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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 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
☐	☒ 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
☐	☒ 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.*
☐	☒ 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 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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ 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

Data collection	Microsoft Excel, Flowjo, Attune Cytometric Software
Data analysis	Microsoft Excel

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

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

The data that support the plots within this paper and other findings of this study are available from the last corresponding author upon reasonable request.

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/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample size of 2 or 3 was used for all experiments unless otherwise noted.
Data exclusions	Only instance of data exclusion pertained to Figure 6a-b. 2 points that showed abnormal aScaf-pScaf assembly were excluded from the structure-performance analyses because the structure of these assemblies was either abnormal or characterized incorrectly.
Replication	Significant trends in were reproduced throughout the manuscript to generate additional figures. Majority of data between figures is from independent experiments.
Randomization	N/A
Blinding	N/A

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-V5-AF647: Invitrogen, Catalog No: 46-1260, Clone: N/A, Lot No: 2042372 Anti-c-Myc PE: R&D Systems, Catalog No: IC3696P, Clone: 9E10 Lot No: ADVP0317111 Anti-His AF647: Biolegend, Catalog No: 362611, Clone: J095G46, Lot No:B278583
Validation	Anti-V5-AF647: Validated in publications: PMID: 24699865 Anti-c-Myc PE: Validated by manufacturer by staining c-Myc epitope of human Jurkat T cell line Anti-His AF647: Validated by manufacturer via ICC staining of HeLa cells transiently expressing His-tag fused protien

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	EBY100 yeast cell line (ATCC MYA-4941)
Authentication	N/A
Mycoplasma contamination	Cell lines not tested for mycoplasma
Commonly misidentified lines (See ICLAC register)	N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Detailed methods are provided in the methods section of the paper.
Instrument	Applied Biosciences Attune Acoustic Focusing Flow Cytometer
Software	Attune Cytometric Software from Applied Biosciences
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Gating strategy is shown in Figure S3 and S5. FSC/SSC Area gating (G1) was used to gate on single yeast cells of comparable size. G1 served as the parent gate for all subsequent analyses. The median fluorescence intensity of aScaf (V5 AF647), pScaf (c-Myc PE), and enzymes (His AF647) was assessed by histogram gating as shown in Figures S3 and S6.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.