

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	Molecular Devices Spectramax M2e plate reader, Beckman Coulter CytoFLEX S Flow Cytometer, BD FACS Aria Fusion, Algorithmic Enumeration of 3-INPUT T-Pro Circuits (Custom), SISO Performance Optimization (Custom). Custom software available in https://github.com/Jayaos/TPro
Data analysis	Microsoft Excel, ApE Plasmid Editor v3.0.8, Graphpad Prism 10.3.1, CytExpert 2.4, Molecular Devices SoftMax Pro v7.0.3, Image Lab 6.0.1, BD FACSDiva 8.0.1, Snapgene v8.1

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 authors declare that all data supporting the findings of this study are available within the paper and its supplementary information. The analyzed data and

source data are available in the Supplementary Information and Source Data. Any other information are available from the corresponding author upon reasonable request. The sequences of the plasmids used in this study are available from Genbank with accession numbers: PV806910-PV807102.

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](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	A sample size of n=6 was chosen for all assays. The sample size was based on previous works from our lab (Rondon et al., 2019), (Groseclose et al., 2020), (Huang et al, 2023) and comparison with standard techniques in literature (typical n=3 biological replicates).
Data exclusions	No data has been excluded from analyses. All data collected is shown on plots and is included in the Source Data file.
Replication	All attempts of replication were successful. All experiments with n=6 replicates were performed on two separate days with n=3 biological replicates per day.
Randomization	For site-saturation mutagenesis libraries and phenotypic screening, colonies were picked at random after PCR and transformation (sampling 3x for a 95% probability to ensure representative coverage). Similarly, colonies obtained after FACS sorting were randomly selected for plating and phenotypic verification. All transformants were included in the FACS process, having been streaked from selection plates. For all cloning experiments—including the construction of genetic logic circuits, reporter systems, and modified transcription factors—colonies resulting from PCR and transformation were randomly picked for sequencing to confirm correct assembly.
Blinding	Blinding was not relevant to this study since knowledge of genotype and plasmid sequence was required for experiments to be performed under the correct conditions.

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

Methods

- 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

- 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

Samples were prepared as detailed in the Methods section for Error-prone PCR library construction, FACS, and Flow cytometry analysis. For all experiments, transformed 3.32 E.coli cells were used. For FACS, cells were grown, pelleted, and resuspended in a sort buffer comprising PBS, 25mM HEPES, and 1mM EDTA. For flow cytometry, cells were diluted 1:50 in PBS with 2mg/ml kanamycin, and incubated at room temperature for 1hour.

Instrument

Beckman Coulter CytoFLEX S, BD Biosciences FACS Aria Fusion

Software

CytExpert Software, BD FACS Diva

Cell population abundance

Multiple consecutive sorts were performed using FACS collecting cells ranging from 200,000 to 700,000. For flow cytometry experiments greater than 10,000 cells were collected for analysis in all experiments.

Gating strategy

Cell gating for both FACS and flow cytometry is detailed in the Methods section. Briefly, cells were gated by FSC-A vs SSC-A, and then by SSC-H vs SSC-A. FITC-H was used to deem cells as GFP positive. A typical threshold for GFP expression was a FITC-H value of greater than 3×10^3 .

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