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# Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

## Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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 |
| ☐ | ☒ | An indication of 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<br>*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 statistics 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<br>*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 |
| ☐ | ☒ | Clearly defined error bars<br>*State explicitly what error bars represent (e.g. SD, SE, CI)* |

*Our web collection on statistics for biologists may be useful.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | X-ray collection: HKL2000. Bio-Tek plate readers: Gen5 (v3.04.17). Microscopy: NIS elements (v4.60), Volocity 3D analysis (Perkin Elmer). |
| Data analysis | Structural analysis: PHENIX PHASER, PHENIX REFINE, and COOT. Statistical analysis: GraphPad Prism 7.0, KaleidaGraph 4.5. Mass spectrometry analysis: MaxQuant (version 1.5.5.1). Image analysis: ImageJ 1.51 (NIH). Pymol (Schrodinger, v2.2). Metabolic analysis: Agilent Masshunter B08. Actin motility: FAST (Fast Automated Spud Trekker) software (Askel et al. Cell Reports, 2015). |

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The X-ray structures (coordinates and structure factor files) of SETD3 with bound actin peptide have been submitted to PDB under accession number 6MBJ (P21),

6MBK (Two complexes in P212121) and 6MBL (one complex in P212121). Source data for this study are provided as supplementary information (Fig. 4d, 5b, 5e, 5f, 5i, 5k; Extended Data Fig. 7e, 9c, 9h). Source gel data provided for cropped images (Fig. 1a, 1d, 1e, 2a, 2c, 3a-f,g, 4c, 5g, 5j; Extended Data Fig. 1b) is provided in Supplementary Figure 1. Mass spectrometry data associated with Fig. 1c/Extended Data Fig. 1h and Fig. 3i are provided as a Supplementary Tables. Additional requests can be made to the corresponding authors.

# Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Sample size | No statistical measure was used to predetermine sample size. The dystocia phenotypic analysis resulted in an almost binary outcome leading to a strong conclusion with the sample size provided. |
| Data exclusions | No data were excluded |
| Replication | Experiments were replicated as indicated in the figure legends. Generally, all experiments were performed with three independent biological replicates. All results repeated during replication. |
| Randomization | No randomization was necessary as only single variables changed per experiment |
| Blinding | Blinding was not necessary as any phenotypic assessment or other measurements was performed using discrete, quantitative measurements |

# Reporting for specific materials, systems and methods

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Unique biological materials |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |

## Methods

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

## Unique biological materials

Policy information about availability of materials

| | |
|---|---|
| Obtaining unique materials | Most materials are freely commercially available from common sources. Anti-actin-H73(3-me) antibody generated in this study is available upon request. |

## Antibodies

| | |
|---|---|
| Antibodies used | Primary: SETD3 (Abcam, #176582, Lot GR283420-5); Actin (Cytoskeleton, #AAN01, Lot 123); Beta-tubulin (Millipore, #05-661 clone AA2, Lot 2834905); H3K4me2 (Cell Signaling, #9725); H3K36me2 (Cell Signaling, #2901); GFP (Invitrogen, A-11122). Anti-actin-H73(3-me) polyclonal antibody was made in-house. Secondary: Alexa488 anti-rabbit antibody (Life Technologies, A-11008), anti-rabbit-HRP (Jackson Immunoresearch, 711-035-152), anti-mouse-HRP (715-035-151) |
| Validation | SETD3 antibody validated by loss of detection after CRISPR/Cas9 knockout with multiple guide RNAs. Actin, beta-tubulin, and secondary antibodies were validated by the vendor across multiple cell types. Anti-actin-H73(3-me) polyclonal antibody was validated by dot blot with unmodified, H73(3-me), and H73(1-me) peptides and western blotting with cell extracts where actin methylation was quantified by mass spectrometry. H3K36me2 and H3K4me2 antibodies were validated by the vendor and independently on a peptide array containing multiple modified histone peptides. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | HEK293T, H1-Hela, HT1080, and primary uterine smooth muscle cells were all acquired from ATCC. Murine embryonic fibroblasts (MEFs) were generated from mice in this study. |
| Authentication | HEK293T, H1-Hela, HT1080, and primary uterine smooth muscle cells were authenticated by ATCC. MEFs were genotyped from residual tissue after embryonic dissection and the presence of SETD3 was probed by western blot in this study. |
| Mycoplasma contamination | Cell lines were declared free of mycoplasma by ATCC. MEFs were not tested. |
| Commonly misidentified lines (See ICLAC register) | no misidentified lines utilized in this study |

## Animals and other organisms

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

| | |
|---|---|
| Laboratory animals | Mice: Strains derived from C57BL/6N - Setd3tm1.1(NCOM)Mfgc/Tcp heterozygous mice. Timed pregnancy matings were performed with C57BL/6N male mice. Male mice began breeding at 8 weeks and female mice at 6 weeks. Typically, mice no older than 6 months old were used in this study. |
| Wild animals | No wild animals used |
| Field-collected samples | No field-collected samples used. |