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Corresponding author(s): Ralph Bock

# 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.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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 |
| ☐ | ☒ | An indication of 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<br>*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 statistics 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<br>*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 |
| ☒ | ☐ | Clearly defined error bars<br>*State explicitly what error bars represent (e.g. SD, SE, CI)* |

*Our web collection on statistics for biologists may be useful.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | No software was used. |
| Data analysis | No software was used. |

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 upon request. 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

The data supporting the findings of this study are available within the paper and its supplementary information files. Annotated sequences of plastid transformation vectors pCH8, pJF1151 and pJF1153 were deposited in GenBank (accession numbers MH590891, MH590893 and MH590894).

# Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

# Life sciences study design

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

| | |
|---|---|
| Sample size | The sample sizes are given where appropriate. A sufficiently large number of samples was bombarded to guarantee isolation of at least three independent transplastomic lines from all transformation experiments with our optimized protocol. |
| Data exclusions | No data were excluded. |
| Replication | All independent plastid transformation experiments with our optimized protocol were successful. All experiments were repeated and gave similar results. The number of replicates is given in the figure legends. |
| Randomization | No randomization was done. It was not required, because the generated transplastomic lines were independently confirmed by molecular methods and inheritance assays. |
| Blinding | No blinding was done. It was not required, because the generated transplastomic lines were independently confirmed by molecular methods and inheritance assays. |

# Reporting for specific materials, systems and methods

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |

## Methods

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

## Unique biological materials

Policy information about availability of materials

| | |
|---|---|
| Obtaining unique materials | Unique materials (acc2 CRISPR/Cas lines, plastid transformation vectors) are available from the authors. |

## Antibodies

| | |
|---|---|
| Antibodies used | anti-GFP antibody: Living Colors® A.v. Monoclonal Antibody (JL-8), TaKaRa Bio, Clontech Laboratories, Mountain View, CA; catalog number 632381, clone name: JL-8, lot number: A5033481; dilution used: 1:1000 |
| Validation | The anti-GFP antibody used is a standard commercial antibody. It was validated by the supplier (see https://www.takarabio.com/products/antibodies-and-elisa/fluorescent-protein-antibodies/green-fluorescent-protein-antibodies?catalog=632475) as follows: "The quality and performance of this lot of Living Colors A.v. Monoclonal Antibody (JL-8) was tested by Western blot analysis using lysate made from a HEK 293 cell line stably expressing AcGFP1. After cells were collected and lysed using SDS sample buffer, the lysate (10 µl; equivalent to 35,000 cells) was electrophoresed on a 12% SDSpolyacrylamide gel and transferred to a nitrocellulose membrane. The blot was probed with the Living Colors A.v. Monoclonal Antibody, JL-8 (diluted 1:1,000), followed by a secondary goat anti-mouse antibody conjugated to horseradish peroxidase (HRP). The HRP signal was detected by chemiluminescence. A band of approximately 30 kDa corresponding to AcGFP1 was observed in the lane loaded with the AcGFP1 cell lysate. A band of this size was not detected in the lysate of untransfected HEK 293 cells." The specificity of the antibody in our study was further confirmed by |

inclusion of negative controls (plants not harboring the yfp transgene).