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Last updated by author(s):	Nov 7, 2019	

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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						
<u></u>	ple size (n) for each experimental group/condition, given as a discrete number and unit of measurement					
	n 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					
A description	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 descripti	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 a	inalysis, information on the choice of priors and Markov chain Monte Carlo settings					
For hierarchical	al and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
Estimates of e	ffect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated					
1	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.					
Software and c	ode					
Policy information abou	ut <u>availability of computer code</u>					
Data collection	SymPhoTime 64 software (PicoQuant, Berlin, Germany), version 2.4					
Data analysis	Matlab R2015b 64-bit (MathWorks, Natick, MA, USA)					
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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.						
Data						
- Accession codes, uni - A list of figures that I	It <u>availability of data</u> nclude a <u>data availability statement</u> . This statement should provide the following information, where applicable: que identifiers, or web links for publicly available datasets have associated raw data restrictions on data availability					
The data that support the findings of this study are available from the corresponding author upon reasonable request.						
Field-speci	fic 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.						
☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences						

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.						
Sample size	Samples were always solutions of fluorescently labeled molecules with well defined molecule concentrations					
Data exclusions	Data from aggregates in the measured sample solutions were removed from the data sets (for details see Methods)					
Replication	Prepared stock solutions of all samples were diluted and aliquoted. Measurements from different aliquoted samples produced reproducible results, number of repeated measurements and a statement whether the repeated measurement was performed with a new (independent sample /sample reaction) are given.					
Randomization	not applicable					
Blinding	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.

Ma	terials & experimental systems	Methods	
n/a	Involved in the study	n/a	Involved in the study
\boxtimes	Antibodies	\boxtimes	ChIP-seq
\boxtimes	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology	\boxtimes	MRI-based neuroimaging
\boxtimes	Animals and other organisms		•
\boxtimes	Human research participants		
\boxtimes	Clinical data		