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Corresponding author(s): Amy Palmer

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	Microscopy images were acquired with Nikon Elements Software.
Data analysis	Analysis of Isothermal titration calorimetry was done in Origin 5.0. Image display and line trace analysis for fluorescence analysis in stress granules was done in ImageJ2 with the Fiji plugin suite. Statistical ANOVA analysis was done in KaleidaGraph version 4.02. The One Way ANOVA test in KaleidaGraph was used with the Tukey's All Pairs Comparison option. In vitro fluorescence measurements were analyzed in Microsoft Excel 2013 to calculate mean and standard deviation.

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 datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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	No statistical method was used to predetermine sample size. The following considerations were used to determine that the sample sizes used for each experiment are sufficient. In vitro biochemical measurements (fluorescence measurements, ITC) were repeated 3 times or more (as indicated in figure legends) for calculation of mean +/- STDEV. The STDEV was typically below 10% for fluorescence measurements, and we concluded that these measurements are sufficient to determine trends of fluorescence quenching and de-quenching for the study. Three measurements were deemed reliable to compare the affinity between Cbl and a representative probe. The Northern blot (Supplementary Fig. 12) was reproducible for two independent experiments. This was deemed sufficient, as the processing phenotype was robust and easily detected. For control experiments involving fixed cells (immunofluorescence and FISH), a large subset of experiments are reproduced from known literature localization patterns as controls for the live cell experiments (as indicated in the main text). For example, beta actin localization to GFP-G3BP1-labelled SGs was demonstrated by diverse methods in different laboratories by different groups. Furthermore, phenotypes were analyzed by orthogonal methods. For example, U-body formation was confirmed via SMN and DDX20 localization; tagged U1 localization to U-bodies was confirmed by testing co-localization to both orthogonal marker proteins. Together, it was deemed sufficient to repeat these experiments on fixed cells once or twice for each specific condition (as indicated in figure legends), and the total number of cells interrogated is noted in each figure legend. Control live fluorescence experiments to determine uptake by bead loading in different cell lines (Supplementary Fig. 13) was done once or twice for the exact conditions shown, and ~50 or more cells were interrogated (as detailed in the figure legend). This was deemed sufficient, as similar localization patterns were observed in non-transfected controls throughout the study. For live cell imaging to assess mRNA colocalization to SGs, at least 2 separate experiments were performed for each condition. Timing of live cell imaging was critical such that cell death upon prolonged arsenite treatment does not interfere with data analysis. With these constraints, >50 stress granules were analyzed per condition. The analysis of these measurements revealed statistical significance. We concluded that our data is robust because we observed consistent trends for imaging with probes of two different colors and for tagging with monomers versus multimers of our RNA tag. For live cell imaging of U-bodies (Fig. 5), we performed four separate experiments and analyzed hundreds of cells per condition. We concluded that these numbers were sufficient as we observed a statistically significant differences.
Data exclusions	For the U-body assay, thapsigargin treatment was done for 3 hours for all experiments reported, but for initial pilot experiments the thapsigargin treatment was longer and more variable (>4 hours). These data were excluded as U-body formation is likely time-dependent and this variable should not be altered whenever possible.
Replication	All attempts at replication were successful, and we have indicated in each figure legend how many times each experiment was independently repeated.
Randomization	Randomization of samples in groups is not relevant to this study as no animal or clinical work was performed.
Blinding	In vitro fluorescence measurements were not blinded as automated analysis on the plate reader on fluorimeter is unbiased. For live cell imaging, blinding was not possible for the following reasons. Quantification of RNA localization to stress granules (Supplementary Figure 18) was developed while data sets were collected. The U-body analysis was not blinded, as several of the transfected datasets were initially collected with the goal to perform FISH on the same fixed cells. While this correlative imaging of live and fixed cells failed for technical reasons, the live cell images were useable for the analysis presented in Fig. 5b. Cells used for the analysis in Fig. 5b were imaged as 'large images' (such as 8x8 scans) to achieve an objective set of cells from the population without 'cherry picking' cells. Immunofluorescence and FISH experiments were not blinded as most experiments are control experiments to validate the live experiments and these fixed immunofluorescence / FISH experiments reproduce localization patterns shown in the literature.

Reporting for specific materials, systems and methods

Materials & experimental systems	
n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	We will deposit plasmids generated in this study (Supplementary Fig. 11) at Addgene upon acceptance of this manuscript for publication. Several synthetic Cobalamin-fluorophore probes were developed in this study. While we may be able to provide small quantities of these materials if requested, we would like to point out that synthesis produced limited quantities of these materials. Detailed methods on how to synthesize these probes are provided in the Supplementary Note.

Antibodies

Antibodies used	Two primary antibodies were used: (1) DDX20 antibody: Gemin3 (12H12): sc-57007, Lot# E0614, Santa Cruz Biotechnology, mouse monoclonal antibody raised against amino acids 368-548 of Gemin3 of human origin, 1:1,000 dilution. (2) SMN antibody: SMN (H-195): sc-15320, Lot# B1512, Santa Cruz Biotechnology, rabbit polyclonal antibody raised against amino acids 1-195 mapping at the N-terminus of SMN of human origin. Two secondary antibodies were used. Goat anti-mouse Alexa 594 antibody: A11005, Lot# 55579A, Invitrogen. Donkey anti-rabbit Alexa 568 antibody: A10042, Lot# 1134929, Invitrogen, 1:1,000 dilution.
Validation	The DDX20 antibody was validated to detect U-bodies (cytosolic puncta upon treatment with various stimuli including thapsigargin) in HeLa cells by Tsaliki J, Tattoli I, Ling A, Sorbara MT, Croitoru DO, Philpott DJ, Girardin SE, JBC, 2015, doi: 10.1074/jbc.M115.659466). This phenotype was reproduced in the present study for validation (Supplementary Fig. 28). Tsaliki et al to validated that a SMN antibody detects U-bodies (cytosolic puncta upon treatment with stimuli) in HeLa cells using a home-made SMN antibody. The commercial SMN antibody used in the present study was validated to detect U-bodies in HeLa cells, resulting in puncta comparable with literature patterns (Supplementary Fig. 28).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HeLa and 293T cells were purchased from ATCC and only used for up to 12 passages upon receipt. U2-OS cells were received from Roy Parker and described previously (Wheeler JR, Matheny T, Jain S, Abrisch R, Parker R, Elife. 2016, doi: 10.7554/eLife.18413.). The U2-OS cell line with genomic Halo-G3BP1 was generated in this study (see methods section for details and Supplementary Figure 33).
Authentication	HeLa and 293T cells were purchased from ATCC and not further authenticated, as they were only used for up to 12 passages upon receipt from ATCC. U2-OS cells were received from Professor Roy Parker (Wheeler JR, Matheny T, Jain S, Abrisch R, Parker R, Elife. 2016, doi: 10.7554/eLife.18413.). U2OS cells were also purchased from ATCC and not further authenticated. Validation of the U2-OS cell line with genomic Halo-G3BP1 generated in this study is presented in Supplementary Fig. 33. Correctly edited cells were confirmed by PCR, Western blot and immunofluorescence using standard protocols.
Mycoplasma contamination	All eukaryotic cell lines (HeLa, 293T, U2-OS) were regularly (at least bi-yearly) tested for mycoplasma contamination by staff at the in-house Cell Culture Core Facility at the BioFrontiers Institute. No mycoplasma contaminations were detected for all three cell lines or other cell lines in the Palmer laboratory throughout the duration of the study.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.