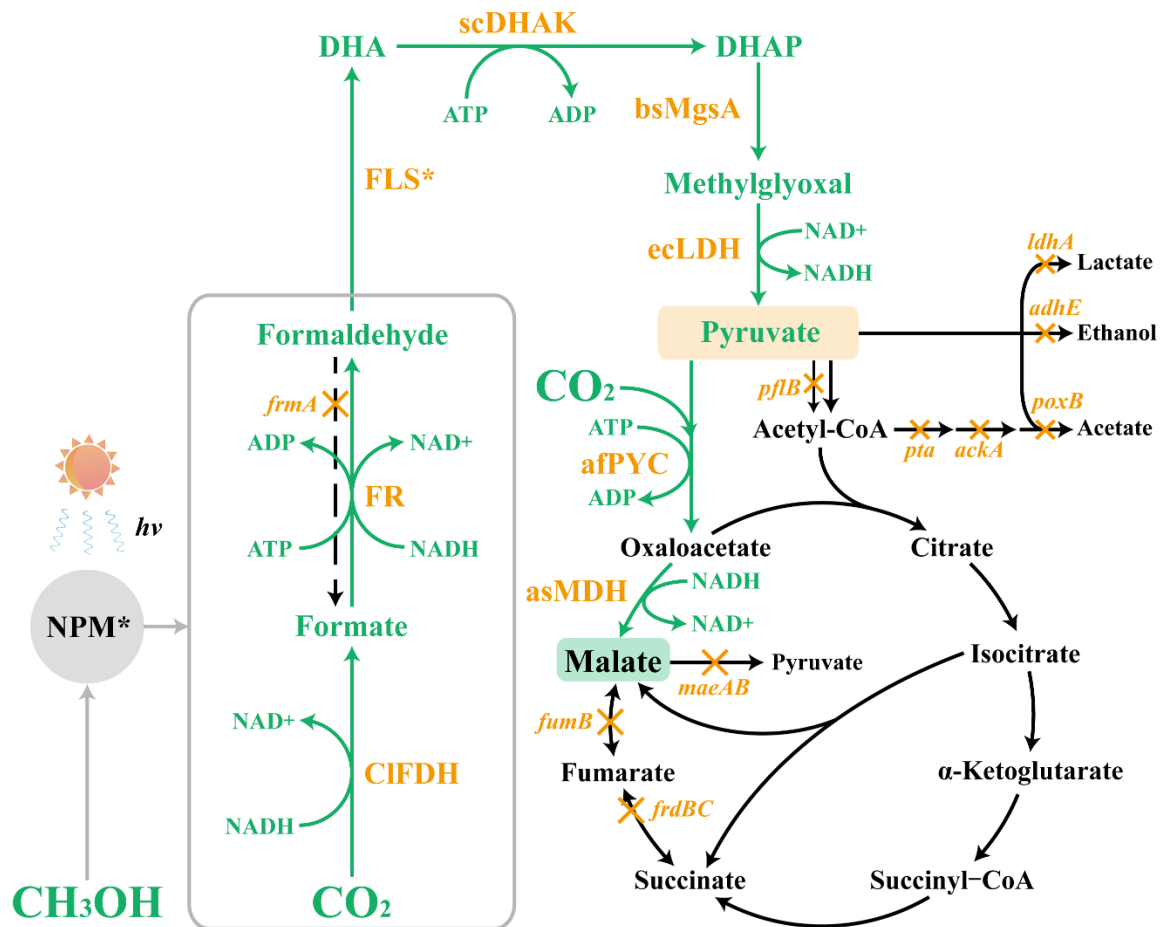
Supplementary Figure 44. Detailed metabolic pathway for ADRP-based acetone synthesis. Abbreviations: CO₂, carbon dioxide; CH₃OH, methanol (as an electron sacrificial agent, its oxidation products are utilized by the C1 module⁸); NPM*, photosystem NuoK*+PufL+MgP; *hν*, photon energy; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; ATP/ADP, adenosine triphosphate/diphosphate; NADH/NAD⁺, nicotinamide adenine dinucleotide reduced/oxidized; ThIA, acetyl-CoA acetyltransferase; YbgC, acyl-CoA thioesterase; Adc, acetoacetate decarboxylase⁹; TCA, tricarboxylic acid cycle.



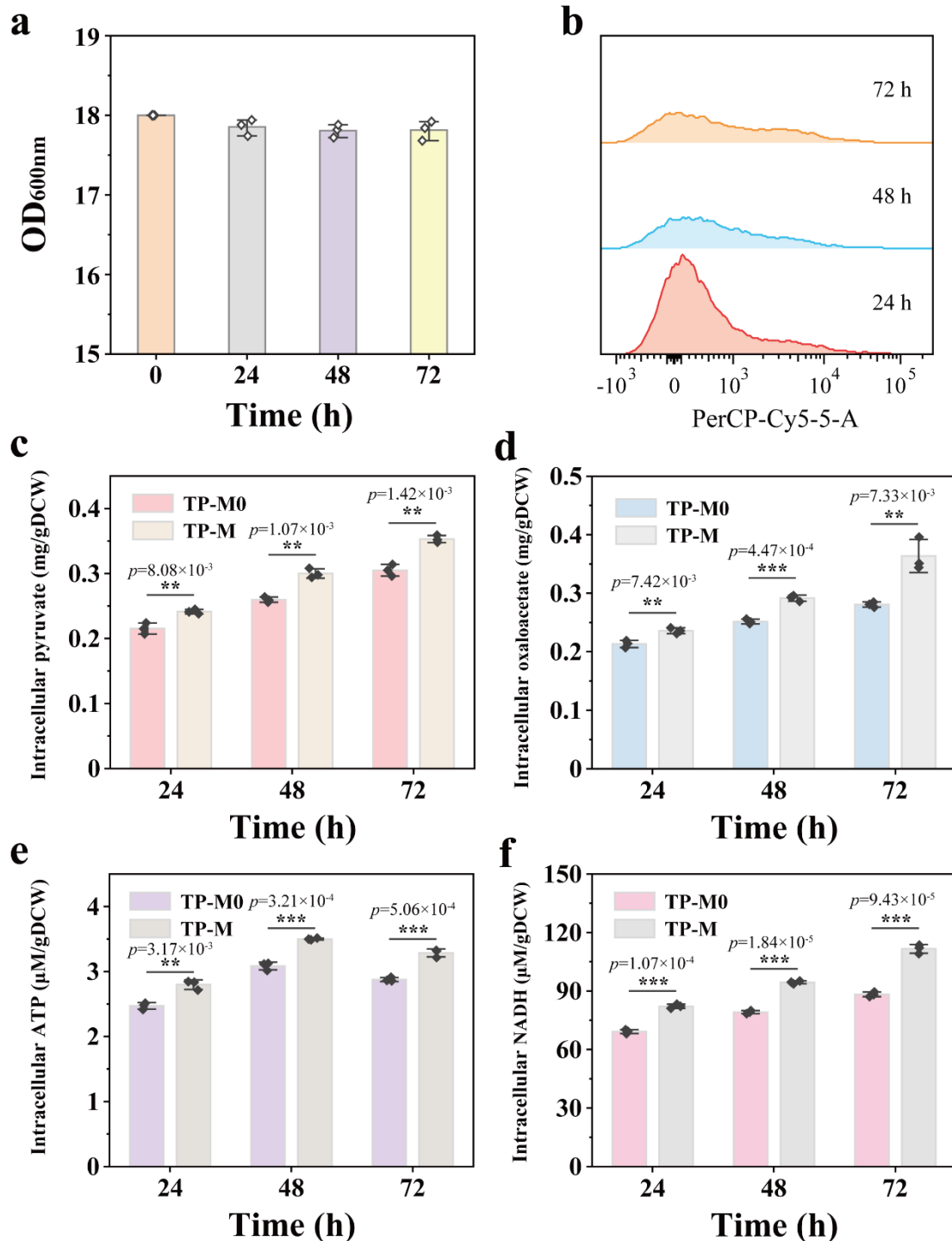
Supplementary Figure 45. Evaluation of OD_{600nm}, cell viability, and intracellular metabolite content for strain TP-A. **a**, The OD_{600nm} value of strain TP-A in the resting cell catalytic stage. **b**, Comparison of cell viability for strain TP-A at different time point during resting cell catalytic stage using flow cytometry. Half offset of histograms. PI signals were measured on the PerCP-Cy5-5-A channel. The mortality of strain TP-A was 20.2%, 29.5%, and 35.5% after 24 h, 48 h, and 72 h resting cell catalysis, respectively. **c-f**, The intracellular pyruvate (**c**), acetoacetate (**d**), ATP (**e**), and NADH (**f**) contents of strains TP-A0 and TP-A at 24 h, 48 h, and 72 h during resting cell catalytic stage, respectively. Values were shown as mean $\pm$ s.d. from three biological replicates (**a**, **c-f**). All experiments were repeated independently for three times with similar results (**b**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided t -test. Source data are provided as a Source Data file.



