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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed		
	\square	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement	
	\square	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly	
\boxtimes		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.	
\boxtimes		A description of all covariates tested	
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons	
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)	
\boxtimes		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>	
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings	
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes	
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated	
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, Cl)	

Our web collection on statistics for biologists may be useful.

Software and code

 Policy information about availability of computer code

 Data collection
 Data was acquired using software supplied by instrument manufacturer (ZEISS ZEN 2011) or the open source software µmanager (Version 1.4): Journal of Biological Methods 2014 1(2):e11 doi:10.14440/jbm.2014.36

 Data analysis
 Open source software Fiji (distribution of ImageJ) was used to process confocal imaging data, it was described in the following publication: Nature methods 9(7): 676-682, PMID 22743772, doi:10.1038/nmeth.2019. Custom analysis software Picasso was used to process raw super-resolution data. The software is open source and was described in detail in the following publication: Nature Protocols volume 12, pages 1198–1228 (2017) doi:10.1038/nprot.2017.024.

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 upon request. 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

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 list of figures that have associated raw data
- A description of any restrictions on data availability

All raw data are available upon request from the authors

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences

Study design

All studies must dis	close on these points even when the disclosure is negative.
Sample size	Localization precision for EGFR imaging experiments were calculated from around 30,000 labeled proteins.
Data exclusions	No data were excluded from analysis
Replication	All experiments were reliably reproduced
Randomization	No results that require randomization are presented in this study
Blinding	n/a since no allocation into groups was performed

Materials & experimental systems

Policy information about availability of materials

n/a Involved in the study				
Unique materials				
Antibodies	Antibodies			
Eukaryotic cell lines				
Research animals				
Human research partic	ipants			
Unique materials				
Obtaining unique materials	All unique materials are readily available from the authors or from standard commercial sources (SomaLogic)			
Antibodies				
Antibodies used	Primary monoclonal Anti-EGFR antibody: Cell Signaling, cat: 4267S, clone D38B1, Lot: 11, dilution 1:200 Primary antibody against extracellular region of EGFR: MA5-13319, clone 119.12, Lot: TD2556544B, dilution 1:50 Primary anti-PMP70 antibody: Abcam, ab211533, clone CL2524, dilution 1:200			
	Secondary polyclonal anti-rabbit antibody conjugated to Alexa Fluor 647: Abcam cat: ab150075, dilution 1:200 Secondary polyclonal AffiniPure Anti-Mouse IgG antibody: Jackson ImmunoResearch cat: 115-005-003			
Validation	All antibodies were validated for IF and human species reactivity by the manufacturer according to their websites.			
Eukaryotic cell lines				
Policy information about <u>cell lines</u>				
Cell line source(s)	A431, A549, and SK-BR-3 cells were purchased from ATCC. mEGFP-Nup107 HeLa Kyoto was obtained from the Ellenberg lab (Reference Otsuka, S. et al. Elife 5 (2016))			

Authentication

Cell lines were not authenticated

Mycoplasma contamination

Commonly misidentified lines (See ICLAC register)

No commonly misidentified cell lines were used

All cell lines were tested negative for mycoplasma contamination

Method-specific reporting

n/a | Involved in the study

ChIP-seq

 \times

Flow cytometry

Magnetic resonance imaging