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Reporting Summary

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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	
☐	☒	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.*
☒	☐	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 statistics 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
☐	☒	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	MetaMorph Advanced Image Acquisition software, version 7.8.13.0, by Molecular devices LLC, was used for confocal imaging.
Data analysis	cutadapt ver 1.8.3 https://pypi.org/project/cutadapt/ Bowtie2 ver. 2.2.5.0 Langmead and Salzberg, 2012 http://bowtie-bio.sourceforge. net/bowtie2/index.shtµl Tophat2 ver. 2.0.13 Kim et al., 2013 http://ccb.jhu.edu/software/tophat/ downloads/ Python ver. 2.7 and ver. 3.4 Python Software Foundation https://www.python.org/downloads/ ImageJ software, ver 1.50i. https://imagej.nih.gov/ij/

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

The selective ribosomal profiling (SeRP) raw data sets relating to figures: 1-4 and extended data figures 1,4,5 and 7 are publicly available, upon publication, accession number GEO: GSE93830. Figure 4 and extended data figure 6 rely also on raw data derived from the publicly available data sets of Ssb1 SeRP experiments, accession number GEO: GSE93830.

Field-specific reporting

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

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

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

Life sciences study design

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

Sample size

No sample size calculation was performed, as our analysis is based on the parallel measurements of billions of yeast cells per experiment (~5.6X10^9 cells, according to O.D measurements at 600nm). This sample size is by definition very high.

Data exclusions

No data were excluded from the analyses. Minimal detection thresholds were used for including each gene in the analysis (A threshold of 64 total counts per gene was chosen as a point where the inter-replicate variation approached its infinite-counts asymptote and counting statistics contributed little. As in: Ingolia, N.T. et al., Science. 10; 324(5924)(2009)).

Replication

We used a minimal of 2 independent biological replicates per experiment. All replication attempts were successful. For each specific experiment the number of replicates is indicated in the text or figure legend. The replicates were highly reproducible as indicated by the shaded area between SeRP replicates, indicating the degree of experimental variation.
Similarly, imaging stability assays and qPCR results all showed high reproducibility.

Randomization

Only relevant for Metagene analysis, Figure 4.
the randomization code:
(based on- https://stackoverflow.com/questions/3996904/generate-random-integers-between-0-and-9)

```
from random import randint
print(randint(A, B))
A = 30 (30 amino acids from the N terminus are ignored because of the tunnel)
B = End of the gene or gene length
```

Blinding

Not relevant for this study, as it does not include animals and/or human research participants. Furthermore, data analysis was performed mainly bioinformaticaly, using dedicated scripts.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	
[ ]	[x]	Unique biological materials
[ ]	[x]	Antibodies
[x]	[ ]	Eukaryotic cell lines
[x]	[ ]	Palaeontology
[x]	[ ]	Animals and other organisms
[x]	[ ]	Human research participants

Methods

n/a	Involved in the study	
[x]	[ ]	ChIP-seq
[ ]	[x]	Flow cytometry
[x]	[ ]	MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials used in this study are readily available from the authors or from standard commercial sources, as described in the Methods for each specific experiment.

Antibodies

Antibodies used	Polyclonal rabbit FAS antibody, S. cerevisiae as described in: Egner, R. et al. JBC 268, 27269-27276 (1993), (a gift from Dieter H. Wolf, Stuttgart University, Stuttgart, Germany), 1:5000 dilution. Polyclonal rabbit GFP antibody (antiserum from rabbit raised against YFP) (Haslberger, T. NSMB 15(6):641-50 (2008). 1:5000 dilution.
Validation	FAS antibody, as described in: Egner, R. et al. JBC 268, 27269-27276 (1993). GFP antibody, as described in: antiserum from rabbit raised against YFP (Haslberger, T. NSMB 15(6):641-50 (2008). Proteins were visualized by enhanced chemi-fluorescence reaction, each data sets contains positive results, as described in the manuscript .

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	Yeast cells were grown to log phase, then cycloheximide (0.5 mg/ml) was added, and aliquots from each time point were taken. GFP levels of fixed cells at each time point were determined by Flow Cytometry analysis performed using FACS.
Instrument	BD FACS Canto II equipped with Lasers 405nm, 488 nm, 635nm.
Software	BD FACSDiva 8.0.1
Cell population abundance	Cell population abundance was determined by using the following detectors: FSC, SSC, 488-E for GFP with filter 530/30.
Gating strategy	Cell population gated on FSC/SSC area dot plot, exclusion of debris and cell aggregates additionally by SSC/FSC height and width.

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