

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	Snaptron data were processed during the creation of recount3 and include sequencing data from TCGA v3.0, GTEx v8, SRA human datasets as of 10/06/2019, and SRA mouse datasets as of 01/08/2020. The ENCODE shRNA-seq data were processed by Snaptron and uploaded 06/2018.
Data analysis	-Early SnapMine interface and visualization scripts are available in the following GitHub repository: https://github.com/irikas/SnapMine_Paper . Code for the SnapMine web application is available at https://github.com/ssec-jhu/snaptron-query . -For RNA-seq analysis, files downloaded using SRA toolkit (fastq-dump). -Other software used: STAR (v.2.7.10a), featureCounts, Megadepth.

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](#)

Publicly deposited RNA-seq data in this study is available on the National Center for Biotechnology Information's Sequence Read Archive under SRA study numbers SRP166282 (58), SRP057819 (11), SRP092413 (125), SRP104405 (69), SRP194266 (126), SRP367696 (127), and SRP079236 (71). NAUC data tables are available on <http://ascot.cs.jhu.edu/> (63). The application to query junction inclusion data is hosted at <https://snapmine.idies.jhu.edu>. RNA-seq data have been deposited in the NCBI Sequence Read Archive. They are associated with BioProject accession PRJNA1242253.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A no human participants
Reporting on race, ethnicity, or other socially relevant groupings	N/A no human participants
Population characteristics	N/A no human participants
Recruitment	N/A no human participants
Ethics oversight	N/A no human participants

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

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](https://www.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 sample size calculation was performed. 2-3+ replicates were conducted for each experiment and most results are binary (cryptic exon is included or not).
Data exclusions	For figure 2, some GTEx data were excluded as per reviewer request. These individuals potentially had undiagnosed TDP-43 pathology and so would skew the results regarding basal level inclusion of cryptic exons in a normal population.
Replication	All results were replicated at least twice in independent experiments.
Randomization	Samples were allocated randomly.
Blinding	Basescope sections were imaged and analyzed blinded to status. Initial RT-PCR experiment results were analyzed blind. Blinding was not relevant for the rest of the experiments.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Alpha-Tubulin-HRP (Proteintech HRP-66031, Lot# 21006026), TDP-43 (Proteintech 10782-2-AP Lot# 00128435), TDP-43 (Proteintech 81350-1-RR Lot# 23002373), Ferritin abcam ab287968), GAPDH-HRP (Proteintech HRP-60004 Lot# 21010938), GPX4 (Proteintech 67763-1-Ig Lot# 10027858)
Validation	Antibodies were validated by commercial manufacturers.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	i3Neurons initially from Dr. Michael Ward's lab at the NIH were derived from WTC11 iPSCs (sex = male). IMR-32 cells from ATCC are male. Human peripheral blood mononuclear cells from ATCC were from a male donor. Human dermal fibroblasts of line 2003-071-056 originally from the Coriell Institute were from male donor. Human Dermal Fibroblasts from ATCC were from female donors. iPSC-derived microglia were from male donor.
Authentication	Cell lines were authenticated by commercial manufacturers.
Mycoplasma contamination	Cell lines were not tested for mycoplasma by authors.
Commonly misidentified lines (See ICLAC register)	N/A

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Mus musculus, strain C57BL/6J, age 4-8 weeks
Wild animals	N/A
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	Animal experiments were conducted as per the regulations of the Animal Care and Use Committee at Johns Hopkins University School of Medicine in accordance with the laws of the State of Maryland and the United States of America.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A