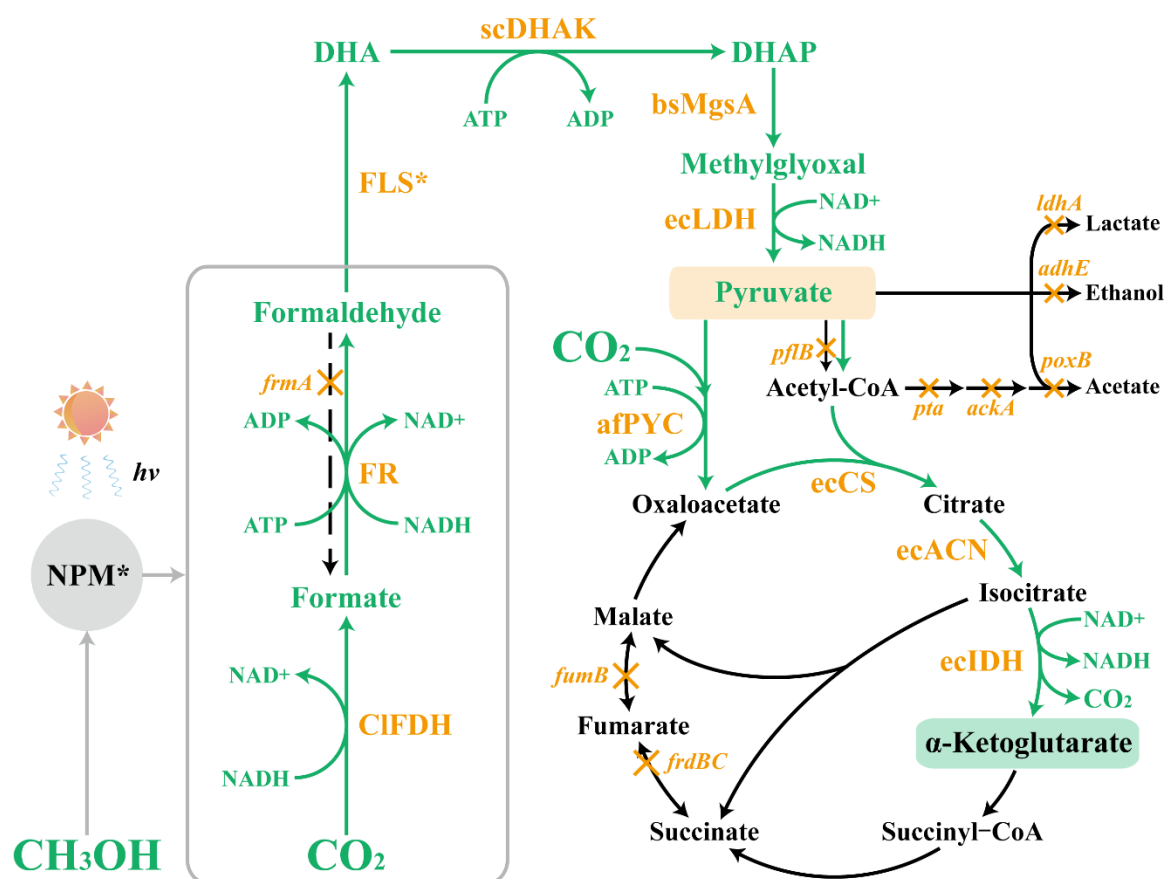
Supplementary Figure 46. Transmission electron microscopy (TEM) micrograph of strain TP-A. TEM micrograph of fixed and sectioned strain TP-A showed the gradual discharge of intracellular protein capacitor during the resting cell catalytic stage. Strains TP-M and TP-K had similar results. The white arrows point to protein capacitor. The white scale bar at the bottom right of microscope image represented 200 nm. All experiments were repeated independently for three times with similar results. Source data are provided as a Source Data file.



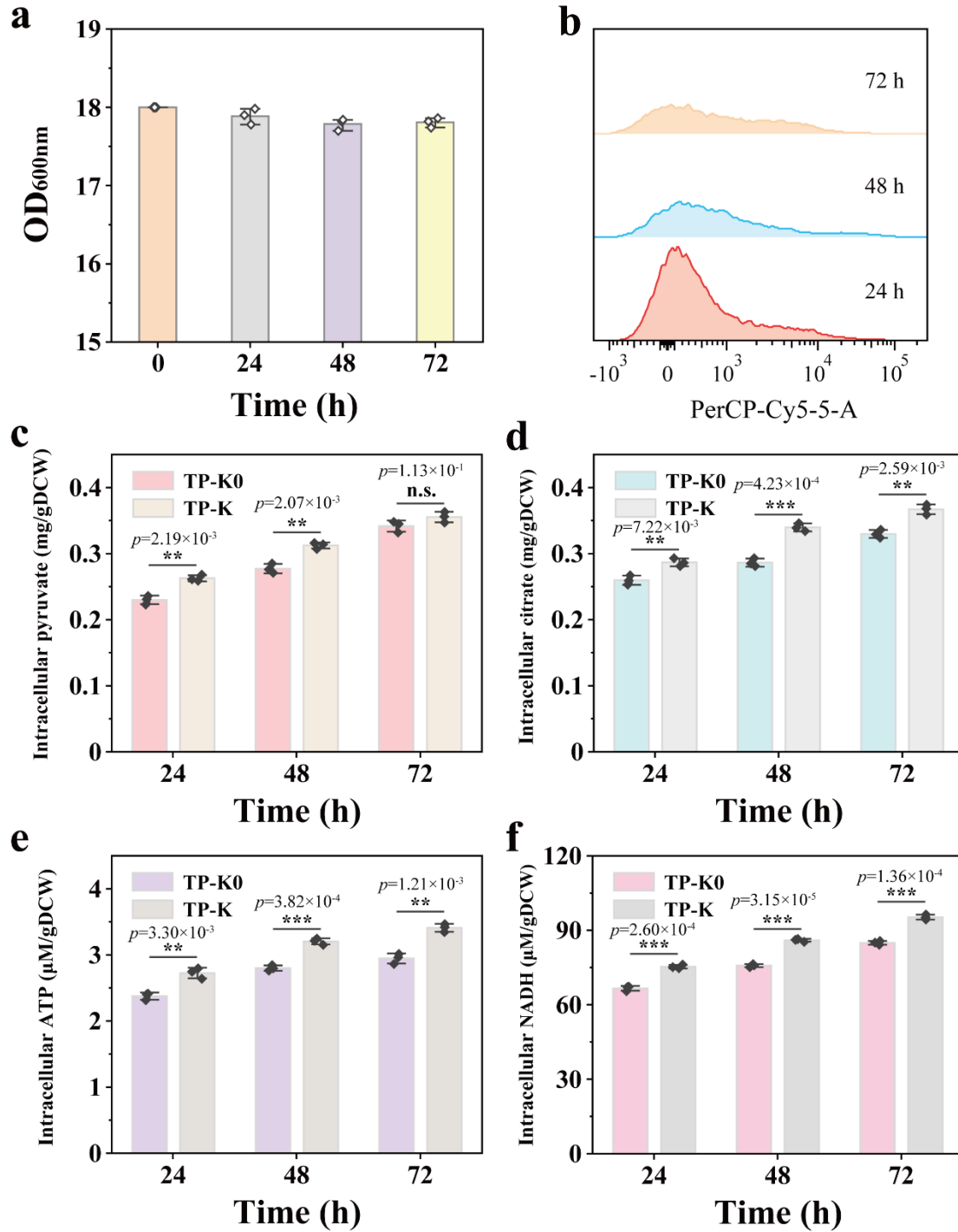
Supplementary Figure 47. Detailed metabolic pathway for ADRP-based malate synthesis. Abbreviations: CO_2 , carbon dioxide; CH_3OH , methanol (as an electron sacrificial agent, its oxidation products are utilized by the C1 module); NPM*, photosystem NuoK*+PufL+MgP; $h\nu$, photon energy; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; ATP/ADP, adenosine triphosphate/diphosphate; NADH/NAD^+ , nicotinamide adenine dinucleotide reduced/oxidized; afPYC, pyruvate carboxylase; asMDH, malate dehydrogenase¹⁰.



