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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	High-throughput Illumina NovaSeq 6000 was used for RNA-seq and RacRIP-seq data collection. Metabolomics data was collected by Agilent 1290II ultra-high-pressure liquid chromatography (UHPLC) system equipped with 6546 quadrupole time-of-flight (QTOF) mass spectrometry. TMT labeling and quantitative proteomics was performed by an Orbitrap Eclipse mass spectrometer (Thermo Fisher Scientific) connected with an Easy-nLC 1200 online.
Data analysis	The custom Perl and R scripts used in this study are available on request to the corresponding authors. To analyze and visualize the RNA-seq and RacRIP-seq data, we used Cutadapt version 4.1, HISAT2 version 2.1.1, SAMtools version 1.16.1, Deeptools version 3.5.2, DESeq2 version 1.40.2, Circlize version 0.4.15, HOMER version 4.11.1, motifStack version 4.3.1, HTSeq version 2.0.2 and IGV version 2.16.0. Metabolomics data was analyzed using Agilent Profinder version 10.0. The TMT labeling and quantitative proteomics data were processed using Proteome Discoverer software v2.5 with Sequest HT search engine using a human database downloaded from UniProt (including 20397 entries, downloaded on 16 October 2022). Proteome Discoverer software v2.2 was used to analyze the LC-MS/MS data of histone acetylation. We used Tanon Image software version 1.00 to quantify the intensity of protein bands and dot blots. GraphPad Prism version 10.1.2 was used for statistical analyses and graphing. ELDA software (https://bioinf.wehi.edu.au/software/elda/) was used to analyze frequencies of LSCs/LICs. CytExpert version 2.4 and FlowJo version 10 were used to analyze the flow cytometry data according to manufacturer's instructions.

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

RacRIP–seq and RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes GSE246505 and GSE246503, respectively. Mass spectrometry data have been deposited in ProteomeXchange with the primary accession code PXD055119. The dataset derived from the TCGA Research Network (http://cancergenome.nih.gov/) that supports the findings of this study is available in cBioPortal for Cancer Genomics (http://www.cbioportal.org/, using TCGA pan-cancer studies). All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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	In this study, we collected samples from five acute myeloid leukemia (AML) patients (three males and two females). Three healthy donors are male.
Reporting on race, ethnicity, or other socially relevant groupings	We recruited 30-70 year-old AML patients derived from Sun Yat-sen University Cancer Center (SYSUCC) or Fujian Medical University Union Hospital, and healthy donors of corresponding ages.
Population characteristics	The leukemic samples (from 3 males and 2 females) were obtained at the time of diagnosis of AML without any treatment. The ages of these sample donors range from 30 to 70 years old.
Recruitment	Patients were recruited when for the first time diagnosed with AML without any treatment.
Ethics oversight	All human samples were collected and approved by the Ethics Committee of Sun Yat-sen University Cancer Center (G2023-108-01 and G2023-285-01) and Fujian Medical University Union Hospital in China (2021KJCX004). Informed consent was obtained from the patients and healthy donors. No compensation was offered to any participant.

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

Life sciences study design

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

Sample size	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (Weng et al., Cancer Cell 2022; Weng et al., Cell Stem Cell 2018). Sample size and number of independent experiments are always clearly stated in the figure legend or in the Methods section.
Data exclusions	No data were excluded from analysis.
Replication	Experiments in the article were reliably reproduced, replication were described in the figure legends.
Randomization	Mice were randomly divided into different groups in the bone marrow transplantation study and drug treatment study. For cell line-based experiments, randomization was not required because cells were manipulated in various ways and all samples were analyzed equally.
Blinding	The investigators were not blinded to allocation during experiments and outcome assessment. Blinding was not performed during allocation as mice were randomly assigned to groups with strict controls. In cell experiments, blinding is impractical due to varied manipulation based on design. During the data collection, our experimental data were relatively objective, so the lack of blinding had little impact on the results.

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

Anti-NAT10 antibody (ab194297, [EPR18663], 1:2000 WB, Abcam)
Anti-N4-acetylcytidine (ac4C) antibody (ab252215, [EPRNCI-184-128], 1:1000 Dot blot, acRIP, Abcam)
SLC1A4 Polyclonal antibody (13067-2-AP, 1:5000 WB, Proteintech)
Menin Rabbit mAb (A3395, [ARC1968], 1:1000 WB, ABclonal)
PHGDH Polyclonal antibody (14719-1-AP, 1:2000 WB, Proteintech)
PSAT1 Polyclonal antibody (10501-1-AP, 1:10000 WB, Proteintech)
PSPH Polyclonal antibody (14513-1-AP, 1:1000 WB, Proteintech)
METTL3 Polyclonal antibody (15073-1-AP,1:1000 WB Proteintech)
HOXA9 Rabbit Polyclonal Antibody (AF7128, 1:1000 WB, Beyotime)
Anti-acetyl Lysine antibody (ab190479, [RM101], 1:1000 WB, Abcam)
Anti-Histone H3 (acetyl K9) antibody (ab177177, [EPR16988], 1:5000 WB, Abcam)
Anti-Histone H3 (acetyl K14) antibody (ab52946, [EP964Y], 1:2000 WB, Abcam)
Anti-Histone H3 (acetyl K27) antibody (ab177178, [EP16602], 1:10000 WB, Abcam)
Anti-Histone H4 (acetyl K16) antibody (ab109463, [EPR1004], 1:1000 WB, Abcam)
Histone H3 XP® Rabbit mAb (D1H2, #4499, 1:2000 WB, Cell Signaling Technology)
Anti-Histone H4 antibody (ab177840, [EPR16599], 1:1000 WB, Abcam)
MLL1 Rabbit mAb (D2M7U, #14689, 1:50 CUT&RUN, Cell Signaling Technology)
Anti-Histone H3 (tri methyl K4)] (ab213224, [EPR20551-225], 1:50 CUT&RUN, Abcam)
Alpha Tubulin Monoclonal antibody (66031-1-Ig, [1E4C11], 1:20000 WB, Proteintech)
GAPDH Monoclonal antibody (60004-1-Ig, [1E6D9], 1:50000 WB, Proteintech)
Beta Actin Monoclonal antibody (66009-1-Ig, [2D4H5], 1:20000 WB, Proteintech)
Anti-CD45.2 Mouse Monoclonal Antibody (PE)(109808,[104], FC, BioLegend)
Anti-CD45.2 Mouse Monoclonal Antibody (APC)(109814,[104], FC, BioLegend)
Normal Mouse IgG (NI03, RIP, Millipore)
Normal Rabbit IgG (NI01, RIP,Millipore)
Monoclonal ANTI-FLAG (F3165, [M2], 1:1000 WB, RIP, Sigma-Aldrich)
Goat Anti-Rabbit IgG, HRP-linked (SA00001-2, 1:10000 WB, Proteintech)
Goat Anti-Mouse IgG, HRP-linked (SA00001-1, 1:10000 WB, Proteintech)

Validation

The antibodies used in the study were validated by the corresponding manufacturers. Commercial antibodies were used in accordance with the manufacturers' recommendations as provided on their official websites. Additional validation was done by the use of negative control and/or positive control (such as knockdown, knockout or overexpression) for FLAG, NAT10, HOXA9, MENIN, SLC1A4, METTL3, PHGDH, PSPH and PSAT1 antibodies.
The validation of the species and application of all the primary antibodies by the manufacturers are provided as follows.
Anti-NAT10 antibody (ab194297, [EPR18663], Abcam), species: Mouse, Rat, Human, applications: Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P;
Anti-N4-acetylcytidine (ac4C) antibody (ab252215, [EPRNCI-184-128], Abcam), species: Species independent, applications: Dot blot;
SLC1A4 Polyclonal antibody (13067-2-AP, Proteintech) , species:human, mouse, rat, applications:WB, IP, IHC, ELISA;
Menin Rabbit mAb (A3395, [ARC1968], ABclonal) , species:Human, Mouse, Rat, applications:WB;
PHGDH Polyclonal antibody (14719-1-AP, Proteintech) , species: human, mouse, rat, applications:WB, IHC, IF/ICC, IP, ELISA;
PSAT1 Polyclonal antibody (10501-1-AP, Proteintech) , species:human, rat, applications:WB, IHC, IF/ICC, IP, ELISA;
PSPH Polyclonal antibody (14513-1-AP, Proteintech) , species:human, mouse, rat, applications:WB, IHC, IF/ICC, IP, ELISA;
METTL3 Polyclonal antibody (15073-1-AP, Proteintech) , species:human, mouse, rat, monkey, applications: WB, IHC, IF/ICC, IP, ELISA;
HOXA9 Rabbit Polyclonal Antibody (AF7128, Beyotime) , species: human, mouse, applications: WB;
Anti-acetyl Lysine antibody (ab190479, [RM101], Abcam) , species: Species independent, applications: ELISA, WB, IHC-P, ChIP, Flow Cyt, IP, ICC/IF;
Anti-Histone H3 (acetyl K9) antibody (ab177177, [EPR16988], Abcam) , species:Mouse, Rat, Human, applications:ICC/IF, IHC-P, WB, PepArr, ChIP-sequencing, ChIC/CUT&RUN-seq, ChIP;
Anti-Histone H3 (acetyl K14) antibody (ab52946, [EP964Y], Abcam) , species:Mouse, Rat, Human, applications:ChIP, WB, IHC-P, ICC/IF;
Anti-Histone H3 (acetyl K27) antibody (ab177178, [EP16602], Abcam) , species:Mouse, Rat, Human, applications:Flow Cyt (Intra), ICC/IF, PepArr, ChIC/CUT&RUN-seq, IHC-P, WB, ChIP, ChIP-sequencing;
Anti-Histone H4 (acetyl K16) antibody (ab109463, [EPR1004], Abcam) , species:Mouse, Rat, Human, applications:Flow Cyt (Intra), WB, IHC-P, ICC/IF, ChIC/CUT&RUN-seq;
Histone H3 XP® Rabbit mAb (D1H2, #4499, Cell Signaling Technology) , species: Human, Mouse, Rat, Monkey, applications:

