nature portfolio

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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	Confocal images were acquired using the Nikon Ti Eclipse spinning disk commercial software
Data analysis	-Electrophysiology (AP morphology, firing rate and Cav2.2 inactivation) data were analyzed using custom MATLAB 2012b (Mathworks). Code is available at: 10.5281/zenodo.15102975. -Confocal images were analyzed using Fiji (Image J, dendritic spines were counted using the Dendritic Spine counter plugin, Pearson's correlation coefficient was calculated using the coloc2 plugin) -Prism Graphpad10 was used for statistical 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

All data is included in the main text or Supplementary Information file. Source Data are provided with this paper.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	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

Life sciences study design

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

Sample size	Required sample sizes were calculated based on power analysis with 90% power to detect a 10% change with (alpha = 0.05). All electrophysiology experiments included a sample size of at least 5 cells
Data exclusions	No data was excluded
Replication	We performed two to four independent replicates for all biochemical studies, confocal imaging experiments, and electrophysiology studies.All data was pooled. All replications were successful.
Randomization	Experiments were not randomized based on the type of experiments. HEK293 cells and neurons were maintained using similar culture condition and cultures were selected at random to be transfected or transduced with relevant palmitoylation probe. Test and control groups were assessed in parallel and isochronically. Electrophysiological experiments were collected in a random order.
Blinding	The study was not blinded as cells typically contained an expression marker noting the presence of the specific actuator. Therefore it was not possible to blind the experimenter,.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Rabbit anti-GFP (Genscript;A01388-40, 1:2000),
Anti-rabbit HRP (Thermofisher,31460, 1:5000),
anti-mouse PSD95 NeuroMab 75-028, 1:500),
ER stain (Abcam 139482, 1:1000),
Anti rabbit RCAS, Cell Signaling 12290, 1:100),
Cell Mask (Thermofisher: C10046, 1:2500),
anti-Rabbit -EEA1 (Cell Signaling, 3288,1:100),
anti-Rabbit- Rab9A (Cell Signaling, 5118,1:50),
anti Rabbit Mcherry (Cell Signaling;43590, 1:1500 for wester and 1:500 for IHC)
Anti rabbit secondary Alexa Fluor 488 (Invitrogen; A11008, 1:1000),
anti Rabbit Alexa Fluor 568 (Invitrogen A11011, 1:1000),
Anti Rabbit Alexa Fluor 647 (invitrogen A27040, 1:1000),
Anti mouse Alexa Fluor 488 (Invitrogen A10680, 1:1000)

Validation

-AntiGFP has been validated by the company in purified GFP /YFp fusion protein and immunoprecipitated samples using western Blots) (Genscript, A01388-40) (1:2000)

-Anti rabbit HRP has been validated by the company in western blots and IHC, (ThermoFisher, catalog #:31460, 1:5000)

-Anti rabbit MCherry has been validated in western blots and immunofluorescent analysis in cells and brain sections (Cell Signaling cat #43590 (1:1500 for western and 1:500 for IHC)

-Anti mouse psd95 validated by company in western blots, immunocytochemistry, immunoprecipitation and analysis, (NeuroMab 75-028, 1:500)

-Cytopainter validated for adherant and suspension cells (Abcam 139482, 1:1000)

-Anti-Rabbit RCas antibody validated for western blots, Immunoprecipitations, Immunofluorescence and flow cytometry (Cell Signaling, #12290, 1:100)

-Cell Mask validated for plasma membrane staining in adherent and suspension cells (ThermoFisher C10046, 1:2500)

-anti Rabbit EEA1 validated for western blots, Immunoprecipitation and immunofluorescence (Cell Signaling 3288, 1:100)

-anti Rabbit Rab9A validated for immunoprecipitation, immunofluorescence and westernblots (Cell Signaling 5118, 1:50)

-Anti rabbit secondary AlexaFLuor 488-validated in multiple cell lines and tissues by company, (Invitrogen, A-11008, 1:1000)

-Anti rabbit secondary AlexaFluor 568-validated by company in cell lines, tissue sections, Invitorgen A-11011, 1:1000)

-Anti mouse secondary Alexa FLuor 488- validated by company in western blots, immunofluorescence and immunocytochemistry using cell lines. (Invitrogen A10680, 1:1000)

anti-rabbit Alexa Fluor 647 validated by company in western blots, immunofluorescence and immunocytochemistry using cell lines. (Invitrogen A27040, 1:1000)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human embryonic Kidney 293 Cell line (ATCC) (CRL-1573)
Authentication	Cell lines were valiated using STR profiling by ATCC

Mycoplasma contamination	The cultures are maintained in medium containing Plasmocin, a broad spectrum anti-mycoplasmic. Routine testing did not show presence of Mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	E18 Primary Sprague Dawley Rat hippocampal neurons were procured from BrainBits
Wild animals	N/A
Reporting on sex	Neuronal cultures included cells from both male and female rats that were provided as aggregates, therefore it was not possible to perform sex-based analysis.
Field-collected samples	N/A
Ethics oversight	Animal protocol #233-08-013 for BrainBits was approved by the Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee on 04/07/2020.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A