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# 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 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<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 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<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 |

*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 | Data for HPLC and LC-MS/MS (proteome) was collected in Metabolomics Australia (Queensland node). Metabolomics Australia (Queensland node) used Agilent HPLC software, Thermo Fisher Chromeleon Chromatography Data System, and Thermo Fisher LC-MS/MS softwares to collect data. We used a BD Csampler software (BD Accuri C6 software version 1.0.264.21) to collect Flow Cytometry data through BD Accuri C6 Flow Cytometry. |
| Data analysis | Metabolomics Australia (Queensland node) used an Agilent HPLC software or a Thermo Fisher Chromeleon Chromatography Data System to process HPLC data, and a Thermo Proteome Discoverer software to process LC-MS/MS data for proteome data analysis. R was used to calculate kernel density. We used Microsoft Office for data analysis. |

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 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

Source data are provided with this paper.

# 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.

| | |
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| Sample size | Sample size was not calculated through a statistical method. Sample sizes were chosen on an empirical or pragmatical base in the field of metabolic engineering and synthetic biology in yeast. The sample size for construction-in-processing strains is one, when we hypothesize that the spontaneous genetic mutation is insignificant and we can confirm the genotype. The samples size for final characterization strains are at least two. |
| Data exclusions | No data were excluded. |
| Replication | Characterization of final yeast strains were performed with biological replicates: two-to-four biological replicates were performed in Figure 1b-d, Figure 2c-h, NLD401 in Figure 3c-l, Figure 4c-j, Figure S2c-g, Figure S3b,c,f-h, and NLD401 in Figure S5. Characterization of the strain NLD128-1 (one of biological replicates for strain NLD128) was performed in two or four independent replicate cultures in Figure 3c-l and Figure 5. Development procedure of strain NLD128-1 was replicated, shown in Figure S3 (NLD128-1 and NLD128H). We did not execute the replication experiment for characterization of construction-in-process strains in Figure 2b, Figure 4b, and Figure 1, and Figure S3a, and other strain construction procedures. |
| Randomization | The biological replicates were chosen randomly. |
| Blinding | Not applicable, due to the first author and the second author designed, performed experiments, and collected data in the current study. |

# 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 |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

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

# Flow Cytometry

## Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

| | |
|---|---|
| Sample preparation | Samples for fluorescence analysis in Yeast cells were directly used for flow cytometry analysis. Samples for analyzing Y-FAST fluorescence in yeast cells were used for flow cytometry analysis after chromophore was added. Samples for cell survival rate were prepared by incubating cells with Propidium iodide. The details have been described in Methods and supplementary |

| | |
|---|---|
| | information. |
| Instrument | Accuri C6 plus |
| Software | Accuri C6 plus sampler |
| Cell population abundance | 10,000 events are analysed for each data point in this study. |
| Gating strategy | FSC thresholding was used to exclude small debris particles during data collection. Other gating strategy was not used. All collected events were included in the analysis. |

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.