nature portfolio

Corresponding author(s): Weidong Wang

Last updated by author(s): 11/17/2021

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	The bcl files were converted to fastq files by bcl2fastq. The quality of fastq files was checked by fastqc 0.11.8. The adapters in the files were trimmed by fastxtoolkit 0.0.14 or cutadapt 2.8. The files obtained were used for alignments.
Data analysis	The following softwares were used in the data analysis: TopHat 2.1.1, Samtools 1.9, Bamtools 2.5.1, bedtools 2.15.0,Proseq2, Bowtie 2-2.4.0, htseq 0.11.2, cufflinks 2.2.1, cuffdiff 2.2.1, edgeR_3.26.8, deeptools 3.5.1, SICER 2-1.0.3, MACS 2.2.6, ChIPseeker v1.26.2, DiffBind3.0, R.3.6.0 and Perl.v5.28.1.

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 the relevant data supporting the key findings of this study are available in the article and its supplemental information files or from the corresponding authors upon reasonable request. The next-generation sequencing data were deposited at GEO database. The access code is GSE188574.

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	*Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data exclusions	Because in this study we focused on transcription and translation of the mRNAs. Therefore, in our data analyses, non-coding RNA and the lowly expressed genes (the genes contain zero fpkm value in RNA-seq or Ribo-seq) were removed from our analyses.
Replication	All the biochemistry experiments, ChIP-seq, PRO-seq, RNA-seq, Ribo-seq, eCLIP-seq, RT-qPCR and Western blot were done at least twice.
Randomization	This is not relevant to our study.
Blinding	This is not relevant to our study.

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	TOP3B, WH0008940M1-100UG, Sigma Aldrich;TDRD3, 5942S, Cell Signaling Technology or made by ourselves;FMRP, MAB2160, Sigma Aldrich; GAPDH, 2118s, Cell Signaling Technology; RNA Pol II, Sigma Cat#: 05-952-I; CHD8, ab114126, Abcam; FAT1, A304-403A, Thermo Fisher; ACTB, ab8226, Abcam.
Validation	These antibodies have been used in other publications.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HCT116 cells were purchased from ATCC.
Authentication	HCT116 cells were obtained from ATCC with certificate.
Mycoplasma contamination	The HCT116 cell lines were found positive for mycoplasma contamination once. We then treated the contaminated cells with mycoplasma-removal agent (BUF035, Bio-Rad). A new test confirmed that the contamination was removed. By RT-qPCR and 3' End-seq, the main findings reported in the manuscript were confirmed using the cells without mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	We have submitted our raw (fastq) and processed (bedGraph, Excel tables) data files to GEO database. The access code is GSE188574.
Genome browser session (e.g. UCSC)	IGV.2.7.2 with hg38 genome.

Methodology

Replicates	For ChIP-seq, we have done each experiment at least twice.
Sequencing depth	about 20~40 million reads
Antibodies	RNA Pol II, Sigma Cat#: 05-952-I;TDRD3, 5942S, Cell Signaling Technology or made by ourselves.
Peak calling parameters	Reads were mapped to hg38 genome using Bowtie2; SICER 2 was used to call peaks (p-value <= 0.05, SICER score >= 200, and false discovery rate <= 0.05); Diffbind or Perl script was used to calculate peak changes.
Data quality	Our RNA Pol II ChIP-seq data are comparable to the published data. The profiles of Pol II were mainly peaked at transcription start sites, similar to the published data, suggesting high data quality. TDRD3 ChIP-seq data also showed clear peaks by two different TDRD3 antibodies and the peaks were significantly reduced in TDRD3-KO cells, indicating that they are high quality data.
Software	Bowtie2; SICER 2-1.0.3, and Perl.v5.28.1.