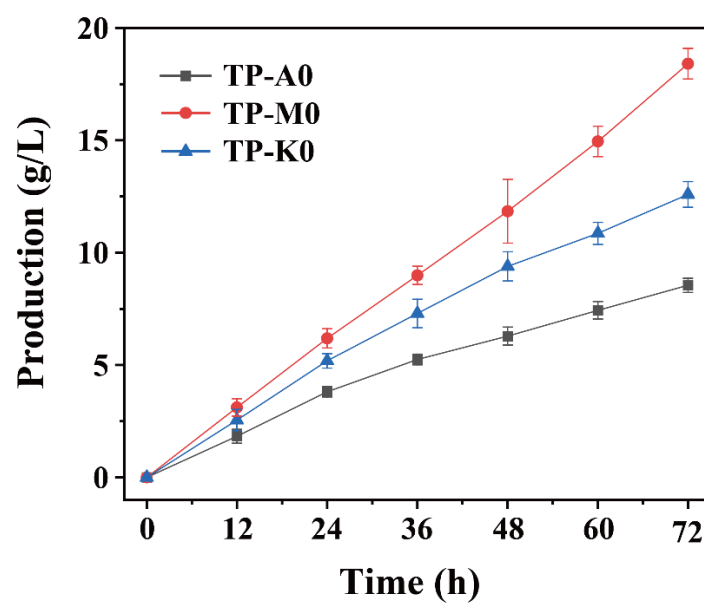
Supplementary Figure 48. Evaluation of OD_{600nm}, cell viability, and intracellular metabolite content for strain TP-M. **a**, The OD_{600nm} value of strain TP-M in the resting cell catalytic stage. **b**, Comparison of cell viability for strain TP-M at different time point during resting cell catalytic stage using flow cytometry. Half offset of histograms. PI signals were measured on PerCP-Cy5-5-A channel. The mortality of strain TP-M was 17.6%, 27.7%, and 32.9% after 24 h, 48 h, and 72 h resting cell catalysis, respectively. **c-f**, The intracellular pyruvate (**c**), oxaloacetate (**d**), ATP (**e**), and NADH (**f**) contents of strains TP-M0 and TP-M at 24 h, 48 h, and 72 h during resting cell catalytic stage, respectively. Values were shown as mean $\pm$ s.d. from three biological replicates (**a**, **c-f**). All experiments were repeated independently for three times with similar results (**b**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided t -test. Source data are provided as a Source Data file.



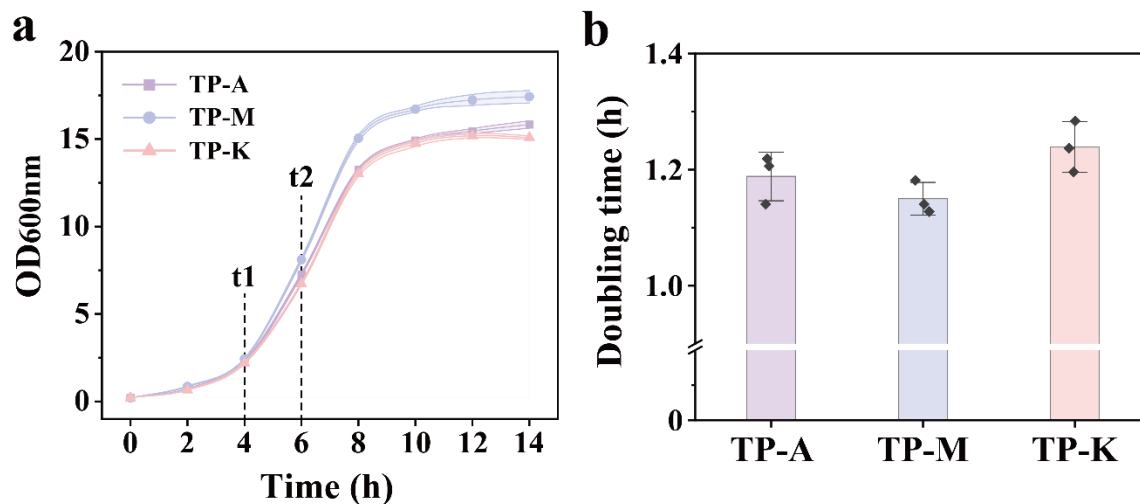
Supplementary Figure 49. Detailed metabolic pathway for ADRP-based α -ketoglutarate synthesis. Abbreviations: CO₂, carbon dioxide; CH₃OH, methanol (as an electron sacrificial agent, its oxidation products are utilized by the C1 module); NPM*, photosystem NuoK*+PufL+MgP; $h\nu$, photon energy; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; ATP/ADP, adenosine triphosphate/diphosphate; NADH/NAD⁺, nicotinamide adenine dinucleotide reduced/oxidized; afPYC, pyruvate carboxylase; ecCS, citrate synthase; ecACN, aconitase; ecIDH, isocitrate dehydrogenase¹¹.



