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	All codes used in MD simulation can be found here https://github.com/kairongdong/camk2_linker_modeling
Data analysis	The missing loop and linker is inserted with MODELLER ver 10.4, the simulation preparation is with CHARMM-GUI (CHARMM36m), and run with GROMACS ver 2019.4, the analysis used Mdtraj ver 1.9.4. HKL-3000, Phaser, Coot Mosfilm 7.4.0; Pointless 1.8.20; Phenix 1.20; Refeyn AcquireMP v2.3; DiscoverMP v2.3; BioXTAS RAW v2.1.4; VMD v1.9.3

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 X-ray diffraction data has been deposited to the RCSB PDB (Accession code: 8USO).

The experimental data that support the findings of this study are available in Figshare with the identifier https://doi.org/10.6084/m9.figshare.27170322.
The MD input files and starting structure can be found here https://github.com/kairongdong/camk2_linker_modeling

The shortgun proteomics data have been deposited to the ProteomeXchange Consortium via the MassIVE server with the dataset identifier MSV000094576.
SAXS data and models have been submitted to SASBDB45 (https://www.sasbdb.org/) - CaMKIIα exon 14 (SASDVU7); CaMKIIα exon 18 (SASDVV7)
SEC-SAXS-MALS have been uploaded to zenodo repository (https://doi.org/10.5281/zenodo.15741768)
SAXS data with Ca2+/CaM have been deposited to Zenodo (https://doi.org/10.5281/zenodo.15785972)
FRET data have been deposited to Zenodo (https://doi.org/10.5281/zenodo.15741933)

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	No sample size calculation was performed for biochemical analyses and structural determination. For the in vivo FRET experiment, two biological replications were performed, and at least 5 oocytes were included in each group.
Data exclusions	For FRET experiment, dead oocytes were excluded from the data analysis, as indicated by large increases in Ca2+ level prior to exposure to treatment.
Replication	Three technical replications were performed for each measurements to provide the means and standard deviations of mean displayed in the graphs.
Randomization	Oocytes were randomly selected for FRET experiment.
Blinding	Blinding was not relevant for the biochemical assay and structural analysis. Blinding for the FRET experiment was not performed as no subjective analysis was involved.

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

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	C57BL/6J (WT, Strain #: 000664) females were mated with DBA/2J male (WT, Strain #:000671) mice to generate BDF1 hybrids. Female BDF1 mice were house to 6- to 10-weeks-old before oocytes collection.
Wild animals	No wild animals were involved.
Reporting on sex	Sex was not a factor in this study. Only female mice were involved as they provide oocytes
Field-collected samples	No field collection was involved in this study
Ethics oversight	All animal procedures were performed according to research animal protocols approved by the University of Massachusetts Institutional Animal Care and Use Committee (IACUC, protocol #4579)

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