natureresearch

DBPR and your manuscri Corresponding author(s): instead of author names.

Double-blind peer review submissions: write DBPR and your manuscript number here instead of author names.

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 are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Cor	firmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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>
\ge		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
	\square	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	\boxtimes	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	Image Lab 5.2.1
	Fractionator Software v8.04
	Typhoon FLA 7000 Control Software
	Illumina HiSeq3000
	Spectra Manager Suite (Jasco)
	CFX Manager 2.0 (Biorad)
	StepOne software (Applied Biosystems)
	Leica TCS SP5
	BD FACSDIVA™ SOFTWARE
Data analysis	Image Lab 5.2.1
,	Coral Draw X8
	Coral PhotoPaint X8
	Adobe Illustrator CS5
	Adobe Illustrator CS6
	Microsoft Excel 2016
	PARalyzer
	MEME suite
	IGV

R studio CFX Manager 2.0 (Biorad) Fiji GraphPad Prism 6 FlowJo

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 <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

PAR-CLIP, ribosome footprinting, and RNA-seq data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the accession number GSE105175.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	All experiments were performed in biological triplicates, except for the PAR-CLIP experiments, which were collected as biological duplicates.
Data exclusions	No data were excluded from the analysis.
Replication	Experiments were performed in biological replicates.
Randomization	Not relevant to the study. No statistical analysis was performed that required allocation to different experimental groups.
Blinding	Not relevant.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
\boxtimes	Unique biological materials
	Antibodies
	Eukaryotic cell lines
\ge	Palaeontology
\boxtimes	Animals and other organisms

Human research participants

Antibodies

Antibodies used

Methods

- n/a Involved in the study
- ChIP-seq
 - Flow cytometry
 - MRI-based neuroimaging
- Primary antibodies
 DHX36 antibody: Mouse DDX36 antibody (B-6), Santa Cruz Biotechnology, ref# sc-377485
 TUBA4A antibody: Monoclonal Anti-α-Tubulin antibody produced in mouse, clone B-5-1-2, Sigma-Aldrich, now Merck, ref# T5168-1000UL
 HA antibody: Anti-HA.11 Epitope Tag Antibody, mouse, Biolegend, previously Covance, ref# MMS-101R
 CANX antibody: Anti-Calnexin antibody, mouse, clone AF18, Abcam, ref# ab31290

