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	Details are described in the Methods. Olympus FV1000, PicoQuant SymPhoTime64 and LabView software.
Data analysis	MatLab(R2013b), Microsoft Excel (Microsoft Office 365), XLSTAT (2021.3.1.1170) The MatLab scripts for the FRET/FLIM analysis together with other codes used in this study are available at; https://github.com/sw-swatanabe/ucar-et-al-2021.

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

Source Data for Figs. 1–4 and Extended Data Figs. 1–4,6,7,9,10 are provided with the paper. All other data are available from the corresponding author upon reasonable request.

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. Sample size was determined based on our previous findings. Similar numbers of data have been utilized in many papers: Including our own Iino el al.(2020)Nature579:555, Matsuzaki et al. (2004)Nature 429:761, or others, Hedrick et al. (2016)538:1024, Havey t al. (2007)450:1195.
Data exclusions	For pushing experiments, data where pushing pipette passed more than the axial line of the axon were excluded from FRET analysis. For spine enlargement experiments, cells with a resting membrane potential above -55 mV during STDP stimulation were excluded.
Replication	Data were replicated in multiple synapses from multiple slice preparations and rats at least during two independent periods, as now described in "Statistics and Reproducibility" section in the Methods.
Randomization	We did not use randomization process, as stated in "Statistics and Reproducibility." Making and testing the samples were unseparable in our experiments as in most in vitro studies where new techniques are developed using only wild type animals.
Blinding	We did not use blind process, as stated in "Statisitics and reproducibility" section, due to the same reason above.

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)	HEK293 (AAV293, stratagene)
Authentication	Not authenticated was done since the cell line was just used for AAV production.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	We did not use commonly misidentified lines listed by ICLIAC.

Animals and other organisms

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

Laboratory animals	6- to 8-day-old Sprague-Dawley male and female rats (Slc:SD rat, Sankyo Labo Service).
Wild animals	The study did not involve wild animals.

Field-collected samples	No field collected sample was used.
Ethics oversight	All mice experiments were complied with the animal care standards provided by the University of Tokyo, and were approved by the Animal Experimental Committee.

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