nature research

Corresponding author(s): Jin Zhang

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

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	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 *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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Live-cell imaging data were collected using METAFLUOR 7.7. Fluorimetry data were collected using FelixGX 4.1.2 (Horiba Scientific).
Data analysis	Raw imaging data were analyzed using METAFLUOR 7.7 or ImageJ as described in the Methods. In vitro fluorimetry data were analyzed using Microsoft Excel. Statistical analyses were performed using GraphPad Prism 7. Bleedthrough correction was performed using standard calculations in MATLAB. No custom code was used.

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

The data that support the findings of this study are available upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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. Sample sizes were similar to those generally employed in the field.
Data exclusions	Cells that did not respond to stimulation or that did not express all of a given set of constructs (for multi-parameter imaging experiments) were excluded from analysis. Neurons that did not exhibit stable baseline intensities were excluded from analysis. All exclusion criteria were pre-determined.
Replication	Unless otherwise noted, all experiments were repeated at least three times. All replication attempts were successful.
Randomization	Cells were randomly seeded into experimental groups after resuspension.
Blinding	Blinding was not performed as it was not necessary. Objective quantitative data was generated and analyzed.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All plasmids will be made freely available by direct request from the investigators, as well as through Addgene.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa, HEK293T, NIH3T3, and PC12 cells were used in this study. All cell lines were obtained from ATCC.
Authentication	Cell line identities were verified via STR analysis by the commercial source and then maintained separately and isolated from one another to avoid cross-contamination.
Mycoplasma contamination	All cell lines were determined to be free of mycoplasma contamination based on weekly DNA staining.
Commonly misidentified lines (See ICLAC register)	HEK cells were used in some experiments. However, the identity of the cells was not central to the outcome of these experiments.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Sprague-Dawley rats at embryonic day 18 were used to obtain primary cortical neurons. Tissue was pooled from both male and female embryos.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.