Supplementary Figure 50. Evaluation of OD_{600nm}, cell viability, and intracellular metabolite content for strain TP-K. **a**, The OD_{600nm} value of strain TP-K in the resting cell catalytic stage. **b**, Comparison of cell viability for strain TP-K at different time point during resting cell catalytic stage using flow cytometry. Half offset of histograms. PI signals were measured on the PerCP-Cy5-5-A channel. The mortality of strain TP-K was 19.3%, 28.5%, and 33.3% after 24 h, 48 h, and 72 h resting cell catalysis, respectively. **c-f**, The intracellular pyruvate (**c**), citrate (**d**), ATP (**e**), and NADH (**f**) contents of strains TP-K0 and TP-K at 24 h, 48 h, and 72 h during resting cell catalytic stage, respectively. Values were shown as mean $\pm$ s.d. from three biological replicates (**a**, **c-f**). All experiments were repeated independently for three times with similar results (**b**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significance, as determined by two-sided t -test. Source data are provided as a Source Data file.



Supplementary Figure 51. The production of acetone, malate, and α -KG for strains TP-A0, TP-M0, and TP-K0, respectively. Values were shown as mean $\pm$ s.d. from three biological replicates. Source data are provided as a Source Data file.



Supplementary Figure 52. Evaluation of growth performance and doubling time for strains TP-A, TP-M, and TP-K. **a**, Growth performance (OD_{600nm}) of strains TP-A, TP-M, and TP-K in TB medium. **b**, The doubling times of strains TP-A, TP-M, and TP-K in TB medium (initial OD_{600nm} = 0.2, 200 rpm, 30°C), respectively. The doubling time of strains could be calculated by reported equation¹². The doubling times of strains TP-A, TP-M, and TP-K in TB medium were 1.19 h, 1.15 h, and 1.24 h, respectively. Values were shown as mean ± s.d. from three biological replicates. Source data are provided as a Source Data file.

Supplementary Note 1. Analysis of transient absorption spectroscopy of MgP in methanol solution.

As shown in supplementary Fig. 9a-b, the transient absorption spectroscopy of MgP in methanol solution revealed the existence of four distinguishable spectral features. An intensive negative signal centered around 417 nm is in the Soret band absorption region so that it could attribute to ground state bleach (GSB). The three positive signals centered around 465 nm, 560 nm, and 600 nm could attribute to excited states absorption (ESA).

Supplementary Note 2. The validation of the photoelectron-generating properties of MgP molecules in methanol

As shown in Supplementary Fig. 11a-b, the photoelectron-generating properties of the MgP molecules were verified by evaluating the photocatalytic activity of MgP for degrading the dyes RhB and congo red under blue light illumination. The absorbance values of RhB and congo red solutions were decreased by 9.9% and 60.0% compared with those of the dark condition after blue light illumination, respectively. As shown in Supplementary Fig. 11c, the absorbance values of RhB and congo red solutions were not significantly different in the absence of MgP molecules after blue light illumination. As shown in Supplementary Fig. 11f, the formaldehyde contents in the dyes RhB and congo red degradation systems were 8.85 mM and 18.19 mM after blue light illumination, respectively. These results showed that the MgP molecules had the property of photoelectron generation (methanol as an electron sacrificial agent) under blue light illumination.

Supplementary Note 3. UV-Vis spectrum scanning and the detection of MgP content of the methanol extract for strains TL-M0 and TL-M1

As shown in Supplementary Fig. 13, UV-Vis spectrum results of the methanol extract of strain TL-M1 showed that the characteristic absorption peak at 394 nm was significantly higher than that of the control strain TL-M0. As shown in Supplementary Fig. 14a, the MgP content in the methanol extract of strain TL-M1 was 19.3-fold higher than that of the control strain TL-M0. These results indicated that MgP molecules were successfully synthesized in *E. coli*.

Supplementary Note 4. Molecular docking analysis of MgP and PufL

To elucidate theoretically that MgP could be integrated into PufL, molecular docking analysis was performed by Autodock Vina. As shown in Supplementary Fig. 15a, natural PufL+BCB model (PDB ID code 5PRC)⁴ indicated that the assembly way of BCB and PufL was through the formation of coordination bonds between Mg²⁺ and the meta-N atom of residues histidine 153 (HIS 153) and histidine 173 (HIS 173). As shown in Supplementary Fig. 15b, PufL+MgP model indicated that MgP (PubChem CID 44229120) lacking the phytol tail could also be integrated into PufL in the same assembly way.

Supplementary Note 5. Transient kinetics analysis of intracellular MgP molecules

As shown in Fig. 2e, the difference spectra represent the transient kinetics of intracellular MgP molecules. According to literature^{13, 14}, when charge transfer occurs, a bi-exponential model function (as follows) was used to fit the decay dynamic of ground state bleach (GSB).

$$N(t) = N_0 + N_1 e^{-t/\tau_1} + N_2 e^{-t/\tau_2} \quad (1)$$

where $N(t)$ is the photoexcited electron population at a pump-probe delay of t , τ_1 and τ_2

represents the charge transfer process and delayed recombination process due to charge transfer.

