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Reporting Summary

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

Statistical	parameters

text	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				
	An indication of 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 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>				
X	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				
	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)				

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Data analysis

For analysis of CRAC data, Illumina sequence reads were trimmed and quality controlled using Flexbar (Dodt et al., 2012) and were mapped to the S. cerevisiae genome using Bowtie2 (Langmead and Salzberg, 2012). For analysis of mass spectrometry data, peak lists were extracted from the raw data using Raw2MSMS software and proteins were identifed using MASCOT 2.4 software (Matrixscience). Details are provided in the references cited in the Online Methods section.

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 <u>availability of data</u>

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

The CRAC datasets of Has1, Mak5, Spb4 and the wild-type yeast control are deposited in Gene Expression Omnibus (GEO) database [http://www.ncbi.nlm.nih.gov/geo/] under the accession code GSE109216. Other data supporting the findings of this study are available from the corresponding authors upon reasonable reques A reporting summary for this Article is available as a Supplementary Information file.

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Field-spe	ecific r	eporting	
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Life scier	ices s	tudy design	
All studies must disclose on these points even when the disclosure is negative.			
Sample size	Not applicab	le e	
Data exclusions	No data were excluded		
Replication	All experiments were performed at least three times with reproducible results		
Randomization	Not applicab	le e	
Blinding	Not applicab	le	
Materials & experimental systems Methods Methods			
Antibodies Antibodies			
Antibodies used Details of the antibodies used in this study are listed in Supplementary Table S6		Details of the antibodies used in this study are listed in Supplementary Table S6	
Validation Besides the validations performed by th		Besides the validations performed by the manufacturers, antibodies were validated in Western blots using cells expressing and	

Besides the validations performed by the manufacturers, antibodies were validated in Western blots using cells expressing and cells not expressing proteins carrying the detected tags (for antibodies that detect tags), or cells that were depleted and cells not depleted of the detected protein (for antibodies that detect endogenous proteins).