

Corresponding author(s): Juana Díez, Mordechai Choder

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 ar	e present in the relevant l	ocation (e.g. figure	legend, table le	gend, mair
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 <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)
	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>
	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 <u>statistics for biologists</u> may be useful.

Software and code

Policy information about availability of computer code

Data collection | Provide a desc.

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

Sequencing data analysis

Mapping of sequencing reads: bowtie 1.0.0, settings: -S -t -p 30 -n 1 -m 1 -l 25 –norc

Statistical data analysis: R v3.4

Analysis of differential translation: Riborex v1.2.3 and DESeq2 v1.14.1 GO term enrichment: gProfileR v0.6.1 / REViGO: http://revigo.irb.hr/

visualization: ggplot2 v2.2.1

Visualization and analysis of data: GraphPad Prism 6.01 Structural modelling: MODELLER, Matchmaker, Chimera, Rosetta

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 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

Ribosome Profiling and Genomic Run-On (GRO) raw data are available under accession GSE109734 and GSE123326 at Gene Expression Omnibus (GEO). All the other data supporting the findings of this study are available within the article, its Supplementary Information files or upon request.

Field-specific reporting			
Please select the be	est 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 t	the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf		
Life scier	nces study design		
All studies must dis	sclose on these points even when the disclosure is negative.		
Sample size	All experiments where performed at least twice, with three different biological replicates.		
Data exclusions	No data were excluded from the analyses.		
Replication	The results were confirmed by performing the experiment multiple times, with sucessful replication.		
Randomization	Data were grouped according to their genotype.		
Blinding	Blinding was not performed in our study.		
Reporting for specific materials, systems and methods			
Materials & expe	erimental systems Methods		
n/a Involved in th			
Unique bio	ological materials ChIP-seq		
Antibodies			
Eukaryotic cell lines MRI-based neuroimaging			
Palaeontology Animals and other organisms			
Human research participants			
Unique biological materials			
Policy information about <u>availability of materials</u>			
Obtaining unique	Obtaining unique materials All unique biological materials are available upon request.		
Antibodies			

Antibodies used

- 1. Anti-2a Antibody. Provided by Paul Ahlquist.
- 2. Anti-Xrn1 Antibody. Provided by Arlen Johnson.
- 3. Anti-PGK1 Monoclonal Antibody clone 22C5D8. Provided by Invitrogen with catalog number #459250. The lot number is #459250/C1665
- 4. Anti S8 antibody. Provided by Jesús de la Cruz.
- 5. Anti L1 antibody. Provided by Jesús de la Cruz.

(6. Anti-FLAG Monoclonal Antibody Clone M2. Provided by Sigma-Aldrich with catalog number #F3165. The lot number is #SLBN8915V.

7. Living Colors® A.v. Monoclonal Antibody. It is produced by hybridoma cells against full-length Aequorea victoria green fluorescent protein (GFP). Provided by Clontech Company with catalog number #632380.

Validation

- 1. Anti-2a Antibody functionality was validated by Immunofluorescence assay by Restrepo-Hartwig M and Ahlquist P 1999; doi:
- 2. Xrn1-antibody functionality was validated in a binding assay performed by Nissan et al 2010; doi: 10.1016/j.molcel.2010.08.025
- 3. Invitrogen has validated the use of the anti-PGK Antibody in Western blot.
- 4. Previous work
- 5. Previous work
- 6. Sigma-Aldrich has validated the use of the Anti-FLAG Monoclonal Antibody in Immunoprecipitation and Western blot.
- 7. Clontech has validated the use of the Anti-GFP Monoclonal Antibody in Immunoprecipitation and Western blot.

Eukaryotic cell lines

Policy information about <u>cell lines</u>	
Cell line source(s)	State the source of each cell line used.
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.
Mycoplasma contamination	Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.
Tick this box to confirm	n that the raw and calibrated dates are available in the paper or in Supplementary Information.

______ not the box to commit that the rail and calls acced acced and available in the paper of in supplementary morn

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research			
Laboratory animals	The study did not involve animals.		
Wild animals	The study did not involve wild animals.		
Field-collected samples	The study did not involve samples collected from the field.		

Human research participants

Policy information about studies involving human research participants

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

ChIP-seq

Data deposition

Recruitment

Confirm that both raw and final processed data have been deposited in a public database such as <u>GEO</u> .	
Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.	

Data access links May remain private before publication	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. <u>UCSC</u>)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.
Methodology	
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.
The axis scales are clearly All plots are contour plots	marker and fluorochrome used (e.g. CD4-FITC). y visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers). s with outliers or pseudocolor plots. mber of cells or percentage (with statistics) is provided.
Methodology	
Sample preparation	Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.
Instrument	Identify the instrument used for data collection, specifying make and model number.
Software	Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.
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	Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.
Tick this box to confirm the	hat a figure exemplifying the gating strategy is provided in the Supplementary Information.
Magnetic resonance	e imaging
Experimental design	

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

 $State\ number\ and/or\ type\ of\ variables\ recorded\ (e.g.\ correct\ button\ press,\ response\ time)\ and\ what\ statistics\ were\ used$ to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition				
Imaging type(s)	Specify: functional, structural, diffusion, perfusion.			
Field strength	Specify in Tesla			
Sequence & imaging parameters	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.			
Area of acquisition	State whethe	r a whole brain scan was used OR define the area of acquisition, describing how the region was determined.		
Diffusion MRI Used	Not used	d		
Preprocessing				
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).			
Normalization	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.			
Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.			
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).			
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.			
Statistical modeling & inference				
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).			
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.			
Specify type of analysis: Whole	e brain	ROI-based Both		
Statistic type for inference (See <u>Eklund et al. 2016</u>)	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.			
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).			
Models & analysis n/a Involved in the study				
Functional and/or effective connectivity		Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).		
Graph analysis		Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).		
Multivariate modeling and predictive analysis		Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.		