

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Plate-based optical density, fluorescence, and luminescence measurements were collected using BioTek Gen5 3.18.17 with a Agilent Biotek Synergy H1 plate reader. HPLC data were collected using Agilent ChemStation (Version C.01.10) software. Scanner images were collected on dual light flatbed scanner (Epson V800). Widefield images were collected with Leica widefield fluorescence microscope (M205 FCA) using the LAS X software. Luminescence images were collected with iBright Imaging System (Invitrogen FL1500). Proteomics data was collected with Orbitrap Eclipse. For flow cytometry the instrument and collection protocol is listed below.
Data analysis	HPLC data were analyzed using Agilent ChemStation (Version C.01.10) software. Graphpad Prism was used to generate plots and calculate standard deviations. Graphs were constructed using GraphPad Prism and Python packages. Flow cytometry histograms were constructed in the NovoExpress software. Unless otherwise specified, significance tests were computed in Prism. Relative IC50 curves were fitted in Prism from median values. For proteomics analysis, MSFragger was used to identify MS/MS spectra and Perseus were used for data processing and statistical analysis. For flow cytometry the software for analysis is in the flow cytometry section below.

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 datasets generated during and/or analyzed during the current study are contained in the published article (and its Supplementary Information) and are available in publicly available databases. Sequence files, processed and unprocessed images and raw data are available as supplementary materials and source data. The Source Data 1 image set is hosted on Zenodo. The proteomics dataset is hosted on PRIDE.

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](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	For bacterial measurements, three biological replicates is standard practice for plate reader experiments (examples include: (i) doi:10.1038/s41467-021-25691-4 and (ii) doi:10.1038/nbt.3372) and we used n=3 accordingly. For HPLC analysis of metabolites produced via fermentation, three replicates is similarly standard practice (examples include: (i) doi:10.1038/ncomms11709 and (ii) doi: 10.1038/s41589-020-00684-4) and we used n=3 accordingly. For proteomics, n=3 is considered standard in the field and we used this sample size. For plant phenotyping, we previously found n=9 T3 seedlings to be sufficient to detect the morphological effects (DOI: 10.1126/science.abo4326) and selected equivalent or larger sample sizes in this study. Across the study, sample sizes were chosen based on convention in each relevant field. No formal statistical power calculations were performed to determine sample sizes.
Data exclusions	No data were excluded for bacterial experiments. For Arabidopsis phenotyping, ungerminated seeds were excluded from root length and lateral root density measurements.
Replication	All experiments were performed at least in triplicate, with the exact number of biological replicates reported in figure legends. For most bacterial experiments, three biological replicates (from independently inoculated cultures) were collected simultaneously. For bacteria and plant assays, replicates were performed and successful across repeated trials. Plant experiments included substantially larger numbers of replicates, as specified in the corresponding figure legends.
Randomization	Experimental samples using the same strain but coming from different groups were generated from the same source. If one strain was exposed to +nsAA/ +producer and -nsAA/ non-producer conditions, a single culture of that strain was split immediately before nsAA was added. For Arabidopsis, seeds allocated to different OMY, amino acid, or beta-estradiol treatments all originated from the same batch sterilized and stratified on the same day. For seedlings transferred to bacterial-agar conditions, seedlings were sequentially transferred from the same batch of seeds moved to the growth chamber at the same time. For this study, randomization was not relevant, as all bacterial cells and plants were analyzed equally.
Blinding	All experiments were unblinded as there would be difficulty fully blinding studies to ensure proper growth and media conditions, but is unlikely researcher bias in would result in replicated effects.

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

Arabidopsis thaliana Col-0 seeds were obtained from Jose Dinneny at Stanford University. Nicotiana benthamiana seeds were obtained from Sue Rhee at Carnegie Institution. Tomato seeds were obtained from Jung-Gun Kim and Mary Beth Mudgett at Stanford University. Poplar cuttings were obtained from Chris Dervins and Matias Kirst at the University of Florida.

Novel plant genotypes

All Arabidopsis transgenic lines were generated via floral dip Agrobacterium-mediated transformation. Experiments were performed with T3 homozygous lines unless otherwise indicated. At least four independent lines were generated for each construct, and at least two lines were analyzed.

Authentication

Segregation analysis was performed to ensure that transgenic lines were all single-locus transformation events.

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

Cells grown in LB were diluted 100x in sterile PBS. To prepare soil samples for flow cytometry, cells were washed by addition sterile PBS and vortexing before filtration through a 10 µm syringe filter and filtrate was collected. Immediately after extraction, cells were run on the flow cytometer.

Instrument

Samples were run on a NovoCyte NovoSampler Pro by Agilent

Software

NovoExpress software from Agilent was used to collect and analyze the flow cytometry data

Cell population abundance

Cells were run to achieve 10,000 events in the gated population.

Gating strategy

Gates were drawn on cells grown in LB in the FSC-SSC space, as shown in Supplemental Fig. 2. Singlets were gated in the FSC-A FSC-H space. These gates were then applied to samples in soil and 10,000 events were collected within these gates. Gates for fluorescent positive cells were determined to contain the top ~5% of wild-type UD1022 cells extracted from soil containing the proUBQ10::MfnG plants within the FITC channel.

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