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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	Molecular simulations were performed using openMM v7.5 (https://openmm.org/).
Data analysis	Simulations were analysed using OVITO Basic (v.3.7), MDTraj (v.1.9.6), and MDAnalysis (v.1.1). Molecular visualisations were generated using VMD (v.1.9.4). Custom code used to generate and analyse data is available at https://github.com/KULL-Centre/_2023_Garcia-Cabau_CPEB4 and at Zenodo (https://doi.org/10.5281/zenodo.13880099). NMR backbone resonance assignments were performed using CcpNmr 2.4.2. Experiments were processed using qMDD 3.2, NMRPipe and Topspin 4.0.8 (Bruker). Diffusion coefficients were obtained using MestreNova v14.2.1-27684.

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 NMR assignments are available on BMRB (ID 51875 and 52346). The simulation data generated in this study are available via https://github.com/KULL-Centre/_2023_Garcia-Cabau_CPEB4. Protein sequences correspond to UniProt IDs Q17RY0 for CPEB4, Q7Z5Q1 for CPEB2, and Q7TN99 for CPEB3. The rest of the data are included in this article as source data.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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 was empirically established in accordance with previous experience with similar experiments and based on the variability of previous similar data. the distribution and reproducibility of the data were also taken into account to choose sample size for each experiment. No statistical test was performed to predetermine sample size. Accordingly, we performed n=3 for the majority of experiments.
Data exclusions	No data were excluded from the analyses.
Replication	Images of primary striatal neurons in Fig. 1c and Extended Fig. 1d are representative of two independent experiments. Live-cell time-lapse movies of striatal neurons in Fig. 1d and Extended Fig. 1e were acquired once and images show representative behavior from all imaged cells. Live-cell acquisition of NTD nCPEB4-GFP in Fig. 1h and Extended Fig. 1i was performed twice. RT-PCRs monitoring CPEB4 splicing in Ext. Fig. 1b and Srrm4 in qRT-PCRs in Ext. Fig 1c were performed twice. qRT-PCRs monitoring nCPEB4 isoforms and splicing factors in cell lines were performed in n >= 1. Ionomycin experiments in Ext. Fig 1c were replicated twice. BioID and RNA competition experiments in Ext. Fig 4b,c were performed once. Live-cell acquisition of NTD nCPEB4-GFP 12A in Fig. 2b and Extended Fig. 2b was performed once and images show representative behavior from all imaged cells. All the other results were replicated at least in three independent biological replicates, as indicated in the manuscript.
Randomization	For the comparison of the fate of the condensates formed by nCPEB4 variants upon depolarization (Fig. 1j, Fig. 3f, Fig. 3l and Extended Data Fig. 2e), all samples were randomly allocated into experimental groups. Moreover, time-lapses from different variants (different experimental groups) were acquired in a different order for different biological replicates to avoid biases due to the time of incubation post-transfection. For all other experiments, all samples were randomly allocated into experimental groups when possible. Moreover, in all other experiments, different experimental groups were simultaneously and equally analyzed.
Blinding	The investigators were blinded to group allocation during data analysis in all experiments involving qualitative analysis of the data of more than one group of data. These experiments include quantification of the percentage of cells displaying cytoplasmic foci after depolarization (Fig. 1j, Fig. 3f, Fig. 3l and Extended Data Fig. 2e) was performed blindly. For the rest of the experiments, quantitative analysis of the data was performed without the need of blinding since specific quantifications were equally applied to all the groups analyzed by the specified used

softwares.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

For WB, we used the following antibodies:
- Primary antibodies: anti-CPEB4 (homemade, mouse monoclonal, clone 149C), anti-GFP (Invitrogen, rabbit polyclonal, A6455), anti-myc (Abcam, goat polyclonal, ab9132), HRP anti-β actin (Abcam, mouse monoclonal, ab20272); or streptavidin (Thermofisher, S911).
- Secondary antibodies: goat anti-mouse (Thermofisher, 31430), goat anti-rabbit (Thermofisher, G-21234), donkey anti-goat (Abcam, ab6885).
For IHC, we used rabbit polyclonal anti-CPEB4 antibody (Abcam, ab83009).
For IF, we used the following antibodies:
- Primary antibody: anti-CPEB4 antibody (clone 149C).
- Secondary antibody: Alexa 488 Donkey anti-mouse (Thermofisher, A-21202).

