

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Visual Basic. NET (Microsoft)
Data analysis	Fiji (ImageJ) software (NIH, USA), Igor Pro9 software (WaveMetrics, USA), OriginPro 2025 software (OriginLab, USA)

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 that support the findings of this study are available from the corresponding author upon request.

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 Sample-size calculations were not performed in this study. HS-AFM observation allows more than 100 molecules to be observed in a single experiment. Observations were repeated at least three times, and molecules that were similar in shape and dynamics in the HS-AFM images were determined to be those that exhibited average CaMKII behavior. Approximately 10 such molecules were analyzed; the analysis of about 10 molecules was deemed sufficient because there was little variation in Dh-k and Rg among the individual molecule.

Data exclusions No data were excluded from the analyses.

Replication All HS-AFM experiments were repeated independently at least three times, yielding consistent results.

Randomization Allocation was random.

Blinding We were blinding group allocation during data collection and analysis.

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

☒ ☐ Clinical data

☒ ☐ Dual use research of concern

☒ ☐ Plants

Methods

n/a Involved in the study

☒ ☐ ChIP-seq

☒ ☐ Flow cytometry

☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used anti-phospho-CaMKII (Thr286) (D21E4; Cell Signaling Technology, USA), anti-phospho-CaMKII (Thr305) (abx012403; Abbeva), anti-

Antibodies used

phospho-CaMKII (Thr306) (p1005-306; PhosphoSolutions), anti-phospho-PKA substrate (RRXS*/T*) (#9624; Cell Signaling Technology), anti-His-tag (27E8; Cell Signaling Technology), anti-pan-CaMKII (D11A10; Cell Signaling Technology), anti-GFP (M048-3; MBL), anti-CaMKII β (ab34703; Abcam), anti-CaMKII α (13730-1-AP; Proteintech), and HRP-conjugated anti-rabbit/mouse secondary antibodies (Jackson ImmunoResearch Laboratories). All primary/secondary antibodies were used at a 1:4000 dilution, except for anti-His-tag (27E8), which was used at a 1:5000 dilution.

Validation

Validated by manufacturer.

Eukaryotic cell lines

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

Cell line source(s)

HEK293 cells were obtained from the American Type Culture Collection (ATCC, CRL-11268).

Authentication

None of the cell lines used were authenticated.

Mycoplasma contamination

The cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a