The transient kinetics of intracellular MgP molecules were fitted to obtain the best-fit bi-exponential model function:

$$N(t) = 1.49756 \times 10^{-4} + 1.06396 \times 10^{-4} \times e^{-t/44.0693} - 8.32917 \times 10^{-5} \times e^{-t/559.33043} \quad (2)$$

where $\tau_1 = 44.07$ ps and $\tau_2 = 559.33$ ps. These results indicated that the observed charge transfer time from MgP to cells is around 44.07 ps which is accompanied by 559.33 ps delayed recombination time.

Supplementary Note 6. Evaluation of growth performance and intracellular ATP and NADH contents for strains TL-NPM and TL-NPM*

As shown in Supplementary Fig. 17a, to drive NPM in the engineered strain TL-NPM to provide electrons for the electron transport chain (ETC), two conserved sites of NuoK were mutated (NuoK^{E36A/E72Q}, NuoK*) to reduce the activity of the NADH dehydrogenase complex¹⁵, generating strain TL-NPM*. As a result, the OD_{600nm} of strain TL-NPM* reached 5.64 at 10 h, which was 30.1% lower than that of the control strain TL-NPM. Its intracellular ATP content (C_{ATP}) was decreased by 26.7% at 12 h, but its intracellular NADH content (C_{NADH}) was increased by 55.5%, compared with that of the control strain TL-NPM (Supplementary Fig. 17b-d). These results showed that NPM* (NuoK*+PufL+MgP) can be successfully used as an alternative electron source for the ETC.

Supplementary Note 7. Evaluation of growth performance, intracellular ATP and NADH content, ROS levels, and flagella motility for strain TL-NPM*

As shown in Supplementary Fig. 20a, the growth of strain TL-NPM* was inhibited under 50 mW/cm², 75 mW/cm², and 100 mW/cm² blue light illumination, and its OD_{600nm} values were decreased by 24.9%, 54.8%, and 90.2% compared with those of dark condition, respectively. The reason might be due to the generation of reactive oxygen species (ROS) from the photoexcitation of metalloporphyrins^{16, 17}.

As shown in Supplementary Fig. 20c, the relative ROS levels of strain TL-NPM* under 50 mW/cm², 75 mW/cm², and 100 mW/cm² blue light illumination were 2.3-, 4.6-, and 6.8-fold higher than those of dark condition, respectively. However, the growth of strain TL-NPM* was not affected under 25 mW/cm² blue light illumination. These results indicated that the potential toxicity induced by 25 mW/cm² blue light illumination was negligible for strain TL-NPM*.

As shown in Supplementary Fig. 20d, when fomesafen was added, the intracellular ATP and NADH levels of strain TL-NPM* were decreased by 29.0% and 18.1% at 25 h, respectively. In addition, when oxyfluorfen was added, the intracellular ATP and NADH levels of strain TL-NPM* were decreased by 40.9% and 25.9% at 25 h, respectively. These results indicated that strain TL-NPM* was more sensitive to oxyfluorfen compared with that of fomesafen.

As shown in Supplementary Fig. 22a-b, the flagella motility of strain TL-NPM* was more active under blue light illumination, and its colony diameter was increased by 33.0% compared with that under dark condition. These results indicated that the light reaction could convert light energy into ATP to promote flagella motility.

Supplementary Note 8. Transcriptomic analysis for strain TL-NPM* under dark condition and blue light illumination

The transcriptional levels of genes associated with ubiquinone biosynthesis (*ubiK* and *ubiV*), electron transport (*fdnI*, *yceJ*, and *yodB*), and cytochrome bd complex (*appB*) were increased under blue light illumination, suggesting that the operation of NPM* could play a positive effect on enhancing the efficiency of electron transfer and proton motive force (PMF) generation in the IM. The transcriptional levels of NAD⁺ biosynthesis-related pathway genes (*nadB*, *nadD*, and *pncC*) were up-regulated, which could provide more precursor NAD⁺ for the generation of NADH by the NPM*. In addition, the transcriptional levels of flagellar assembly-related genes (*fliD*, *fliI*, *fliM*, *fliN*, *flgF*, and *flgJ*) were significantly up-regulated, suggesting that the increase of intracellular ATP levels could promote flagella motility (Supplementary Note 18).

Supplementary Note 9. Screening of target genes that regulate ATP and NADH generation

As shown in Supplementary Fig. 24, based on transcriptomic analysis, the genes *appB*, *fdnI*, *ubiK*, *ubiV*, *yceJ*, and *yodB* for ATP supply were obtained and then overexpressed in strain TL-A (harboring ATP fluorescence sensor QUEEN2m) to generate a series of strains TL-A1~TL-A6, respectively. The intracellular QUEEN2m fluorescence signal of strain TL-A4 (overexpressing *ubiV*) was increased by 34.3% under blue light illumination compared with that of the control strain TL-A0 (Supplementary Fig. 24a). Moreover, the genes *nadB*, *nadD*, and *pncC* for NADH supply were overexpressed in strain TL-N (harboring NADH fluorescence sensor Frex) to obtain a series of strains TL-N1~TL-N3, respectively. The intracellular Frex fluorescence signal of strain TL-N2 (overexpressing *nadD*) was increased by 94.0% under blue light illumination compared with that of the control strain TL-N0 (Supplementary Fig. 24b). Thus, *ubiV* and *nadD* were selected as target genes to regulate the efficiency of ATP and NADH generation, respectively.

