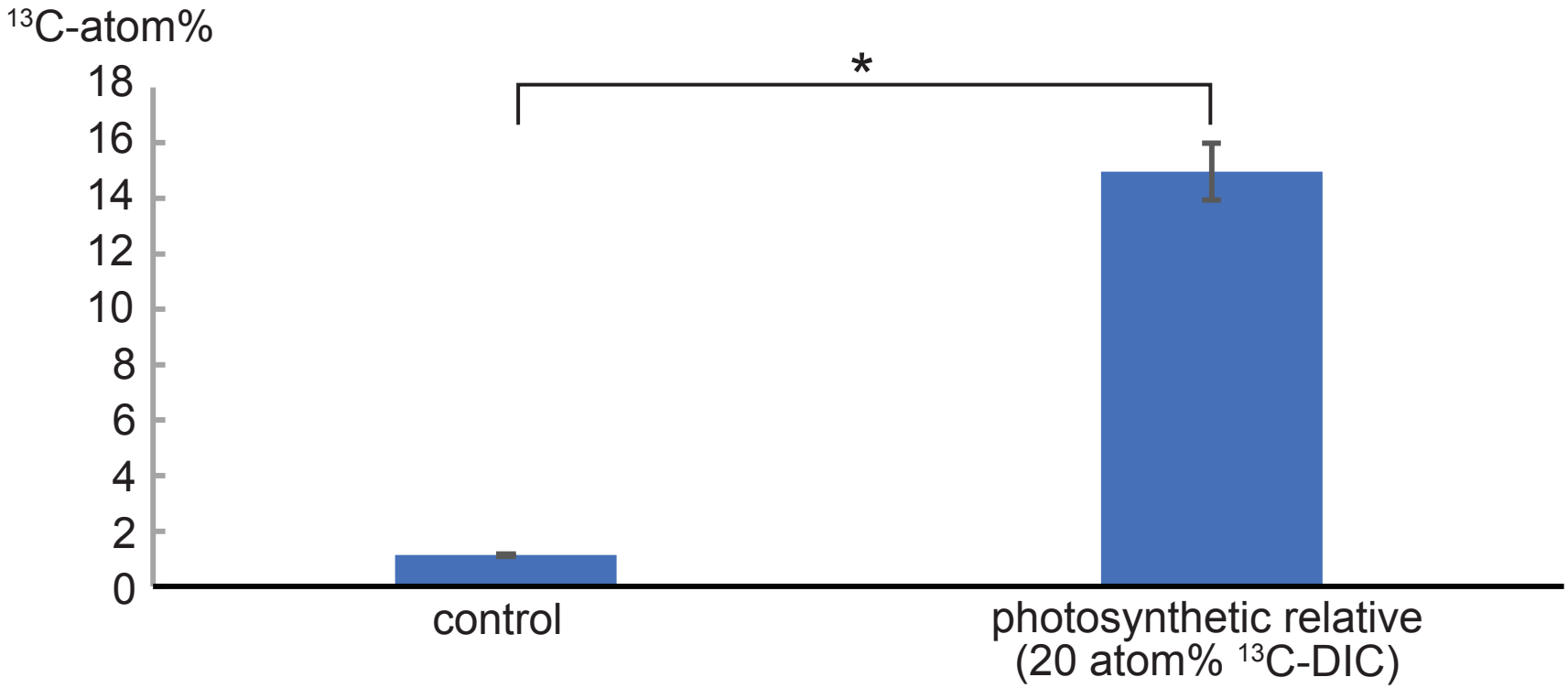
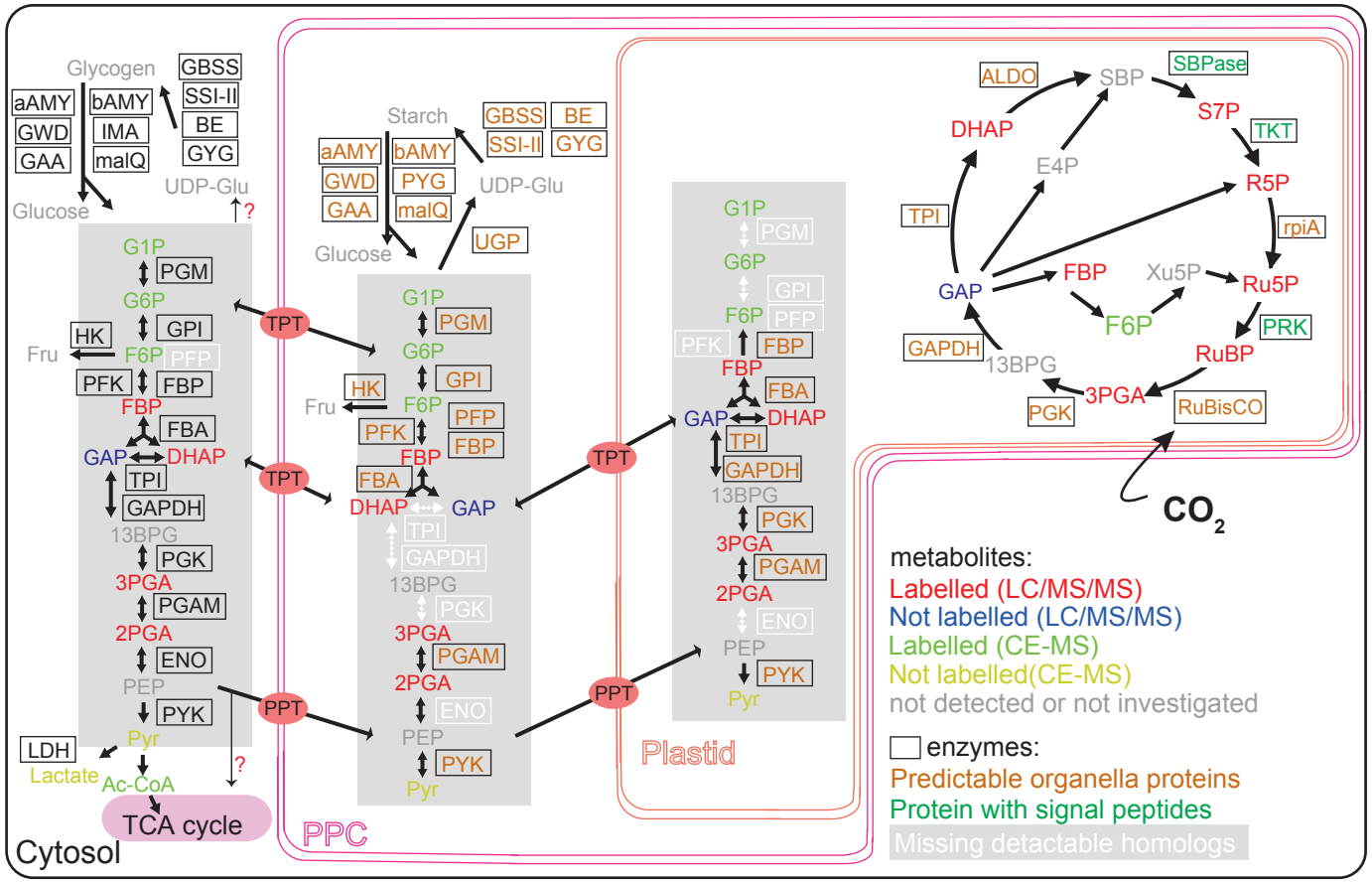


Extended Data 2. EA/IRMS analyses using photosynthetic relative, *Cryptomonas curvata* CCAP979/52. Cells were cultured for 30 days in AF6 medium containing either no ^{13}C-labelling (control) and 20 atom% ^{13}C-DIC.

