

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	RT-qPCR data were collected with StepOnePlus or QuantStudio 3 Real time PCR systems (Thermo Fisher). Western blotting data were collected with FUSION FX imaging system (VILBER). NMR experiments were performed on Bruker 600 MHz and Varian INOVA 600 MHz spectrometers equipped with cryogenic probes. Tryptophan fluorescence spectra were recorded on a Fluoromax-3 spectrofluorometer (HORIBA). X-ray diffraction data were collected at the ALS 4.2.2 beamline, Berkeley and on Rigaku Micromax 007 high-frequency microfocus X-ray generator in CU Anschutz X-ray crystallography core facility.
Data analysis	All statistical data were analyzed with Prism 8 software (GraphPad v8 software). Software for structure determination include XDS, HKL2000, COOT, Phaser, CCP4 v7.1 and PHENIX 1.18-3855, as listed in method section. Fluorescence data were analyzed using GraphPad Prism v8.

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](#)

ChIP-seq data have been deposited to the DDBJ (DNA Data Bank of Japan) Sequence Read Archive as fastq files and as WIG files under accession numbers DRA012473, DRA010819, DRA016087, E-GEAD-446, E-GEAD-401, and E-GEAD-604[https://ddbj.nig.ac.jp/public/ddbj_database/dra/fastq/] and [https://ddbj.nig.ac.jp/public/ddbj_database/gea/experiment/E-GEAD-000/] (Supplementary Table 4). Coordinates and structure factors have been deposited in the Protein Data Bank under accession numbers 8GDX and 8GEO. All other relevant data supporting the key findings of this study are available within the article, its Supplementary Information or from the corresponding authors upon reasonable request.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	The chosen sample sizes are based on the numbers used for previous publications and are present in relevant figure legends
Data exclusions	no data exclusions
Replication	present in relevant figure legends
Randomization	Randomization was not a relevant feature as we applied a uniform set of techniques.
Blinding	Blinding was not a relevant feature as we applied a uniform set of techniques and tests were repeated two or more times by different individuals.

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

Methods

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

Antibodies

Antibodies used

For immunoblots, anti-Flag M2 conjugated to horse radish peroxidase (HRP, Sigma) was used at a 1:10,000 dilution, Anti-ING5 (Sigma HPA042685, rabbit polyclonal), anti-MEAF6 (Abcam Ab42472, rabbit polyclonal), anti-MLL-N (CST Cat# 14689, rabbit monoclonal), anti-MLL-C (CST Cat#14197, rabbit monoclonal), anti-Histone H3 (CST Cat# 4499, Rabbit monoclonal), RNAP2 Ser5-P (Millipore Cat#05-623, Mouse monoclonal), anti-FLAG (Sigma Cat# F7425, Rabbit polyclonal), anti-Xpress (Sancacruz Biotech Cat#sc-499, mouse monoclonal) and anti-HBO1 (Abcam Ab70183, rabbit polyclonal) antibodies were used at a 1:1,000 dilution. HRP conjugated secondary goat anti-rabbit antibody (Sigma, or Bethyl Laboratories Cat#A120-201P), anti-rat IgG (Bethyl Laboratories Cat#110-305P), or anti-mouse IgG (bethyl Laboratories Cat#A90-516P) was used at a 1:10,000 dilution. For immunofluorescence, anti-HA 3F10 (Roche, 11867423001) and anti-Flag M2 (Sigma, F1804) were used at a 1:300 dilution, AlexaFluor 594 conjugated anti-rat for HA (Invitrogen A11007) and FITC conjugated anti-mouse for Flag (Invitrogen A21202) were used at a 1:100 dilution.

Validation

All antibodies validation are available on the manufacturers' websites.

Eukaryotic cell lines

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

Cell line source(s)

HEK293T cells were purchased from ATCC. Isogenic K562 cell lines expressing 3xFlag2xStrep-tagged JADE1 and JADE1S constructs were generated by integration at the AAVS1 safe harbor locus after DSB induction and recombination targeted by co-transfection with a ZFN expression plasmid.

Authentication

HEK293T and K562 cells were authenticated by the manufacturer and using STR (Short Tandem Repeat) analysis by the JCRB cell bank.

Mycoplasma contamination

HEK293T and K562 cells were tested negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in the study.

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

Mouse: C57BL/6Jcl CLEA Japan, Inc. C57BL/6Jcl

Wild animals

N/A

Reporting on sex

Female mice were used.

Field-collected samples

N/A

Ethics oversight

The experiment was approved by the institutional review board at the National Cancer Center and performed by abiding by ethical regulations

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

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

ChIP-seq and CIRA-seq data have been deposited to the DDBJ (DNA Data Bank of Japan) Sequence Read Archive under

Data access links*May remain private before publication.*

accession numbers and sample IDs listed in Suppl. Table 4.

https://ddbj.nig.ac.jp/public/ddbj_database/dra/fastq/DRA012/DRA012473/
https://ddbj.nig.ac.jp/public/ddbj_database/dra/fastq/DRA010/DRA010819/
https://ddbj.nig.ac.jp/public/ddbj_database/dra/fastq/DRA016/DRA016087/
https://ddbj.nig.ac.jp/public/ddbj_database/gea/experiment/E-GEAD-000/E-GEAD-446/
https://ddbj.nig.ac.jp/public/ddbj_database/gea/experiment/E-GEAD-000/E-GEAD-401/
https://ddbj.nig.ac.jp/public/ddbj_database/gea/experiment/E-GEAD-000/E-GEAD-604/

Files in database submission

accession numbers and sample IDs are listed above and in Suppl. Table 4.

Genome browser session(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology**Replicates**

Most of the ChIP-Seq data are from one experiment and its reproducibility was confirmed by ChIP-qPCR analysis on representative target loci. ChIP analysis of the chromatin for JADE3 was done in two biological replicates.

Sequencing depth

Total number of reads, Uniquely mapped reads, Length of reads, Paired or single-end
 293T-fanChIP-INPUT(21T0226-IN) DRA012473 SAMD00393839 E-GEAD-446 28,759,722 25,285,507 50 single-end
 293T-fanChIP-JADE3 rep1 (21T0226-PHF16) DRA012473 SAMD00393842 E-GEAD-446 32,138,983 27,663,295 50 single-end
 293T-fanChIP-JADE3 rep2 (20T0108-PHF16) DRA010819 SAMD00247197 E-GEAD-401 27,590,253 23,623,049 50 single-end
 293T-fanChIP-MLL (21T0226-MLL) DRA012473 SAMD00393840 E-GEAD-446 34,752,818 29,627,695 50 single-end
 293T-fanChIP-ING4 (21T0226-ING4) DRA012473 SAMD00393843 E-GEAD-446 30,423,675 26,093,823 50 single-end
 293T-fanChIP-HBO1(21T0226-HBO1) DRA012473 SAMD00393841 E-GEAD-446 27,211,386 23,983,025 50 single-end
 293T-fanChIP-RNAP2 non-P (21T0226-RNAP2 non-P) DRA012473 SAMD00393846 E-GEAD-446 34,258,133 30,680,875 50 single-end
 293T-fanChIP-RNAP2 Ser5-P(21T0226-RNAP2 Ser5-P) DRA012473 SAMD00393847 E-GEAD-446 30,850,929 27,719,450 50 single-end
 293T-fanChIP-MEAF6 (21T0226-MEAF6) DRA012473 SAMD00393844 E-GEAD-446 25,985,182 22,782,710 50 single-end
 293T-fanChIP-IN(22T0810 _IN) DRA016087 SAMD00594505 E-GEAD-604 83,446,708 64,561,362 100 single-end
 293T-fanChIP-pCMV5-FLAG(22T0831_pCMV5_M2) DRA016087 SAMD00594508 E-GEAD-604 47,552,788 35,273,941 100 single-end
 293T-fanChIP-fPHF16-FLAG(22T0831_fPHF1_M2) DRA016087 SAMD00594506 E-GEAD-604 60,780,707 48,276,143 100 single-end
 293T-fanChIP-fPHF16 d169-369-FLAG(22T0831_fPHF16d169-369_M2) DRA016087 SAMD00594507 E-GEAD-604 42,249,679 30,790,297 100 single-end

Antibodies

FLAG Sigma-Aldrich F3165/M2 (1:400), JADE3 (PHF16) Abcam Cat#129495(1:400), MLL CST Cat# 14689 (1:400), MEAF6 STJ 116836 (1:400), ING4 Abcam 108621 (1:400), HBO1(KAT7) Abcam Cat#:70183 (1:400), RNAP2 non-P Abcam 8WG16/ab817 (1:400), RNAP2 Ser5-P Millipore CTD4H8/05-623 (1:400)

Peak calling parameters

Peak calling was not performed in this study.

Data quality

All sequenced reads that cleared the illumina's pass filter were mapped to human genome assembly hg19 using BWA 0.7.5.

Software

The alignment tags were counted and ppm was calculated every 25 bp from TSS and the ChIP signal distribution was plotted using NGSplot 2.61. The BAM alignment was converted to the bBigWig coverage files using bam2wig 1.6. and wigToBigWig.