

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

IDBA-UD v1.1.1, Megahit v1.2.8, CD-Hit v4.8.1, MAFFT v7.419, TrimAl v1.2, RAXML-HPC BlackBox v8.2.10, CCP4 Xia2 v7.0.077, PHASER v2.7.0, Phenix v1.17.1-3660, COOT v0.8.9.2, MOLPROBITY v4.5, SCATTER v3.2e, Wyatt ASTRA 7 v7.1.4, MODELLER ModWeb v. r213, FoXS IMP v2.12.0, Agilent Quikchange Primer Design Tool (online @ www.chem.agilent.com/store/primerDesignProgram.jsp)

Data analysis

Protein Thermal Shift v1.3, PROMALS3D server, CCP4 CONTACTS v7.0.077, CCP4 PISA v7.0.077, UCSF Chimera v1.13.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Form I' RbcL amino acid sequences are included as a supplementary file (Supplementary data 1). Sequences used to generate Fig. 1a were uploaded to figshare (DOI: 10.6084/m9.figshare.9980630) along with the associated phylogenetic tree. Representative MAG genbank scaffolds are included as a supplementary file (Supplementary data 2). Site-directed mutagenesis primers and synthesized candidate Form I' rbcL genes are included as a supplementary file (Supplementary data 3). The structural coordinates of 2CABP-bound P. breve Rubisco have been deposited in the PDB under the accession ID 6URA. The crystal structure of Syn6301 Rubisco can be found on the PDB under the accession ID 1RBL. Two Chloroflexi genomes identified in this study are available at: https://ggkbase.berkeley.edu/Chloroflexi_Rubisco_PatrickShih/organisms. Publicly available databases used in this study include: PDB (www.rcsb.org), pfam (www.pfam.xfam.org), TIGRfams (www.tigrfams.jcvi.org), and KEGG database (www.genome.jp/kegg/kegg1.html).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes for data presented in Figure 5, Table 1 and ED Figure 9 were chosen at random. No experiment was replicated less than three times.
Data exclusions	No data was excluded from analyses reported in the current study.
Replication	Experimental replicates were chosen at random. No experiment was replicated less than three times.
Randomization	Randomization was not required in this study because bias was not a concern.
Blinding	Blinding was not intentionally used in this study because the research was discovery-based (i.e. it was impossible to be biased).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging