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
- [x] A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [x] 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.
- [x] A description of all covariates tested
- [x] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [x] 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)
- [x] 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.
- [x] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [x] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [x] 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

| Datacollection | Collection of microscopy images was performed using microManager software version 2.0 (https://micro-manager.org). |
|---------------|------------------------------------------------------------------------------------------------------------------|
| Dataanalysis  | Data were analyzed with GraphPad Prism version 8.4. Image analysis was performed with custom code described in Supplementary Note 1 and deposited at a public repository (https://github.com/jmsung/smr-analysis). |

For manuscripts utilizing custom algorithms or software that are not yet published, submit the code, algorithm or software as a supplementary file, or describe method details sufficient to allow reproduction of the results.

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 video and single-molecule analysis data generated in this study are available on a public repository (https://zenodo.org/record/5726046). Source data for Fig. 1 and Supplementary Figs. 1, 2, 5, 6 are provided with this paper.
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/re-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: As is the standard in the field, at least two independent videos were obtained for each ligand-protein combination. The list of replicate movies is provided in Supplementary Table 1. For each independent replicate movie, the standard in the field is to analyze at least 100 binding events, but we analyzed several hundred and sometimes thousands of events per movie, resulting in very small errors in mean dwell times.

Data exclusions: No data exclusions.

Replication: All experiments and video analyses were replicated independently at least once, demonstrating the reproducibility of all results.

Randomization: Our study involves biochemical analyses of purified proteins. It does not include statistical comparisons between groups of subjects or other samples. Thus, sample or subject randomization is not applicable to this work.

Blinding: Our study does not involve statistical comparisons of groups of experimental subjects or other samples, and therefore blinding is not applicable.

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 |
| ☑   | Human research participants |
| ☑   | Clinical data         |
| ☑   | Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChiP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

Antibodies

Antibodies used: Biotin-conjugated mouse monoclonal anti-Strep-Tag II antibody (LSBio, Cat. No. LS-C203632-100). Biotin-conjugated rabbit polyclonal anti-GFP antibody (Rockland, Cat. No. 600-406-215).

Validation: Specificity of both antibodies was confirmed by control experiments showing minimal background with antibody alone in all experiments, presented in Supplementary Figs. 3, 7, 8.