nature research

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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined. — No Sample size calculations

2. Data exclusions

   Describe any data exclusions. — Nematode proteins with cytosine methyltransferase domains that were bacterial contaminants were removed from analysis

3. Replication

   Describe whether the experimental findings were reliably reproduced. — All attempts at replication were successful

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups. — There was no randomization procedures

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis. — No blinding was performed

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

   Describe the software used to analyze the data in this study. — DNA methyltransferase annotation: hmmer (version 3.1) freely available

from hmmer.org/; blast+ (version 2.2.30) freely available from https://blast.ncbi.nlm.nih.gov; Phylogenetic tree construction: MUSCLE v3.8.31 for alignment, Gblocks 0.91b for curation and PhyML 3.1 for maximum likelihood phylogeny, all provided via www.Phylogeny.fr. Bisulfite alignment and mapping bismark version 0.14.2 freely available from https://www.bioinformatics.babraham.ac.uk/projects/bismark/; bowtie2 (version 2.1.0) freely available from bowtie-bio.sourceforge.net/bowtie2/; Methylextract version 1.9 freely available from https://github.com/bioinfoUGR/methylextract?files=1. Bedtools (version 2.19.0) was used for data integration; freely available from http://bedtools.readthedocs.io/en/latest/.
Coevolution analysis: blast+ version 2.2.30
All statistical analysis was carried out using R (version 3.1.0); freely available from https://www.r-project.org/.
Custom perl scripts (Perl version 5.16) used for intermediate processing DNA methylation data are available on request.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Mouse monoclonal primary antibody against ALKBH2 (C-9) was purchased from Santa Cruz catalogue number sc-515789. Validation via identification of correct protein size and disappearance of band after disruption of the gene in mouse embryonic stem cells. Secondary antibody was Rabbit anti-mouse HRP conjugated purchased from Abcam (ab6728). Tested for hybrization against mouse antibodies and not against rabbit antibodies to validate antibody. Anti-3meC used for dot-blots was from Active Motif (61180) and was validated by testing for reactivity with a template treated with MMS for 30 minutes at room temperature and for lack of reactivity with a PCR product made with entirely 5meC.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

J1S mouse embryonic stem cells from ATCC
TKO mouse embryonic stem cells from Riken RBC

b. Describe the method of cell line authentication used.

PCR was used to validate gene deletions

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines tested negative for mycoplasma contaminations repeatedly (c once per month)

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animal or animal-derived material was used

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human research participants