Validation

All commercial antibodies were validated by the provider and extensively used in previous studies. Home-made anti-CPEB4 antibody was validated by CPEB4 knock-down or knock-out in multiple eukaryotic cell lines and mice models.

Antibodies used for WB:
- Primary antibodies: anti-CPEB4 antibody (homemade, mouse monoclonal, clone 149C) as detailed above, was validated by CPEB4 knock-down or knock-out in multiple eukaryotic cell lines and mice models; anti-GFP antibody (Invitrogen, rabbit polyclonal, A6455) was validated by the provider in western blot by detection of different proteins fused with GFP that were transiently transfected in human cells; anti-myc antibody (Abcam, goat polyclonal, ab9132) reactivity was validated by the provider in WB with myc tag samples and was used in previous studies including Duran-Arqué et al., Genome Biology, 2022; HRP-anti-β actin (Abcam, mouse monoclonal, ab20272) was validated by the provider for WB in mouse, human and rat and was used in previous studies including Yu et al., Cell, 2021; streptavidin (Thermofisher, S911) was validated for WB in previous studies including Branon et al., 2018, Nat Biotechnol. by addition of exogenous biotin to human cell lines for similar purposes to the ones in this study (e.g. TurboID).

Antibodies used for IHC:
Rabbit polyclonal anti-CPEB4 antibody (Abcam, ab83009) was validated by CPEB4 knock-down or knock-out in multiple eukaryotic cell lines and mice models.

Antibody used for IF: anti-CPEB4 antibody (homemade, mouse monoclonal, clone 149C) was validated as indicated in the WB section.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Neuro-2a (N2a) cell line was purchased from ATCC (CCL-131).
NmumG cell line was a gift from Antonio García de Herreros.
C2C12 cell line was a gift from Antonio Zorzano.

Authentication

None of the cells used were further authenticated, since they are commercial cell lines and were used at low passages.

Mycoplasma contamination

Mycoplasma contamination was checked bi-monthly and all cell lines always tested negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines in accordance with the ICLAC database were used in this 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	For striatal neurons extraction, C57BL/6J mice over 6 weeks of age were crossed in timed matings. For RNA extraction from tissues, B6.129 mice over 6 weeks of age were used. For CPEB4 IHC in mice, C57BL/6J mice embryos at embryonic day 13.5 were used. For BioID and competition experiments, adult Xenopus laevis (over 36 months of age) were used. For the in vivo conformation of CPEB4 studies we used - Homozygous CPEB4 KO mice in C57BL/6J background. - TgCPEB4Δ4 mice in C57BL/6J background. Animals of 1.5 and 6 months were used. All mice were bred and housed in the CBMSO animal facility. Mice were grouped four per cage with food and water available ad libitum and maintained in a temperature-controlled environment on a 12 h–12 h light–dark cycle with light onset at 08:00 and a relative humidity of 55 ± 10%. Animal housing and maintenance protocols followed local authority guidelines.
Wild animals	The study did not involve wild animals.
Reporting on sex	For striatal neurons extraction, mice females with an increment over 3 grams up to 18 days after a positive plug were euthanized and embryos were collected at E18.5. Striatal neurons from all mice embryos were extracted regardless of their sex. For BioID and competition experiments, stage VI oocytes were obtained from adult Xenopus laevis females as previously described in [de Moor and Richter, EMBO, 1999]. For the in vivo conformation of CPEB4 studies both male and female mice were included in similar proportions.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All experiments with mouse models and Xenopus laevis were approved by the the Animal Ethics Committee at the Barcelona Science Park (PCB) and by the Government of Catalonia. For the in vivo conformation of CPEB4 studies Animal experiments were performed under protocols approved by the CBMSO Animal Care and Utilization Committee (Comité de Ética de Experimentación Animal del CBMSO, CEEA-CBMSO), and Comunidad de Madrid (PROEX 247.1/20). In accordance with the current regulation, all requirements for the investigation were assessed by the Animal Testing Commission as the organization authorized by the Government of Catalonia, including the information related to the use of anesthesia during the project, the origin of the animals, information related to housing, zootechnical and care conditions of the animals in the project, the information related to the euthanasia method, the information presented in relation to the accreditation/training of the personnel participating in the project, the compliance with the 3Rs and the damages and benefits of the project were assessed in the frame of ethical balance.

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