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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 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	Data was collected with custom built software that had been described previously: Single fusion assay: Domanska et al THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 284, NO. 46, pp. 32158–32166, November 13, 2009. sdFLIC assay: Liang et al PNAS 110(48) 2013.
Data analysis	Software to analyze the data was custom built and had been described previously. Single fusion assay: Domanska et al THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 284, NO. 46, pp. 32158–32166, November 13, 2009. sdFLIC assay: Liang et al PNAS 110(48) 2013. The FLIC fitting software is based on software that was kindly provided by Armin Lambacher and is described in detail in J. Opt. Soc. Am. B,Vol. 19, No. 6/June 2002. For data illustration Igor Pro 7.0 from Wavemetrics 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

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 from the corresponding author on 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	Results are reported as mean from repeated experiments. The number of experiments, mean and standard errors for all experiments are reported in tables as Supplementary Tables. For fusion assays, we also report the total number of recorded events. SDFLIC statistics is explained in detail in the Methods part. Briefly, results are reported as mean +/-std from repeats. Each repeat includes 4-5 acquired images from one sample. On each image ~100 areas were analyzed. The distribution of results from one (example) sample are shown as Supplementary Figures for all conditions.
Data exclusions	No data was excluded.
Replication	See above. All experiments were repeated at least 3 times. Number of repeats are listed in Extended Data tables.
Randomization	Experimental conditions are defined in the paper. No randomization or further allocation was necessary.
Blinding	Experimental data was analyzed by software. No data was excluded, no blinding was necessary.

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

Obtaining unique materials	All unique materials are available from the authors upon reasonable request.

Eukaryotic cell lines

Cell line source(s)	Rat pheochromocytoma PC12 cell line obtained from Edwin Chapman, University of Wisconsin.
Authentication	Use of amperometry to detect depolarization-dependent noradrenalin secretion (Liu et al (2005) Mol. Biol. Cell 16: 4463-4472 PMID 9487127); use of cell fractionation to purify large dense core vesicles containing secretogranin II, a known stored secretory product (Liu et al (2002) Mol. Biol. Cell 13: 4266-4278 PMID 12475951); use of electron microscopy to analyze compound exocytosis of large dense core vesicles (Zhang et al (2011) Traffic 12: 600-614 PMID 21272170).
Mycoplasma contamination	Cell lines routine tested for mycoplasma 2002-2017
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.