nature portfolio

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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	Illumina HiSeq, HiSeq X and NextSeq platforms were used to collect sequencing data in this study.
Data analysis	Already existing pipelines and softwares were utilized for data analyses. For the analyses of sequencing reads: UNIX commands, UNIX-based Flexbar v2.5, pyCRAC v1.3.2, seqtk v1.3 (https://github.com/lh3/seqtk), Bowtie v1.2.3, BEDTools v2.28.0., STAR aligner v2.7. For further data processing and quantification: GraphPad Prism v9.0.1, R packages (edgeR v3.36.0), R packages (drc v3.0-1), WebGestalt (http://www.webgestalt.org/).

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

All raw and processed data generated in this study are available at the NCBI Gene Expression Omnibus (GEO) with GSE196043 accession number. Published datasets used in this study are also available at the NCBI GEO with the following accession numbers: GSE69352, GSE49831, GSE99978, GSE141507, GSE52662. Genome build: human (hg38), mouse (mm10), Drosophila (dm6).

FANTOM5 CAGE-seq:
human: https://fantom.gsc.riken.jp/5/datafiles/reprocessed/hg38_latest/extra/CAGE_peaks/
mouse: https://fantom.gsc.riken.jp/5/datafiles/reprocessed/mm10_latest/extra/CAGE_peaks/

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 chosen to ensure the reproducibility of the results. All experiments reported in this study were performed at least twice, but often three or more times unless otherwise indicated.
Data exclusions	No data were excluded.
Replication	All attempts to replicate experiments in this study were successful. Sequencing experiments were performed in two replicates at minimum, with the exception of TSS-seq dataset from mES and MCF-7 cells. The validity of the TSS-seq datasets was confirmed by comparison to the FANTOM5 CAGE-seq datasets. Replicates from sequencing experiments were in good agreement for each reported method in this study. Sequencing-based findings in this study were routinely cross-validated and replicated using different, orthogonal approaches.
Randomization	The random allocation is not relevant to our study; all experiments were performed under controlled experimental conditions.
Blinding	The investigators were not blinded. The experiments were performed and the results were analyzed by the experimenter.

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-FTSJD1 polyclonal antibody (Invitrogen, PAS-61696); 1:500 dilution in 3% milk-TBST Rabbit anti-RIG-I (D14G6) monoclonal antibody (Cell Signaling, 3743T); 1:1,000 in 3% BSA-TBST) Rabbit RIG-I/DDX58 [EPR18629] monoclonal antibody (Abcam, ab180675); 1:2,000 in 3% milk-TBST Rabbit anti-MDA-5 (D74E4) monoclonal antibody (Cell Signaling Technology, 5321T); 1:1,000 in 3% BSA-TBST Rabbit anti-IFIT1 (D2X9Z) monoclonal antibody (Cell Signaling Technology, 14769S); 1:1,000 in 3% milk in TBST Rabbit anti-IFITM3 (D8E8G) XP monoclonal antibody (Cell Signaling Technology, 59212T); 1:1,000 in 3% milk-TBST Mouse anti-NLRP1 monoclonal antibody (Biolegend, 679802); 1:1,000 in 3% BSA-TBST Rabbit anti-MAVS polyclonal antibody (Cell Signaling Technology, 3993T); 1:1,000 in 3% milk-TBST Rabbit anti-OAS3 polyclonal antibody (Abcam, ab154270); 1:1,000 in 3% milk-TBST Mouse anti-GAPDH monoclonal antibody (GeneTex, GT239); 1:10,000 in 3% milk-TBST Mouse anti-Beta Actin monoclonal antibody (Thermo, AM4302); 1:4,000 in 3% milk-TBST anti-Bromodeoxyuridine antibody (MBL Life Science, MI-11-3); for IP 0.5-1 micrograms antibody per 10 micrograms RNA) Mouse anti-puromycin (12D10) monoclonal antibody (Millipore Sigma, MABE343); 1:5,000 in 3% milk-TBST HRP-conjugated donkey anti-rabbit IgG (Cytiva, NA934); 1:5,000 in 3% milk-TBST or 3% BSA-TBST HRP-conjugated sheep anti-mouse IgG (Cytiva, NA931); 1:5,000 in 3% milk-TBST or 3% BSA-TBST
Validation	All antibodies used in this study were compared to previously published results and antibody manufacturers data.

Validation	Rabbit anti-FTSJD1 polyclonal antibody for CMTR2 was validated by western blots on protein samples extracted from CMTR2 knockout cells (Extended Data Figures 1&7), as well as through an overexpression of the NeonGreen-tagged CMTR2 (Extended Data Figure 9). Antibody validation source for primary antibodies used in this study: https://www.cellsignal.com/products/primary-antibodies/rig-i-d14g6-rabbit-mab/3743 https://www.abcam.com/rig-iddx58-antibody-epr18629-ab180675.html https://www.cellsignal.com/products/primary-antibodies/mda-5-d74e4-rabbit-mab/5321 https://www.cellsignal.com/products/primary-antibodies/ifit1-d2x9z-rabbit-mab/14769 https://www.cellsignal.com/products/primary-antibodies/ifitm3-d8e8g-xp-rabbit-mab/59212 https://www.biolegend.com/fr-fr/products/purified-anti-nlrp1-antibody-12213 https://www.cellsignal.com/products/primary-antibodies/mavs-antibody/3993 https://www.abcam.com/oas3-antibody-ab154270.html https://www.genetex.com/Product/Detail/GAPDH-antibody-GT239/GTX627408 https://www.thermofisher.com/antibody/product/beta-Actin-Antibody-clone-AC-15-Monoclonal/AM4302 https://www.mblbio.com/bio/g/dtl/A/index.html?pcd=MI-11-3 https://www.emdmillipore.com/US/en/product/Anti-Puromycin-Antibody-clone-12D10,MM_NF-MABE343

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Mouse embryonic stem cells were a gift from J. Hanna and S. Geula (Weizmann Institute of Science). HEK293T (ATCC CRL-3216), MCF-7 (ATCC HTB-22), A549 (ATCC CRM-CCL-185).
Authentication	mES cells were previously described in Geula, et. al. 2015. HEK293T, MCF-7, and A549 cells were purchased from ATCC. The authors did not authenticate cell lines.
Mycoplasma contamination	All cell lines used in this study tested negative for mycoplasma contamination. Mycoplasma contamination was routinely tested with Hoechst staining.
Commonly misidentified lines (See ICLAC register)	None of the ICLAC cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C. elegans, adult; Zebrafish, AB line, 48 h old larvae; Mice, C57BL/6, female, 16 weeks old
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	The mice and zebrafish species were maintained in compliance with Weill Cornell Medicine Institutional Animal Care and Use Committee (IACUC) protocols.

Note that full information on the approval of the study protocol must also be provided in the manuscript.