

| Corresponding author(s): | Jerome Bonnet |
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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 Authors & Referees and the Editorial Policy Checklist.

### Statistical parameters

| text, | , or | Methods section).   |
|-------|------|---|
| n/a   | Со   | onfirmed  |
|       | ×    | The exact sample size ( ) for each experimental group/condition, given as a discrete number and unit of measurement       |
|       | x    | An indication of 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   |

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

| Only common tests should be described solely by name; describe more complex techniques in the M | ethods section |
|---|----------------|
|---|----------------|

| 1 |  |  |
|---|--|--|
| x | A description of all covariates tested |  |

| x |  | A description of any ass | umptions or corrections | , such as tests of | normality and ac | djustment for multipl | e comparisons |
|---|--|--------------------------|-------------------------|--------------------|------------------|-----------------------|---------------|
|---|--|--------------------------|-------------------------|--------------------|------------------|-----------------------|---------------|

|   |   | A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND |
|---|---|---|
| ᆀ | ^ | 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 note |
|---|
| Give P values as exact values whenever suitable.  |

|  | For Bayesian | analysis, | information o | n the choice | of priors and | Markov | chain Monte | Carlo settings |
|--|--------------|-----------|---------------|--------------|---------------|--------|-------------|----------------|
|--|--------------|-----------|---------------|--------------|---------------|--------|-------------|----------------|

| $\overline{}$ | For hierarchical and complex design  |                       |                        |                       |                       |
|---------------|--------------------------------------|-----------------------|------------------------|-----------------------|-----------------------|
| Y I           | For hierarchical and compley design  | anc identification    | of the appropriate lev | al for tacts and full | reporting of outcomes |
| ~             | Tot file at child and complex design | 5113, lucilillication | of the appropriate lev | CITOI (C3t3 and run   | reporting or outcomes |

| X |  | Estimates of | effect sizes | (e.g. C | Cohen's <i>d,</i> | Pearson's r | r), indicatin | g how the | ey were cal | culated |
|---|--|--------------|--------------|---------|-------------------|-------------|---------------|-----------|-------------|---------|
|---|--|--------------|--------------|---------|-------------------|-------------|---------------|-----------|-------------|---------|

| $\neg$ | v | Clearly defined error bars<br>State explicitly what error bars represent (e.g. SD, SE, Cl, |
|--------|---|--|
| -1     | _ | State explicitly what error bars represent (e.g. SD, SE, Cl,                               |

Our web collection on statistics for biologists may be useful.

#### Software and code

Policy information about availability of computer code

Data collection

Flow cytometry data collected using the Attune NxT software version 2.7 and BD LSR Fortessa (Bencton Dickinson) with FACSDiVa v9.0 software.

Plate reader data collected the Biotek Gen5 software

Data analysis

Flow cytometry: Flowjo (treestar). Custom python scripts for generating plots available at github (https://github.com/synthetic-biology-group-cbs-montpellier/OSIRiS) CALIN (https://github.com/synthetic-biology-group-cbs-montpellier/calin)

Plate reader: microsoft excel. (Fluorescence data was normalized by OD using excel software). Omero software version 5.5.0

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/reviewers upon request. 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 for main text figures, along with DNA sequences for all constructs are provided in supplementary materials.

see supplementary excel files and data availabilty section.

## Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Ecological, evolutionary & environmental sciences Behavioural & social sciences

For a reference copy of the document with all sections, see <a href="mailto:nature.com/authors/policies/ReportingSummary-flat.pdf">nature.com/authors/policies/ReportingSummary-flat.pdf</a>

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size     | Each experiment was performed in triplicates. For flow cytometry we analyzed 20000 cells / sample.  |
|-----------------|---|
| Data exclusions | none  |
| Replication     | All measurements performed in triplicates three times on three different days. All attempts at replication were successful, and reliable replicated |
| Randomization   | No randomization was done. The randomization was not relevant for our study.  |
| Blinding        | No blinding was done. Blinding was not done because it was not conducive to this study  |

# Reporting for specific materials, systems and methods

#### Materials & experimental systems

|   | Involved in the study       |
|---|-----------------------------|
| × | Unique biological materials |

Antibodies

x

Eukaryotic cell lines

X Palaeontology

X Animals and other organisms

Human research participants

### Methods

Involved in the study

ChIP-seq

🗶 Flow cytometry

MRI-based neuroimaging

# Flow Cytometry

## **Plots**

Confirm that:

- $\overline{\mathbf{x}}$  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

We worked using E. coli cells DH5alphaZ1, which were grown in Azure Hi-Def medium details for analysis are described in materials and methods.

Instrument

Attune Nxt Flow cytometer and BD LSR Fortessa.

Software

For data collection, Attune NxT software version 2.7 and FACSDiVa software. Data analysis FlowJo V10.

Cell population abundance

We analyzed 20000 single cells after having performed gating for removing debis and doublets ( see below).

Gating strategy

Quantification of expression levels in all strains was performed using an Attune NxT flow-cytometer (Thermofisher) equipped with an autosampler. Experiments were performed on 96 wells plates with 3 replicates per plates. For flow cytometry measurements, 20 000 bacteria events were analysed. A gate was previously designed based on forward and side scatter graphs to remove debris from the analysis. Doublet discrimination were set with a gate based on SSC-A vs SSC-H. Experiment on Attune NxT were performed in 96 well plates with setting; FSC: 200V, SSC: 380V, green intensity BL1: 460V (488 nm laser and a 510/10 nm filter), red intensity YL2: 460V (561 nm laser and a 615/25 nm filter). Setting for experiments on Fortessa were FSC: 400V, SSC: 300V, green intensity GFP: 580V (488 nm laser and a 530/30 nm filter), red intensity mCherry: 565V (600nm laser and a 610/20 nm filter) and blue intensity V1: 460V (405nm laser and a 450/50 nm filter)

Data were analyzed and presented using the Flow-Jo (Tristar) software.

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