	ibody: Anti-Histone H2B antibody -ChIP grade, rabbit, Abcam, ref# ab1790
	dy: Linder, B. et al. Tdrd3 is a novel stress granule-associated protein interacting with the Fragile-X syndrome P. Hum Mol Genet 17, 3236-46 (2008).
	rdy: Ribosomal Protein L22 (52), mouse, Santa Cruz Biotechnology, ref# sc-136413
	dy: Moosolinal Hotelin EEE (52), model, sand end biotechnology, fell se 130 Hosel dy: Monoclonal ANTI-FLAG® M2 antibody produced in mouse, clone M2, Sigma-Aldrich, now Merck, ref#
F1804-200UG	
	., Tannahill, D., McCafferty, J. & Balasubramanian, S. Quantitative visualization of DNA G-quadruplex structures in
	Nat Chem 5, 182-6 (2013). Intonio, M., Tannahill, D. & Balasubramanian, S. Visualization and selective chemical targeting of RNA G-quadruple
	the cytoplasm of human cells. Nat Chem 6, 75-80 (2014).
	body: Anti-alpha 1 Sodium Potassium ATPase antibody [464.6], Abcam, ref# ab7671
	dy: Anti-ACTB antibody produced in rabbit, Sigma Aldrich, now Merck, ref# AV40173-50UG
FLAG-Antiboo	dy for immunofluorescence experiments: DYKDDDDK Tag Antibody (Binds to same epitope as Sigma's Anti-FLAG®), Cell signaling, ref# 2368
Secondary an	ntibodies
,	use IgG-HRP (Cruz Marker), Santa Cruz Biotechnology, sc-2031
	bit IgG-HRP Cruz Marker™compatible, Santa Cruz Biotechnology, sc-2030
	ouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488, Life technologies, ref# A11001
Goat anti-Rak	bbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Cyanine3 Life technologies, ref# A10520
HA antibody:	ganisms (e.g., human, sea urchin, Chlamydomonas). []. The product is useful in immunoblotting, []. The HA tag (hemagglutinin) is an amino acid sequence derived from the human influenza hemagglutinin surface
	, corresponding to amino acids 98-106. It is commonly used as a tag to facilitate detection, isolation, and purificati The full amino acid sequence is: YPYDVPDYA
	dy: Reacts with: Mouse, Human. This antibody gave a positive signal in the following, in WB - HeLa whole cell lysat 3; in IF - HeLa cells; in IHC - human liver carcinoma; in FC - HeLa.
	ibody: Synthetic peptide conjugated to KLH derived from within residues 100 to the C-terminus of Human Histone e for: WB, IHC-P, ChIP, ICC/IF, IP
FMR1 antibo	dy: Linder, B. et al. Tdrd3 is a novel stress granule-associated protein interacting with the Fragile-X syndrome
1	P. Hum Mol Genet 17, 3236-46 (2008). ody: Ribosomal Protein L22 (52) is recommended for detection of Ribosomal Protein L22 of mouse, rat, human and
	by Western Blotting [].
•	dy: The ANTI-FLAG M2 mouse, affinity purified monoclonal antibody binds to fusion proteins containing a FLAG
	ence. The antibody recognizes the FLAG peptide sequence at the N-terminus, Met-N-terminus, C-terminus, and
	of the fusion protein. For highly sensitive and specific detection of FLAG fusion proteins by immunoblotting,
	ipitation, immunohistochemistry, immunofluorescence and immunocytochemistry.
	., Tannahill, D., McCafferty, J. & Balasubramanian, S. Quantitative visualization of DNA G-quadruplex structures in
	Nat Chem 5, 182-6 (2013). Intonio, M., Tannahill, D. & Balasubramanian, S. Visualization and selective chemical targeting of RNA G-quadruple
	the cytoplasm of human cells. Nat Chem 6, 75-80 (2014).
	body: Reacts with: This antibody is specific for Na,K-ATPase alpha 1 subunit. Mouse, Rat, Sheep, Rabbit, Dog, Hum
	laevis, Monkey
ACTB antibod	dy: Anti-ACTB (anti- β -Actin) antibody recognizes an internal sequence of human β -actin.
FI 4 6 4 11	

FLAG-Antibody for immunofluorescence experiments: DYKDDDDK Tag Antibody detects exogenously expressed DYKDDDDK proteins in cells. The antibody recognizes the DYKDDDDK peptide (the same epitope recognized by Sigma's Anti-FLAG[®] antibodies) fused to either the amino- or carboxy-terminus of targeted proteins.

Eukaryotic cell lines

Validation

Policy information about <u>cell lines</u>					
Cell line source(s)	Flp-In™ T-REx™ 293 Cell Line, Thermo Fisher Scientific, ref# R78007. All other cell lines used in this study are generated out of this paternal cell line				
Authentication	Generated cell lines in this study were verified by Western blot, fluorescence activated cell sorting, and/or sequencing of genomic DNA				
Mycoplasma contamination	All used cell lines are free of mycoplasma contamination				
Commonly misidentified lines (See <u>ICLAC</u> register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.				

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	For mCherry expression, cell lines with stably integrated pcDNA5-FRT-GFP-mCherry-3pGW reporter constructs were induced with 500 ng ml-1 tetracycline (Merck) for 15 h. Cells were trpysinized, washed twice with PBS, and filtered through a 35 μ m nylon net.			
Instrument	LSRFortessa™ BD Biosciences			
Software	BD FACSDIVA™ SOFTWARE and FlowJo			
Cell population abundance	Over 90%			
Gating strategy	FSC/SCC was gated to exclude cell duplets and to ensure a homogenous cell population			
Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.				