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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

The GC-MS analysis was performed using Agilent 8890-5977B GC/MSD Ultra (Agilent) equipped with Agilent 190915-433. JW HP-5ms GC Column. Compound identification was performed by comparison of mass spectra with the NIST Mass Spectral Library.
AlphaFold3 was employed to predict the structure of the Saccharomyces cerevisiae Pdrl homodimer, which served as the receptor formolecular docking with the GA ligand. The Pdr1 sequence was obtained from NCB, while the two-dimensional structure of the GA ligand was sourced from PubChem. Molecular docking, scoring, and ranking were performed using AutoDock Vina software to simulate theinteraction between GA and Pdr1. During docking, Pdr1 served as the receptor and GA as the ligand. The docking region center coordinatesfor the design box were set to center x = 0.561, center y= -0.452, center 2 = 0.258. The docking box dimensions were set to size x= 71.75, size_y = 70.61, size_z = 71.75.
The raw sequencing data of single-cel RNA-seq was analyzed by FastQC. The Mobivision M software was used to quantiy the expressionlevels of each gene in each cell. MobiVision-M automaticlly ran following analysis adaptor removing, low quality reads filtering, barcoding, alignment, adustment of multi-mapped reads, filtering low quality microbes, generating of report and expression matrix. Generally, following the pipeline of normal single-cell RNA-seq, the cluster analysis was performed by Seurat. The microbes with relatively low Bene count or UMIcount was filtered. The list of high variable genes was selected by "FindVariableFeatures" function after normalization. Then, the expression matrix was scaled. The PCA analysis was performed based on variable genes. The cluster was separated by "FindNeighbors" and"FindCusters" functions. The results were visualized by uniform manifold approximation and projection (UMAP) algorithms. "FindAllMarkers"and "FindMarkers" functions were used to analysis differentially expressed genes (DEGs). The enrichment analysis started by creating customized database. The pathway or term related to each gene were annotated by eBBNOG-mapper and egBNOG database. Based on theannotation, the customized database was generated by R package "Annotation Forge". The GO and KEGG enrichment analysis were performedby R package "clusterProfiler". The enrichment results were visualized by R package "ggplot2". The analysis was based on the gene expressionmatrix with UMI count. The data was believed to follw a negative binomial distribution. The genes expressed in less than 10 microbes were removed. The genes used for analysis were identified by "FindAllMarkers" function of R package Seurat with P -value lower

than 0.05.
Flow cytometry analysis and cell sorting were performed using a BD FACS Aria SORP flow cytometer (BD Biosciences, USA). Yeast cells werecollected after cultivation, washed three times with sterile lxPBS, and diluted to OD600 0.5. The cell suspension was filtered through a 200-mesh sieve to remove clumps and large debris. For gating, the main yeast population was selected using FSC-A and SSC-A parameters. Doublets and cell aggregates were excluded using FSC-A versus FSC-H, selecting the linear distribution of single cels. Green fuorescence intensity was measured using the 488 nm laser (FITC channel). The top 2% of cels with the highest fuorescence intensity were gated as the target subpopulation, and a control population was gated from cell with low fluorescence intensity and a concentrated distribution of events. For each sorted population, 20,000 cells were collected. Data acquisition and analysis were performed using FlowJo v10.9 software.

Data analysis: Data were processed with Excel or GraphPadPrism.

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

Source data are provided with this paper. The bulk RNA-seq data generated in this study have been deposited in the NCBI database under BioProject accession codes PRJNA1146062 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1146062] and PRJNA1415461 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1415461]. The single-cell RNA-seq data generated in this study have been deposited in the NCBI database under BioProject accession code PRJNA1415668 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1415668]. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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 not determined by formal statistical power calculations. Instead, sample sizes were selected based on standard practice in the field, with at least three independent biological replicates analyzed for each condition. The reproducibility of the results across replicates supports the adequacy of these sample sizes.
Data exclusions	No data was excluded from the analysis.
Replication	All attempts at replication were successful.
Randomization	The experiments were not randomized.

Blinding	The investigators were not blinded to allocation during experiments and outcome assessment.

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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	Yeast cells were collected after cultivation, washed three times with sterile 1xPBS, and diluted to OD600 0.5. The cell suspension was filtered through a 200-mesh sieve to remove clumps and large debris.
Instrument	Flow cytometry analysis and cell sorting were performed using a flow cytometer (Bio-Rad S3eTM Cell Sorter) and BD FACSAria SORP flow cytometer (BD Biosciences, USA), respectively.
Software	Data acquisition and analysis were performed using FlowJo v10.9 software.
Cell population abundance	A total of 50000 and 20000 cells were counted for analysis and sorting per sample, respectively.
Gating strategy	For gating, the main yeast population was selected using FSC-A and SSC-A parameters (Supplementary Fig. 9b). Doublets and cell aggregates were excluded using FSC-A versus FSC-H, selecting the linear distribution of single cells. Green fluorescence intensity was measured using the 488 nm laser (FITC channel). The top 2% of cells with the highest fluorescence intensity were gated as the target subpopulation, and a control population was gated from cells with low fluorescence intensity and a concentrated distribution of events.

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