

## 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

Data analysis

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 data supporting the findings of this study are available within the article and supplementary information, and are available from the corresponding authors upon reasonable request. Source data are provided with this paper. The plasmids developed in this study will be available from Addgene.

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

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://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 calculations were performed. All experiments were replicated (biological replicates) at least three times to ensure reproducibility. When relevant, the sample size (n) is provided in the corresponding figure captions. Sample sizes were chosen to support meaningful conclusions.

Data exclusions

No data were excluded

Replication

The number of replicates for each individual experiments is indicated in the figure legends. All attempts at replication were successful.

Randomization

The study does not require the allocation of samples into different experimental groups. Hence, randomization was not needed or performed.

Blinding

The study does not involve any comparisons between experimental groups, so the samples were not allocated into different experimental groups. Therefore, no blinding was needed.

## 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                                     |
|-------------------------------------|-----------------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                       |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |

### Methods

| n/a                                 | Involved in the study                              |
|-------------------------------------|----------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

HeLa (ATCC CRM-CCL2), HEK 293T (ATCC CRL-3216) and U2OS (ATCC HTB-96) cell lines were purchased from ATCC.

|                                                                      |                                                                                     |
|----------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Authentication                                                       | None of the cell lines were authenticated after reception from ATCC.                |
| Mycoplasma contamination                                             | All cell lines were tested for the presence of mycoplasma. All tests were negative. |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | No commonly misidentified cell lines were used.                                     |

## 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        | To prepare samples, cells were first grown in cell culture flasks, then transiently co-transfected 24 hours after seeding using Genejuice (Merck) according to the manufacturer's protocol for 24 hours. After 24 hours, cells were centrifuged in PBS with BSA (1 mg/ml) and resuspend in PBS-BSA supplemented with the appropriate amounts of compounds.                                                     |
| Instrument                | Flow cytometry in HEK 293T cells was performed on a MACSQuant analyzer equipped with 405 nm, 488 nm and 635 nm lasers and seven channels.                                                                                                                                                                                                                                                                      |
| Software                  | Data were analyzed using FlowJo v10.7.1                                                                                                                                                                                                                                                                                                                                                                        |
| Cell population abundance | For each experiment, 20,000 cells positively expressing mTurquoise2 (Ex 434 / Em 450 $\pm$ 25) and iRFP670 (Ex 638 nm / Em 660 $\pm$ 10 nm) were analyzed with the following parameters: Ex 488nm, Em 525 $\pm$ 20 nm.                                                                                                                                                                                         |
| Gating strategy           | The plasmids allowing the expression of FRB-FIREmate and FKBP-FIREtag also allowed the expression of mTurquoise2 and iRFP670, respectively, for transfection reporting. Measurement of the green fluorescence of doubly transfected cells enabled to quantify the complementation efficiency of FRB-FIREmate and FKBP-FIREtag. A figure presenting the gating strategy is presented in supplementary figure 4. |

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