

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	Fluorescence microscopy data were collected through the Nikon Elements software (version 5.21.03). Fluorescence correlation spectroscopy data were collected using PicoHarp 300 software (version 3.0.0.3).
Data analysis	Microscopy images were analyzed using ImageJ2 (version 2.9.0/1.53t). Single-molecule images were analyzed using and the Track Mate plug in (version 7.10.2). Data analysis was performed using MATLAB (version 2022b Update 5) and GraphPad Prism (version 10.4.0). Kinetic modeling was performed using SimBiology (version 6.4, MathWorks). The custom MATLAB script used for TrackMate data processing, association kinetics, and step size distribution analysis is available on GitHub at https://github.com/youngkwanglee-mem/SPT_Kon_StepSize . The specific version of the code used for the analysis in this study has been deposited in Zenodo with the DOI https://doi.org/10.5281/zenodo.18126517 .

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 authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. The raw image data are available from the corresponding author upon reasonable request. These image data are not deposited in a public database because of their large file sizes. Source Data are provided with this paper. The structures of the CRAF/MEK1/14-3-3 complex in the autoinhibited conformation and the CRAF RBD-CRD/KRAS complex were obtained from the Protein Data Bank under accession codes 9MMP and 6XI7, respectively.

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	Presented data included technical replicates ranging from two to twelve (n=2-12) and experimental replicates up to N=4.
Data exclusions	In single-molecule experiments, we excluded low RAS protein density samples <180 per square micrometer due to low reproducibility.
Replication	Reproducibility was verified through independent experiments, as indicated in the figure captions. The observed trends were highly reproducible across experiments, although absolute values varied slightly between experimental days. Individual measurement results or means and standard deviations are provided in the main text and Supplementary Information to display their distribution.
Randomization	Randomization is not necessary or appropriate in these biochemical and biophysical experiments.
Blinding	Blinding is not necessary or appropriate in these biochemical and biophysical experiments.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A