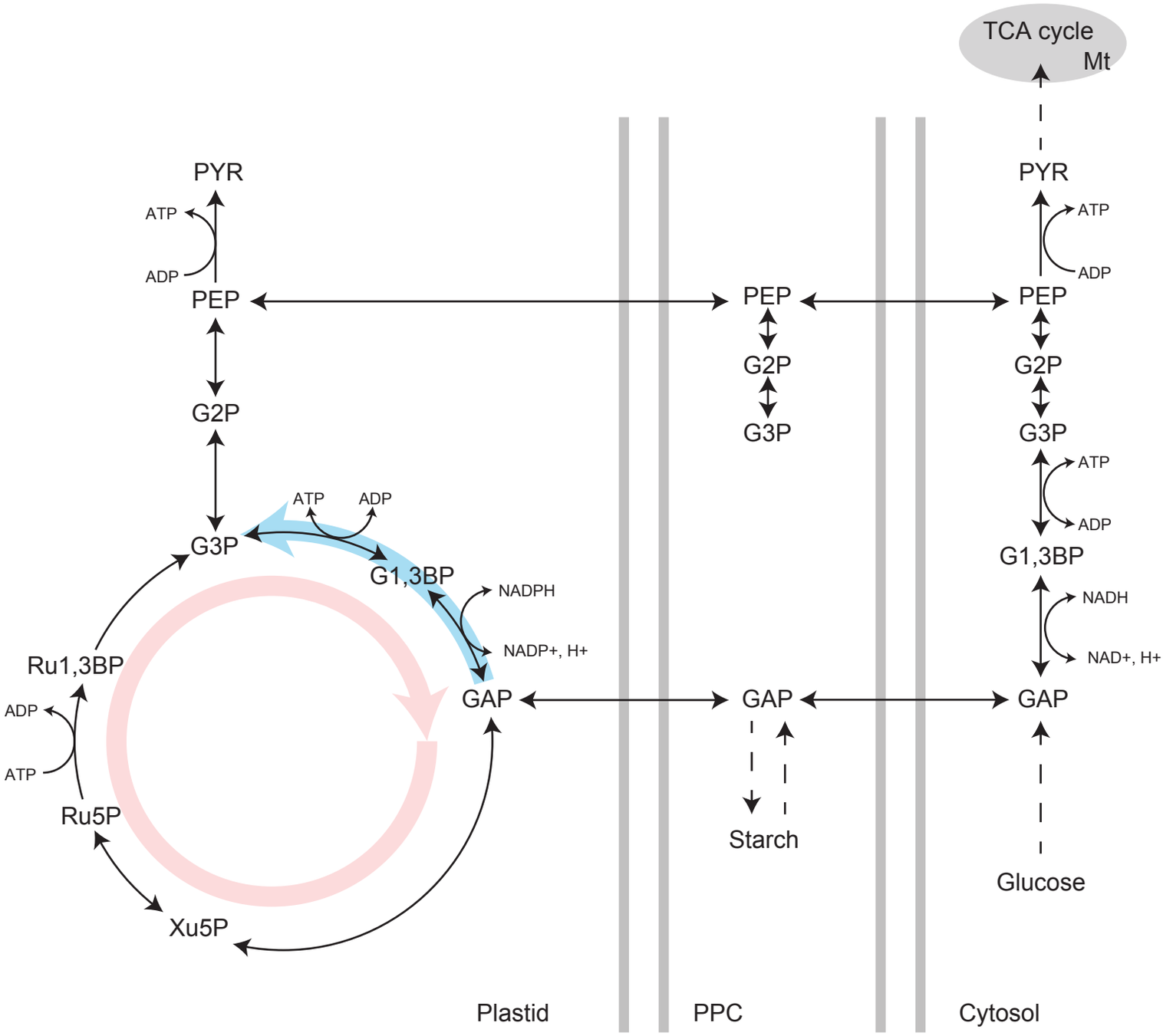


Extended Data 3. Predicted metabolic map of Calvin cycle and glycolysis pathway in *Cryptomonas paramecium*. Starch metabolism related proteins predicted based on KEGG and Cazy are also shown. Map showing enzymes predicted in the cytosol, periplastidial compartment (PPC), and plastid. Abbreviation for metabolites: RuBP, Ribulose-1,5-bisphosphate; 3PGA, Glycerate 3-phosphate; 13BPG, 1,3-Bisphosphoglycerate; GAP, Glyceraldehyde 3-phosphate; DHAP, Dihydroxyacetone phosphate; SBP, Sedoheptulose-1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; Ru5P, Ribulose 5-phosphate; R5P, Ribose 5-phosphate; FBP, fructose-1,6-bisphosphate; F6P, Fructose 6-phosphate; Xu5P, D-Xylulose 5-phosphate; E4P, Erythrose 4-phosphate, G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; PEP, phosphoenolypyruvate; Pyr, Pyruvate; Fru, Fructose; UDP-Glc, UDP-glucose. Abbreviation for enzymes: RuBisCO, Ribulose 1,5-bisphosphate carboxylase/oxygenase; PGK, Phosphoglycerate kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TPI, Triosephosphate isomerase; ALDO, aldolase; SBPase, Sedoheptulose-1, 7-bisphosphatase; TKT, Transketolase; RpiA, Ribose-5-phosphate isomerase A; PRK, Phosphoribulokinase; PGM, phosphoglucomutase; GPI, glucose-6-phosphate isomerase; HK, hexokinase; PFP, diphosphate—fructose-6-phosphate 1-phosphotransferase; PFK, 6-phosphofructokinase; FBP, fructose-bisphosphatase; FBA, fructose-bisphosphate aldolase; PGAM, phosphoglycerate mutase; ENO, phosphopyruvate hydratase; PYK, pyruvate kinase; aAMY, alpha-amylase; bAMY, beta-amylase; GWD, glucan / phosphoglucan water dikinase; PYG, 1,4-alpha-glucan phosphorylase; GAA, lysosomal alpha-glucosidase; IMA, oligo-1,6-glucosidase; malQ, 4-alpha-glucanotransferase; GBSS, granule-bound starch synthase; SSI-II, starch synthase type I-II; BE, 1,4-alpha-glucan branching enzyme; GYG, UDP-glucose-glycogen glucosyltransferase; UGP, UTP—glucose-1-phosphate uridylyltransferase.

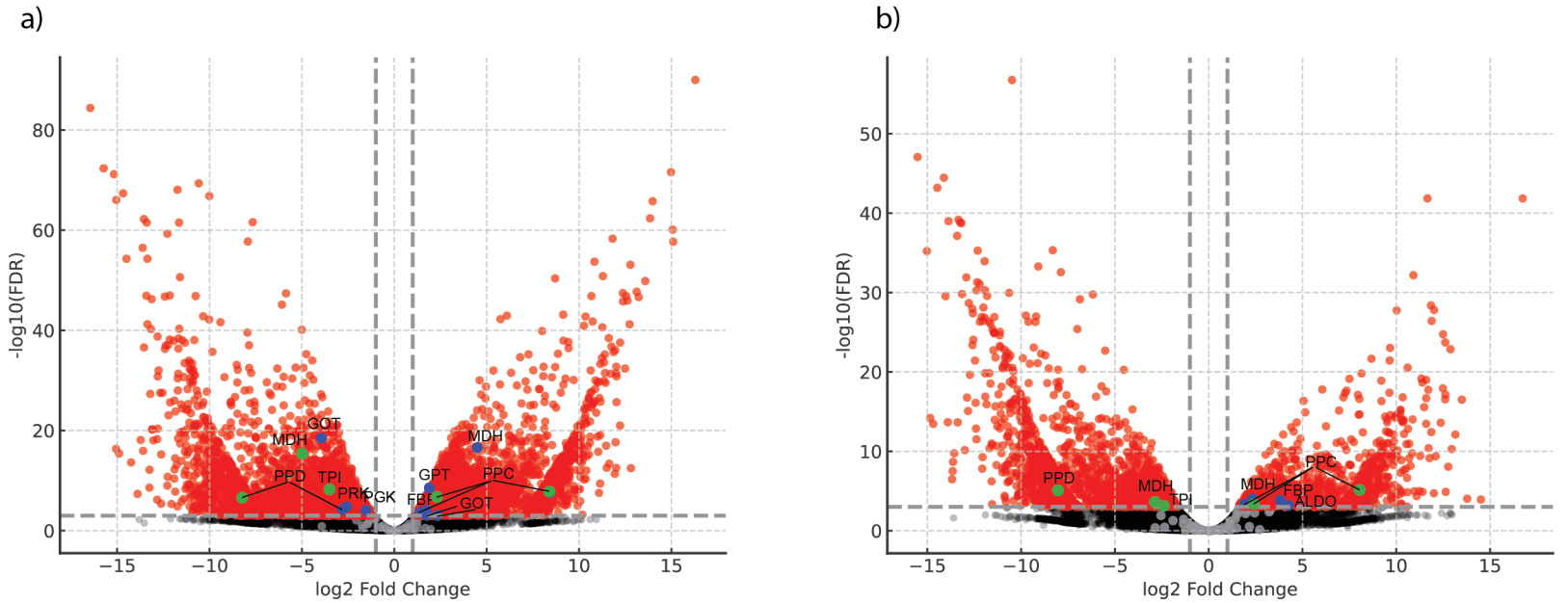
Extended Data 4. ^{13}C-atom% of Calvin cycle metabolites detected by CE-MS analyses

	^{13}C-atom%	
Compounds	#1	#2
G6P	2.52	2.55
F6P	2.57	2.98
G1P	3.03	3.56
Pyruvate	1.54	0.97
Lactate	2.07	0.98
Succinate	1.94	2.13
Malate	1.88	2.01
PEP	n.d.	n.d.