Supplementary Note 10. Evaluation of intracellular ATP content for strain TC-E1 and intracellular NADH content for strain TC-E2

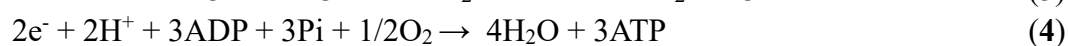
As shown in Supplementary Fig. 37a, the intracellular ATP content of strain TC-E1 were 1.16 and 0.89 $\mu\text{M/gDCW}$ after 2 h and 4 h induction with IPTG, compared with those of the control strain TC-E0 (1.52 and 1.49 $\mu\text{M/gDCW}$) decreased by 23.3% and 40.6%, respectively. As shown in Supplementary Fig. 37b, the intracellular NADH content of strain TC-E2 were 36.8 and 31.9 $\mu\text{M/gDCW}$ after 2 h and 4 h induction with IPTG, compared with those of the control strain TC-E0 (46.2 and 43.2 $\mu\text{M/gDCW}$) decreased by 20.3% and 26.1%, respectively. These results indicated that strains TC-E1 and TC-E2 were constructed as ATP- and NADH-deficient strains by overexpressing ATP sulfurylase (ATPS)¹⁸ and NADH dehydrogenase (NuoE)¹⁹, respectively.

Supplementary Note 11. The nine candidate genes in strains TC-E1 and TC-E2

As shown in Supplementary Fig. 38a-b, nine candidate genes with significantly up-regulated expression levels were identified in strains TC-E1 (*alsK*, *atoB*, *galM*, *gdhA*, *nrfA*, *rpiB*, *sdhC*, *serA*, and *ttdA*) and TC-E2 (*dmlA*, *efeO*, *entC*, *entD*, *fecE*, *fepA*, *fhuF*, *putA*, and *ydbK*), respectively (Supplementary Note 18).

Supplementary Note 12. Theoretical analysis of ATP and NADH generation by NPM*

For wild type *E. coli* cells, one equivalent of NADH provides two equivalents of electrons for the biosynthesis of three equivalents of ATP in oxidative phosphorylation in theory (equation (1))^{20, 21}. In engineered *E. coli*, NPM* replaces NADH to provide two equivalents of electrons to generate three equivalents of ATP in light reaction in theory (equation (2)). Meanwhile, NPM* provides two equivalents of electrons to convert one equivalent of NAD⁺ into one equivalent of NADH in theory (equation (3)). Thus, ATP generation by NPM* is more efficient than NADH generation in theory.

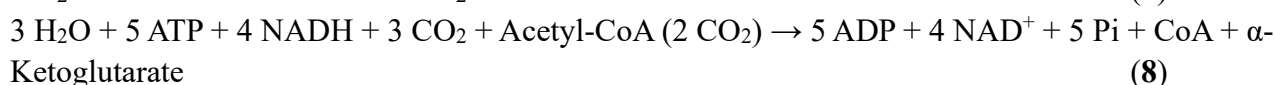


Supplementary Note 13. The ESI value of strains TP-1~TP-9

To verify these hypotheses, a series of strains TP-1~TP-9 were generated by introducing a protein capacitor with different discharging regulators into strain TL-NPMS[#], respectively. As a result, the ESI values of strains TP-1, TP-4, TP-5, TP-7, TP-8, and TP-9 were 1.58, 2.72, 2.12, 5.07, 3.54, and 2.78, respectively, and they were classified as ESI > 1; the ESI values of strains TP-2 and TP-6 were 0.90 and 1.12, respectively, and they were classified as ESI ≈ 1; the ESI value of strain TP-3 was 0.59, and it was classified as ESI < 1. These results indicated that photosynthesis system in *E. coli* could be programmed to achieve the following three modes: ABDM (ESI > 1), UDM (ESI ≈ 1), and NBDM (ESI < 1).

Supplementary Note 14. Theoretical analysis of energy demand index

The ratio of theoretical ATP equivalent to NADH equivalent consumed by the biosynthetic pathway for CO₂ utilization was defined as the energy demand index (EDI) (Fig. 7b). By analyzing the eQuilibrator pathway model, the net reaction equation of the ADRP-based acetone biosynthesis pathway is equation (4), and the EDI value was 8/8 = 1 (Supplementary Fig. 44). The net reaction equation of the ADRP-based malate biosynthesis pathway is equation (5), with an EDI value is 5/6 < 1 (Supplementary Fig. 47). The net reaction equation of the ADRP-based α-ketoglutarate (α-KG) biosynthesis pathway is equation (6), with an EDI value is 5/4 > 1 (Supplementary Fig. 49).



Supplementary Note 15. Definition of CO₂ utilization rate in this study

It should be noted here that the CO₂ utilization rate represents the rate at which CO₂ and methanol are only stored in bioproducts. In addition, to calculate the negative carbon footprint of bioproduction using iPBRC strategy, one equivalent of methanol was equated to one equivalent of CO₂ to calculate the CO₂ utilization rate in resting catalytic (RC) stage.

Supplementary Note 16. Calculation method of CO₂ utilization rate

To determine the CO₂ utilization rate during acetone, malate, and α-ketoglutarate production, NaH¹³CO₃ and ¹³CH₃OH were used for ¹³C-labeling analysis. The CO₂ utilization rate was calculated as: CO₂ stored in bioproducts (mg)/DCW (g)/time interval (h).

