nature research

Corresponding author(s): Michaela Müller-McNicoll, François McNicoll

Last updated by author(s): 2.1.2020

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	no software was used for data collection
Data analysis	Analysis of iCLIP and piCLIP sequencing data was done using the iCount package (https://github.com/tomazc/iCount). Sequence logos were produced using WebLogo (http://weblogo.berkeley.edu/logo.cgi). Browsershots and Sashimi plots were generated with IGV https://software.broadinstitute.org/software/igv/ and UCSC browser http://genome.ucsc.edu/cgi-bin/hgGateway. RNA-seq and Ribo-Seq reads were trimmed with Cutadapt and mapped to the mm10 assembly using STAR or separately to the SRSF7-PCE isoform using Bowtie2. Aligned reads were counted into genic regions using (HTSeq). Quality control and PCA was done with the DESeq2 package in R. MS Data were analyzed by Peaks7 Proteomics software (Bioinformatics solution). Custom FISH probes were designed using the Stellaris® FISH probe designer (www.biosearchtech.com/support/tools/design-software/stellaris-probe-designer). FIJI was used to quantify the sizes of fluorescence bead aggregates using maximum projection and the 'analyze-particles' option and micrographs. Line scans were performed using the line scan and fluorescence measure from ZEN 2012 (black edition; 8.0.5.273; ZEISS) software using the Profile definition tool (arrow) and the results in distance (x-axis) in pixels to intensity (y-axis) were depicted in graphs. GO term enrichment analyses were conducted using the functional annotation tool of the DAVID webserver (https://david.ncifcrf.gov). The Split-ORF pipeline is implemented using python and is available under: https://github.com/SchulzLab/SplitOrfs.

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

iCLIP, RNA-Seq and Ribo-Seq data have been deposited at NCBI GEO (accession number GSE142802). Raw data for figures 1B, 1C, 1G, 3E, 3F, 4A, 5A, 5C, 6C, 6E, 6F, 6I, 6K, 7C are provided in supplementary Data Set 1. The mass spectrometry data and a detailed method description have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (http://www.ebi.ac.uk/pride) with the dataset identifier PXD016871 and PXD016884.

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 was not determined. All experiments follow established guidelines in the RNA field. Sample sizes are indicated throughout the manuscript.
Data exclusions	No data were excluded.
Replication	All attempts of replication were successful.
Randomization	Does not apply.
Blinding	Blinding was not relevant for the study.

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

name:SRSF6 C-terminal (aa250-300):rabbit α-SRSF6; supplier:LSBio; cat.Nr.LS-C290327; clone:polyclonal; Lot:64192
name:SRSF6 N-terminal (1-100aa):rabbit α-SRSF6; supplier:LSBio; cat.Nr.LS-C749604; clone:polyclonal; Lot:165830
name:rabbit α-SRp40; supplier:Merck Millipore; cat.Nr.06-1365; clone:polyclonal; Lot:2807743
name:rabbit α-SRSF7; supplier:Assay Biotech; cat.Nr.C18943; clone:polyclonal; Lot:3118943
name:goat α-GFP; supplier:D. Drechsel, MPI-CBG, Dresden, Germany; cat.Nr. N/A; clone:polyclonal; Lot:N/A
name:goat α–NXF1; supplier:Santa Cruz Biotechnology, Inc.; cat.Nr.sc-32319; clone:53H8; Lot:G2915
name:rabbit α–PABPN1; supplier:Abcam; cat.Nr.ab75855; clone:EP3000Y; Lot:GR32937-I6
name:mouse α–GAPDH; supplier:Santa Cruz Biotechnology, Inc.; cat.Nr.sc-32233; clone:6C5; Lot:BO514
name:mouse mAb104; cat.Nr.CRL_2067 ATCC
name:rabbit α–Beta-catenin; supplier:Abcam; cat.Nr.ab2365; clone:polyclonal; Lot:GR218792
name:mouse α–PRP8; supplier:Santa Cruz Biotechnology, Inc.; cat.Nr.sc-55533; clone:E-5; Lot:DO114
name:rabbit α–U170k; supplier:Sigma; cat.Nr.av40276; clone:polyclonal; Lot:QC9623

name:mouse α−U2AF65; supplier:Santa Cruz Biotechnology, Inc.; cat.Nr.SC-53942; clone:MC3; Lot:N/A
name:rabbit α−UPF1; supplier:Abcam; cat.Nr.ab109363; clone:EPR4681; Lot:GR50468-14
name:mouse α−PolII; supplier:Cell Signalling; cat.Nr.2629; clone:4H8; Lot:3
name:rabbit α−Tubulin; supplier:Abcam; cat.Nr.ab176560; clone:EPR13478(B); Lot:GR177622-30
name:rabbit α−Histone H3; supplier:Abcam; cat.Nr.ab1791; clone:polyclonal; Lot:GR277860-1
name:mouse α−SRSF3 supplier:Sigma; cat.Nr.WH0006428M8-100UG; clone:2D2; Lot:E8251-2D2

Validation

Most primary antibodies were validated in knockdown studies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Supplier:Sigma / European Collection of Authenticated Cell Cultures (ECACC); Name:P19 Cell Line from mouse; Acc No:95102107; Lot:13D028; Date: 12.08.2013

Authentication

The cell line was purchased from the ECACC.

Mycoplasma contamination

All cell lines are regularly tested for mycoplasma contaminations.

Commonly misidentified lines
(See ICLAC register)

No such line was used in this study.