Acetyl CoA	4.32	4.51
Acetoacetyl CoA	n.d.	n.d.
Aspartate	n.d.	n.d.
α-Ketoglutarate	n.d.	n.d.

The results of the two independent experiments are shown (#1 and #2). Abbraviations: G6P, Glucose-6-phosphate; F6P, Fructose 6-phosphate; G1P, glucose-1-phosphate; PEP, phosphoenolypyruvate.



Extended Data 5. Hypothetical energy conservation and consumption of the Calvin cycle enzymes in Cryptomonas paramecium
Arrows show the estimated directions of the enzymatic reactions; two-way arrows indicate that enzymes catalyze reversible reactions. Reactions of the canonical Calvin cycle requiring ATPs and NADPHs are highlighted in the light red arrow while a possible direction under the ATP- and NADPH-deficient conditions is highlighted in the light blue arrow. In the latter case, energy conservation would occur through conversion from GAP to 3PGA, and resultant 3PGA would be converted to 2PGA and further to PEP in the plastid. PEP and GAP could be exchanged between subcellular compartments through membranes depicted as light grey lines, since purified cryptophyte plastid membranes were demonstrated to translocate PEP and triose phosphates (Haferkamp et al. 2006). When GAP, a part of which are transferred from the plastid, and/or glucose are present in the cytosol, substrates for the mitochondrial TCA cycle, which produces NADH for oxidative phosphorylation-based ATP production, would be generated through glycolysis.
TCA: tricarboxylic acid cycle, Mt: mitochondrion, PPC: periplastidal compartment, ATP: adenosine triphosphate, ADP: adenosine diphosphate, NADP+/NADPH: nicotinamide adenine dinucleotide phosphate, PYR: pyruvate, PEP: phosphoenolpyruvate, 2PGA: Glycerate 2-phosphate, 3PGA: Glycerate 3-phosphate, RuBP: ribulose 1,5-bisphosphate, Ru5P: ribulose 5-phosphate, Xu5P: xylulose 5-phosphate, GAP: Glyceraldehyde 3-phosphate, and 1,3BPG: 1,3-bisphosphoglycerate.



Extended Data 6. Volcano plots illustrating differential gene expression derived from RNA-seq analysis comparing organic-poor (AF6 or Waris-H) and organic-rich conditions. a) Organic-poor AF6 versus organic-rich conditions; b) organic-poor Waris-H versus organic-rich conditions. The horizontal axis represents the log2 fold-change (logFC) in gene expression between organic-poor and organic-rich conditions. The vertical axis shows statistical significance as -log10 (FDR). Dashed lines indicate significance thresholds (FDR = 0.001 and logFC = ±1). Colored points indicate specific isoform categories: Green, isoforms assigned to carbon fixation by the Calvin cycle (KEGG map 00710) that commonly and significantly differentially expressed in both AF6 and Waris-H comparisons; Blue, other isoforms assigned to carbon fixation by the Calvin cycle that showed significant differential expression; Gray, isoforms associated with carbon fixation but not significantly differentially expressed; Red, significantly differentially expressed isoforms not annotated as involved in carbon fixation by the Calvin cycle; Black, non-significantly differentially expressed isoforms. ALDO, aldolase (EC 4.1.2.13); FBP, fructose-bisphosphatase (EC 3.1.3.11); GOT, glutamic-oxaloacetic transaminase (EC 2.6.1.1); GPR, glutamic-pyruvic transaminase (EC 2.6.1.2); MDH, malate dehydrogenase (EC 1.1.1.40); PGK, phosphoglycerate kinase (EC 2.7.2.3); PPD, pyruvate, phosphate dikinase (EC 2.7.9.1); PPC, phosphoenolpyruvate carboxylase (EC 4.1.1.31); PRK, phosphoribulokinase (EC 2.7.1.19); TPI, triose-phosphate isomerase (EC 5.3.1.1).