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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 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	Simulation data were generated and collected using MATLAB R2020a. For acquisition of microscopy images, LAS X Life Science Microscope Software was used.
Data analysis	Data analysis was implemented through Graphpad Prism9. Images were analyzed using ImageJ Version 1.53 and LAS X Life Science built-in module (LEICA). Matlab R2020a was used for simulating ordinary differential equations and fitting cellular time-point data. Matlab code is available publicaly via the You Lab Github respository. LASSI 2020 was used for all polymer simulations. LASSI is available publicaly via the Pappu Lab Github respository.

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 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

Raw data can be accessed publicly at https://github.com/youlab. All data and material request should be made to you@duke.edu

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	No sample size calculation was performed and a minimum of 3 biological repeats are standard practice in life science. In each experiment, we captured at least three independent replicates in the form of separate measurements and separate trails for cellular measurements.
Data exclusions	No data were exclueded.
Replication	Both biological replicates and technical replicates were performed for different experiments. Experiments were proved to be repeatable with three independent measurements. Replication statements appear with each corresponding figure.
Randomization	Randomization is not relevant in synthetic biology experiments as samples are genetically identical.
Blinding	Blinding is not relevant to this study. No sample grouping was performed in this study.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi 293 from Thermo Fisher Scientific
Authentication	Cell lines were not authenticated as they were used right after receiving from the manufacturer.
Mycoplasma contamination	Cell lines were presumed to be not contaminated and were not tested for mycoplasma contamination as they were used right after receiving from the manufacturer and under mammalian culture environments.
Commonly misidentified lines (See ICLAC register)	There are no lines on this registry used in our study.

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	0.5 mL of single cell suspension of HEK293 cells was centrifuged and the pellet was resuspended in 1 mL Hank's balanced salt solution to achieve a final concentration. Cells were filtered through a 35micron nylon filter immediately before recording the samples.
Instrument	Three laser (Violet, Blue and Red) BD FACSCanto-II Cytometer.
Software	BD FACSDiva software was used for both data acquisition and analysis.
Cell population abundance	Not applicable as no cell sorting was performed.
Gating strategy	All events were plotted on an FSC-A vs SSC-A bivariate pseudocolor dot plot to exclude debris and to gate on healthy cells (P1 gate). Doublet discrimination was performed hierarchically on P1 population by first plotting SSC-W vs SSC-H followed by FSC-W vs FSC-H. Single cells were then analyzed for Citrine fluorescence by plotting Citrine area signal (log scale) against autofluorescence (log scale). Positive control was utilized to set the threshold for gating Citrine+ population. Percentages of Citrine+ cells along with the mean and median fluorescence intensities (MFIs) for Citrine+ population were analyzed. The mean fluorescence intensity per cell was used as the data point. A sample workflow is shown in Supplementary Fig. 12.

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