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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies 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	Xcalibur v2.2 was used for mass spectrometry data collection. CLARIOstar (BMG Labtech) was used for absorbance assays to determine protein concentration and for CellTiterGlo (luminescence). Bio-Rad ChemiDocTM XRS imager was used for chemiluminescence Western blot and fluorescence gel data collection. Quantigene beads were measured with FLEXMAP3D. High-content imaging was performed on a Cell Voyager 7000 (Yokogawa, Tokyo, Japan) using a 40x dry objective lens (NA=0.95). RNA-sequencing data was acquired on an Illumina HiSeq 4000 and eCLIP-sequencing was acquired on an Illumina NovaSeq 6000 platform. Immunofluorescence microscopy images were acquired on a Zeiss LSM 880 instrument with a 63x/1.4 oil plan-apochchromat objective. 1H and 13C NMR spectra were obtained on a Bruker 300 or 400 MHz NMR. Small molecule HRMS were performed on a Waters Xevo G2-XS TOF.

Data analysis	IP2 v6.5.5 and CIMAGE were used to analyze MS-ABPP and whole proteomics data. IP2 v6.5.5 was used to analyze quantitative TMT data. QGProfiler (https://qgprofiler.openanalytics.eu/app/QGprofiler) was used to analyze Quantigene data. High-content imaging data was analyzed with Phaedra (www.phaedra.io). For RNA-sequencing analys, transcript abundance was quantified using Salmon [v1.3.0] with GENCODE v37 annotation, gene level quantification was performed using tximeta [v1.8.4], differential gene expression was analyzed by DESeq2 [v1.30.1], and gene set enrichment analysis (GSEA) was implemented in fgsea [v1.16.0] using gene sets from MsigDB [v7.1]. Immunofluorescence microscopy images were processed with ZEN 3.0 (blue edition) software (Zeiss). Alternative splicing (AS) analysis was completed using rMATs (v3.2.5). For eCLIP-sequencing data analysis, mapping was performed with STAR (v2.4.0i) and identification of usable reads was performed with CLIPper11 (https://github.com/YeoLab/clipper/releases/tag/1.0). eCLIP peak regional distribution was performed with a custom annotator (https://github.com/YeoLab/annotator), binding motif analysis was done with a custom script (https://github.com/YeoLab/clip_analysis_legacy), and repetitive element mapping was done with Repbase (https://github.com/YeoLab/repetitive-element-mapping). eCLIP metagene plots were generated with a custom script (https://github.com/YeoLab/Metadensity) and eCLIP reads were correlated to alternative splicing analysis with RBP-maps (https://github.com/YeoLab/rbp-maps). GraphPad Prism, v9.2.0 was used to perform statistical tests and generate all quantitative figures and graphs. NMR spectra were processed using MestReNova (v14.2.1) software. Microsoft software packages were used to analyze all other data.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Proteomics datasets have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier dataset identifiers PXD039283 (cysteine MS-ABPP with 6 h compound treatment and FLAG-NONO IP-MS) and PXD032087 (all other experiments). Raw RNA-sequencing and eCLIP-sequencing data has been deposited to NCBI under GEO with accession codes GSE198419 and GSE198212 respectively. All other data is available by written request to authors.

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	No statistical methods were used to pre-determine sample size. All reported data is based on cultured human cell lines. In most cases experiments were performed in biological triplicate (n=3) or quadruplicate (n=4) unless otherwise stated. Reproducibility of data between replicates is reported in extended data figures and main text and supports the chosen sample sizes.
Data exclusions	No data was excluded.
Replication	The exact number of experiment replications and biological replicates for experiments performed on primary cells or mice are noted in the figure legends.
Randomization	Randomization was not relevant to the study, as all experiments were done using human cell lines, genetically identical and derived from the same stock for all sample groups.
Blinding	NONO foci from 22Rv1 cells treated with compounds were counted in a blinded manner (see Figure 5b). Blinding was not relevant to other experiments because they did not involve allocation of different samples, organisms, or participants into experimental groups.

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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Rabbit anti-NONO Antibody, Bethyl Laboratories, catalog no. A300-587A; Mouse anti-NONO Antibody, BD Biosciences, catalog no. 611278; Rabbit anti-Androgen Receptor antibody, Cell Signaling Tech, catalog no. 5153S; anti-actin-HRP antibody, Santa Cruz Biotechnology, catalog no. sc-47778 HRP; Mouse anti-PSPC1 antibody, Sigma-Aldrich, catalog no. SAB4200503; Mouse anti-SFPQ antibody, Sigma-Aldrich, catalog no. WH0006421M2; Mouse anti-FLAG antibody, Sigma-Aldrich, catalog no. F1804; Rabbit anti-fibrillarin antibody, Cell Signaling Tech, catalog no. 2639S; anti-mouse HRP antibody, Cell Signaling Tech, catalog no. 7076S; anti-rabbit HRP antibody, Cell Signaling Tech, catalog no. 7074S; anti-rabbit Alexa 488, Invitrogen, catalog no. A11034; anti-mouse Alexa 488, Invitrogen, catalog no. A32723; anti-rabbit Alexa 647, Invitrogen, catalog no. A32733; anti-mouse Alexa 647, Invitrogen, catalog no. A21236; anti-Androgen Receptor Alexa 488, Cell Signaling Tech, catalog no. 7395

Validation

Mouse anti-NONO Antibody was validated for WB and IF by BD Biosciences in Jurkat cells. Rabbit anti-Androgen Receptor antibody was validated for WB by Cell Signaling Tech in LNCaP, VCaP, and 22Rv1 cells. anti-actin-HRP antibody was validated for WB by Santa Cruz Biotechnology in HeLa, NIH/3T3 and KNRK cells. Mouse anti-PSPC1 antibody was validated for IF in HeLa cells and WB in mouse Hepa1-6 cells by Sigma-Aldrich. Mouse anti-SFPQ antibody was validated for WB and IF by Sigma-Aldrich in HeLa cells. Mouse anti-FLAG antibody was validated for WB by Sigma-Aldrich in CHO cells. anti-rabbit and anti-mouse IgG, HRP-linked antibodies were validated by Cell Signaling Tech in WB against other host-reactive primary antibodies. Rabbit anti-fibrillarin antibody was validated for WB in HEK293, Neuro2A, PC12, and COS cells and for IF in HeLa cells by Cell Signaling Tech. anti-Androgen Receptor Alexa 488 was validated for IF by Cell Signaling Tech in LNCaP cells. anti-rabbit Alexa 488, anti-mouse Alexa 488, anti-rabbit Alexa 647, and anti-mouse Alexa 647 were validated for IF by Invitrogen in HeLa cells. Data generated in this study was consistent with manufacturer reports. NONO antibody (Bethyl) was validated for enhanced cross-linking immunoprecipitation (eCLIP) as part of the ENCODE Project (https://www.encodeproject.org/antibodies/ENCAB467FVY/).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

22Rv1 (ATCC, #CRL-2505), MCF7 (ATCC, #HTB-22), HT1080 (ATCC, #CCL-121), HeLa (ATCC, #CRM-CCL-2), HEK 293T (ATCC, #CRL-3216)

Authentication

All cell lines were authenticated by short tandem repeat loci (STRs) profiling by vendors.

Mycoplasma contamination

All cell lines used in this study were tested negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.