

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

DataMax Ver 2.2 (Jobin Yvon); FluorEssence™ (Jobin Yvon); Typhoon FLA 9500 (GE); Image Studio v5.2 (Li-Cor Biosciences); μ s-ALEX data was collected with a National Instruments PCI-6602 and custom made labView (v.7.1) based acquisition software.

Data analysis

ImageQaunt Mac v1.2; KaleidaGraph v 4.03; Berkeley Madonna v 9.1.3; Quantity One v 4.6.6 ; All μ s-ALEX data analysis was performed using FRETbursts (v0.7+47.gc51b088, available at <https://github.com/harripd/FRETbursts>) and phconvert (v0.9+43.g3a86e58, available at <https://github.com/Photon-HDF5/phconvert>). All mpH2MM analyses in this study were performed using the Python packages, burstH2MM (Available at <https://github.com/harripd/burstH2MM>) and H2MM_C (Available at <https://github.com/harripd/H2MMpythonlib>).

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](#)

All relevant data are presented in the manuscript main text, Supplemental information (including Supplemental Figures S1-S8, Supplemental Table S1), and **Appendix 1. Supplementary Dataset 1**

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	<input type="text" value="n/a"/>
Reporting on race, ethnicity, or other socially relevant groupings	<input type="text" value="n/a"/>
Population characteristics	<input type="text" value="n/a"/>
Recruitment	<input type="text" value="n/a"/>
Ethics oversight	<input type="text" value="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](https://www.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	<input type="text" value="Determined according to established standards. Biochemical measurements are independently repeated at least twice. ALEX data were corrected until sufficient data points (n≥3000) were gathered for subsequent analyses."/>
Data exclusions	<input type="text" value="None."/>
Replication	<input type="text" value="The number of replicates are specified in the figure legends for every experiment."/>
Randomization	<input type="text" value="n/a"/>
Blinding	<input type="text" value="n/a"/>

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

Methods

n/a	Involvement
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

n/a	Involvement
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

THE™ NWSHPQFEK (Strep) Tag Antibody, mAb, Mouse (Genscripts, Cat# A01732)
 THE™ His Tag Antibody, mAb, Mouse (Genscripts, Cat# A00186)
 IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody (LiCor, Cat#926-32210)

Validation

All antibodies are validated by the manufacturer.
 Genscripts, Cat# A01732: https://www.genscript.com/antibody/A01732-THE_NWSHPQFEK_Tag_Antibody_mAb_Mouse.html
 Genscripts, Cat# A00186: https://www.genscript.com/antibody/A00186-THE_His_Tag_Antibody_mAb_Mouse.html
 LiCor, Cat#926-32210: <https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody>