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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies 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	Sanger sequencing and Next-Generation Sequencing (NGS) were outsourced to Eurofin Genomics and Macrogen Japan, respectively. NGS data were obtained by using the Illumina Novaseq platform. Leaf areas were measured with Image J. All the cryoEM image datasets were collected using SerialEM ver. 4.0, YoneoLocr ver. 1.0, and CRYO ARM 300 or CRYO ARM 300 (JEOL).
Data analysis	Data of Sanger sequencing and NGS were analyzed by using Geneious Prime v2024.0.7. Rubisco kinetics were analyzed by using the KaleidaGraph data analysis software. Statistical analyses were conducted by using Prism v. 8.0.1 software. CryoEM data and structures were analyzed by using RELION ver. 4.0, Coot ver. 0.9.8.96, PHENIX ver. 1.20.1, Servalcat ver. 0.4.126, MolProbity ver. 4.5.2, UCSF Chimera ver. 1.16, PyMOL ver. 2.5.0, and ImageJ ver. 1.53q. Figures were prepared by using Microsoft Excel 365, Adobe Illustrator 2025, Adobe Photoshop 2025, UCSF Chimera ver. 1.16, CLUSTALX ver. 2.0, ESPript ver. 3.0, and PyMOL ver. 2.5.0.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data supporting the findings of this study are available in the article and the supplementary information. Vector sequences have been deposited in Addgene. Accession numbers of the reference sequences of the plastid genome are included in the Supplementary Table. Cryo-EM atomic coordinates and maps of Col-0, M309I, and D397N Rubisco have been deposited in the Protein Data Bank (PDB) and the Electron Microscopy Data Bank (EMDB) under the accession codes 9L58 and EMD-62824, 9L5D and EMD-62829, and 9L5I and EMD-62834 for D4 symmetry, respectively. The other coordinates used in this study are available from PDB. Source data are provided in this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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

Life sciences study design

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

Sample size	Sample sizes were determined to ensure they were adequate for conducting statistical analyses.
Data exclusions	No data were excluded.
Replication	All experiments were performed using independent biological replicates (n = 6).
Randomization	All samples were selected randomly.
Blinding	Blinding was not implemented as all samples underwent identical treatment conditions.

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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

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

Antibodies

Antibodies used: Primary antibody: Rabbit anti-rice rubisco activase antibody; Secondary antibody: anti-rabbit IgG HRP-linked antibody (GE Healthcare, NA934, Amersham ECL IgG, HRP-linked whole Ab from donkey, Polyclonal)

Validation: same antibody was used and proved qualified in previous study: https://doi.org/10.1111/pce.14051

Plants

Seed stocks: Arabidopsis thaliana Col-0 seeds have been stocked in the laboratory from the past. Mutant seeds were obtained in this study.

Novel plant genotypes: Plastid-targeted base editors, ptpTALECD and ptpTALECD_v2mod, were employed to edit rbcL. A binary vector encoding these base editors was introduced into the nuclear genome of A. thaliana Col-0 via floral dipping.

Authentication: Sanger sequencing and NGS were performed to investigate whether the target base was modified. NGS was also employed to assess off-target mutations.