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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

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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
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
×	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. <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>
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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
So	ftware and code
Polic	cy information about <u>availability of computer code</u>

Data analysis FalconViewer based on Igor Pro-9 (WaveMetrics: ver 9.01), AlphaFold2 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

$reviewers. \ We strongly \ encourage \ code \ deposition \ in \ a \ community \ repository \ (e.g. \ GitHub). \ See \ the \ Nature \ Portfolio \ \underline{guidelines \ for \ submitting \ code \ \& \ software} \ for \ further \ information.$

Data

Data collection

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. 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

Eagle software package 1.0.2.7 by RIBM in an Igor Pro-6 (WaveMetrics: ver 6.37)

Any data relating to the findings presented in this article are available within the article and its supplementary information files. All source data are provided with this paper as Source data file.

Human resea	arch partio	cipants			
Policy information about studies involving human research participants and Sex and Gender in Research.					
Reporting on sex an	nd gender	Not applicable. Because no human research in this study			
Population characte	eristics	Not applicable. Because no human research in this study			
Recruitment		Not applicable. Because no human research in this study			
Ethics oversight		Not applicable. Because no human research in this study			
Note that full informa	tion on the appro	oval of the study protocol must also be provided in the manuscript.			
Field-spe	cific re	porting			
Please select the or	ne below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.			
x Life sciences	Ве	ehavioural & social sciences			
For a reference copy of the	he document with a	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces stu	ıdy design			
All studies must dis	close on these p	points even when the disclosure is negative.			
Sample size	(Figure 3d and s	ethods were used to predetermine the sample size. Number of the particle for the classification of Slh1 particle by HS-AFM upplementary figure 4) was chosen based on our preliminary experiments; more than 100 particles were established as saffy the major two different orientation of the particles on the mica surface.			
Data exclusions	No data exclusio	ons			
Replication	The experiment	eriments were performed at least twice with consistent results.			
Randomization		mrization. Our study compared proteins dynamics by HS-AFM, and protein function analyzed in vitro and in vivo using yeast clones in ferent genes were deleted or modified in parallel.			
Blinding	The investigator	The investigators were not blinded during data collection.			
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 Involved in the study					

Antibodies

Antibodies used

The details of all antibodies used in this study were described in the supplementary table 4.

Anti-HA antibody (Roche, RRID: AB_390917, Cat#: 12013819001, 1:10000 dilution)
Anti-Flag antibody (Sigma-Aldrich, RRID: AB_262044, Cat#: F1804-1MG, 1:5000 dilution)

Anti-ubiquitin antibody (Santa Cruz Biotechnology, RRID: AB_628423, Cat#: sc-8017, 1:1000 dilution)

Antiubiquitin (linkage-specific K48) antibody (Abcam, RRID: AB_2783797, Cat# ab140601, 1:1000 dilution)

Antiubiquitin (linkage-specific K63) antibody (Abcam, RRID: AB_2895239, Cat# ab179434, 1:1000 dilution)

Anti-eEF-2 antibody (Lab.stock, N/A, 1:20000 dilution)

Anti-mouse IgG, horseradish Peroxidase (Cytiva, RRID:AB_772210, Cat# NA931-1ML, 1:5000 dilution)

Anti-rabbit IgG, horseradish Peroxidase (Cytiva, RRID:AB_772206, Cat# NA934-1ML, 1:5000 dilution)

Validation

The validations of all antibodies refer to as follow,

Anti-HA antibody (https://www.sigmaaldrich.com/JP/ja/product/roche/12013819001)

Anti-Flag antibody (https://www.sigmaaldrich.com/JP/ja/product/sigma/f1804)

Anti-ubiquitin antibody (https://datasheets.scbt.com/sc-8017.pdf)

Antiubiquitin (https://www.abcam.co.jp/ubiquitin-linkage-specific-k48-antibody-ep8589-ab140601.html)

 $Antiu biquit in \ (https://www.abcam.co.jp/ubiquit in-linkage-specific-k63-antibody-epr8590-448-ab179434.html)$

Anti-eEF-2 antibody (Ikeuchi et al., 2019 EMBO J: PMID: 30609991)

Anti-mouse IgG, horseradish Peroxidase (https://www.sigmaaldrich.com/JP/ja/product/sigma/gena9311ml)

Anti-rabbit IgG, horseradish Peroxidase (https://www.sigmaaldrich.com/JP/ja/product/sigma/gena9341ml)