

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 |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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 No software was used for data collection.

Data analysis PRECOG (v1.0), Scanomatic (v2.2), LRSDAY (v1.0.0), LRSDAY_Patch (<https://github.com/nicolo-tellini/LRSDAY-Patch>), SMRT Analysis pipeline (v2.3.0), Canu (v1.5), pbalgn (distributed with GenomicConsensus v2.0.0), Quiver (distributed with GenomicConsensus v2.0.0), Pilon (v1.22), MUMmer (v3.23), IGV (v2.3.68), bwa mem (v0.7.12), picard tools (v2.8.0), SAMtools (v1.2), freebayes (v0.9.5), R (V4.2.3), VCFtools (v0.1.16), Variant Effect Predictor (v87), mutfunc (v1.0), Unipro UGENE (v52.1), clustal omega, bwa mem (v.0.7.17-r1198-dirty), SAMtools (v1.19), bcftools (v1.19), vcf2phylyp.py (v.2.8), seqkit rmdup (v.2.4.0), bedtools (v2.17.0), DIA-NN (v1.8), CytExpert (v2.5). Custom Bash and Perl scripts used in this study are available at https://github.com/mdangiolo89/Humanized_yeasts_project.

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](#)

The genome sequencing generated in this study are available at Sequence Read Archive (SRA), NCBI under accession codes BioProject ID PRJNA985049, Biosample ID SAMN35786841-SAMN35786899. The phenotyping and proteomics data are available at: https://github.com/mdangiolo89/Humanized_yeasts_project and within the supplementary information files. All the strains generated in this work are available upon request. Raw and processed mass spectrometry data have been deposited at the ProteomeXchange consortium via the PRIDE partner repository with the dataset identifier PXD064562 and are publicly available.

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

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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](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

No statistical methods were used to predetermine sample size. Both number of MALs, AELs to propagate was chosen based on the maximum number of lines that could be handled and based on previous bibliography showing they were enough to detect variation in mutation rates (for MALs) and discovery of adaptive solutions (for AELs).

Data exclusions

There was no data exclusion and all available strains were used.

Replication

Population doubling time measurements were performed in triplicate. Survival rate measurements were performed in one replicate. Relative doubling time measurements were performed in 24 replicates for SC and 12 replicates for all the other conditions. Protein abundance measurements were performed in triplicate for all strains except the wild-type MAL at 100 scb, where they were performed in one replicate.

Randomization

Randomization was not relevant because there was only one experimental group.

Blinding

Blinding was not relevant because there was no group allocation.

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
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

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

Ploidy: cells were incubated in 100 μ L of Leucin/Tryptophan dropout liquid medium in a 96-well plate and left two nights at 30 °C. After that, 3 μ L were incubated in 100 μ L of cold 70% ethanol for 3 h at 4 °C, washed twice with PBS, and resuspended in 100 μ L of staining solution (15 μ M PI, 100 μ g/ml RNase A, 0.1% v/v Triton-X, in PBS). After that, samples were incubated for 3 h at 37 °C in the dark.

Survival rate: strains were grown two nights in liquid Leucin/Tryptophan dropout medium, diluted 100x in 200 μ L of fresh Leucin/Tryptophan dropout in a 96-well plate and incubated at 30 °C for 15 days. We measured viability at the beginning of the stationary phase when cultures reached saturation (3 days), and then every 4 days until day 15. Data are shown by days post-saturation, meaning that day 0 post-saturation corresponds to day 3 after the inoculation of the culture, while day 12 post-saturation corresponds to day 15 post-inoculation. At each time point, 5 μ L of cells were transferred in 100 μ L of staining solution (phosphate-buffered saline-PBS + 3 μ M propidium iodide + 200 nM YO-PRO-1) in a 96-well plate and incubated 10 minutes in the dark at 30 °C.

Instrument

FACS-Calibur flow cytometer using the HTS module for processing 96 well plates for ploidy measurements.
CytoFLEX flow cytometer for survival rate measurements.

Software

CytExpert (v2.5)

Cell population abundance

No sorting was applied.

Gating strategy

Cell debris and heavily damaged old necrotic cells were first removed based on forward scatter and side scatter at day 0. Then, cells were excited with the 561nm Yellow/Green laser and fluorescence was read with the Y610-mCHERRY filter for propidium iodide and with the B610-ECDA filter for YO-PRO-1. Non-fluorescent cells were considered viable whereas fluorescent ones were considered dead. The final viability was calculated as the product of "Intact cells" and "Viable cells".

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