The formula for calculating the CO₂ utilization rate of acetone production is as follows:

$$R_A = (\sum_{i=1}^3 \frac{P_A * V}{M_A} * F_i * i) * M_{CO_2} / (0.33 * OD_{600nm} * V) / T \quad (9)$$

where R_A represents the CO₂ utilization rate of acetone production, P_A represents the production of acetone at 72 h, M_A represents the molar mass of acetone, V represents the working volume, F_i represents the labelled fraction of acetone with i ¹³C atom(s) ($i = 1, 2$, and 3), i represents the number of ¹³C atoms, M_{CO_2} represents the molar mass of CO₂, OD_{600nm} represents the optical density (600 nm) of catalytic mixture at 72 h, and T represents the time interval.

The formula for calculating the CO₂ utilization rate of malate production is as follows:

$$R_M = (\sum_{i=1}^4 \frac{P_M * V}{M_M} * F_i * i) * M_{CO_2} / (0.33 * OD_{600nm} * V) / T \quad (10)$$

where R_M represents the CO₂ utilization rate of malate production, P_M represents the production of malate at 72 h, M_M represents the molar mass of malate, V represents the working volume, F_i represents the labelled fraction of malate with i ¹³C atom(s) ($i = 1, 2, 3$, and 4), i represents the number of ¹³C atoms, M_{CO_2} represents the molar mass of CO₂, OD_{600nm} represents the optical density (600 nm) of catalytic mixture at 72 h, and T represents the time interval.

The formula for calculating the CO₂ utilization rate of α -ketoglutarate production is as follows:

$$R_K = (\sum_{i=1}^5 \frac{P_K * V}{M_K} * F_i * i) * M_{CO_2} / (0.33 * OD_{600nm} * V) / T \quad (11)$$

where R_K represents the CO₂ utilization rate of α -ketoglutarate production, P_K represents the production of α -ketoglutarate at 72 h, M_K represents the molar mass of α -ketoglutarate, V represents the working volume, F_i represents the labelled fraction of α -ketoglutarate with i ¹³C atom(s) ($i = 1, 2, 3, 4$, and 5), i represents the number of ¹³C atoms, M_{CO_2} represents the molar mass of CO₂, OD_{600nm} represents the optical density (600 nm) of catalytic mixture at 72 h, and T represents the time interval.

Supplementary Note 17. Semi-quantitative estimation of solar-to-chemical energy efficiency

To semi-quantitatively estimate the solar-to-chemical energy efficiency (SCEE) of this system, we performed calculations based on the formula reported in relevant literature²². The SCEE of this system was calculated using

$$SCEE (\%) = (R(\text{chemical}) \times \Delta G_r) / (P \times S) \times 100 \quad (12)$$

where $R(\text{chemical})$, ΔG_r , P , and S represent the rate of chemical production ($R(\text{acetone}) = 0.0181$ mol/72 h, $R(\text{malate}) = 0.0184$ mol/72 h, $R(\alpha\text{-KG}) = 0.0104$ mol/72 h) during the resting catalytic stage in a 200 mL serum bottle, the estimated Gibbs free energy change (298K, 1atm) of the reaction (ΔG_r (1.185 NaHCO₃ + 1.815 CH₃OH $\rightarrow$ C₃H₆O + 1.185 NaOH + 0.63 H₂O) = 543.7 kJ/mol, ΔG_r (1.58 NaHCO₃ + 2.42 CH₃OH $\rightarrow$ C₄H₆O₅ + 1.58 NaOH + 1.84 H₂O) = 168.5 kJ/mol, ΔG_r (1.975 NaHCO₃ + 3.025 CH₃OH $\rightarrow$ C₅H₆O₅ + 1.975 NaOH + 3.05 H₂O) = 111.2 kJ/mol), the energy intensity of blue light illumination (25 mW/cm²), and the irradiated sample area (approximately 133.45 cm², the surface area of culture medium in a 200 mL serum bottle cylinder. The shape of the culture medium is cylindrical, and the diameter and height are about 5 cm and 6 cm, respectively).

Based on this, we put the relevant data into the formula and calculated that SCEE (acetone), SCEE (malate), and SCEE (α -KG) are about 1.14%, 0.36%, and 0.13%, respectively.

It is worth noting that the P and S parameters can only be estimated, especially S . Here, we take the maximum value of the liquid surface of the culture medium in the cylinder for estimation, but this cannot accurately reflect the actual light energy utilized by the cells. One possibility is that the actual utilization may be smaller than this estimated value. Another possibility is to estimate the amount of light energy absorbed from the cell surface by calculating the surface area of the cell (the surface area of all cells). The number of cells in the culture medium can be estimated, but it is difficult to determine whether all cells have absorbed light energy on their surfaces, it is also difficult to estimate. Therefore, the estimates of P , S , and SCEE are only for readers' reference.

Supplementary Note 18. Transcriptome sequencing analysis in this study

Transcriptome sequencing analysis: The corresponding strains were collected by centrifugation (5000 rpm at 4°C for 5 min) and then snap-frozen using liquid nitrogen. The transcriptome analysis was carried out according to the previous study²³. The sequences were processed and analyzed by Suzhou Genewiz Biotechnology and Shanghai Majorbio. The significance of the differential expression of genes was defined by the bioinformatics service of Genewiz and Majorbio according to the absolute value of $\text{Log}_2\text{FC} \geq 1$ ($\text{FC} \geq 2$)²⁴. Accession number: the RNA-seq raw reads were submitted to NCBI under BioProject number PRJNA1054475, and the Sequence Read Archive (SRA) entries were SRR27292978, SRR27292979, SRR27292980, SRR27292981, and SRR27292982.

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