WB, IHC, IF, Flow Cytometry;
Anti-Histone H4 antibody (ab177840, [EPR16599], Abcam) , species:Mouse, Rat, Human, Drosophila melanogaster, Recombinant fragment, applications:PepArr, ChIP, IHC-P, WB, ICC/IF;
MLL1 Rabbit mAb (D2M7U, #14689, Cell Signaling Technology) , species:Human, Mouse, Rat, Monkey, applications: WB, IP, C&R, C&T;
Anti-Histone H3 (tri methyl K4)] (ab213224, [EPR20551-225], Abcam) , species:Mouse, Rat, Human, applications: ChIP-sequencing, Flow Cyt (Intra), ChIP, WB, ICC/IF, Dot blot, PepArr, IP, ChIC/CUT&RUN-seq;
Alpha Tubulin Monoclonal antibody (66031-1-Ig, [1E4C11], Proteintech) , species: human, mouse, rat, canine, applications: WB, IHC, IF/ICC, FC (Intra), IP, ELISA;
GAPDH Monoclonal antibody (60004-1-Ig, [1E6D9], Proteintech) , species:human, mouse, rat, pig, zebrafish, yeast, plant, applications: WB, IF/ICC, FC (Intra), IP, ELISA;
Beta Actin Monoclonal antibody (66009-1-Ig, [2D4H5], Proteintech) , species: human, mouse, rat, pig, rabbit, canine, monkey, chicken, zebrafish, hamster, applications: WB, IHC, IF/ICC, FC (Intra), IP, ELISA;
Anti-CD45.2 Mouse Monoclonal Antibody (PE)(109808, [104], FC, BioLegend) , species: Mouse, applications: FC;
Anti-CD45.2 Mouse Monoclonal Antibody (APC)(109814, [104], FC, BioLegend), species:Mouse, applications:FC;
Monoclonal ANTI-FLAG (F3165, [M2], Sigma-Aldrich), species: all, applications: immunoblotting, immunoprecipitation, immunocytochemistry, immunofluorescence, ELISA, EIA, chromatin immunoprecipitation, electron microscopy, flow cytometry, supershift assays;

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T (CRL-3216), U937 (CRL-1593.2), THP-1 (TIB-202), Kasumi-1 (CRL-2724), C1498 (TIB-49) and HL-60 (CCL-240) were purchased from the American Type Culture Collection (ATCC). MOLM13 (ACC-554), MonoMac6 (ACC-124), NB4 (ACC-207) were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ). MLL-ENL-ERtm, an immortalized hematopoietic cells with an inducible MLL-ENL derivative (Zeisig et al. Mol. Cell Biol, 2004.), was a kind gift from Dr. Jianjun Chen (City of Hope).
Authentication	STR analyses have been conducted in all cell lines used in this study for the authentication.
Mycoplasma contamination	All cell lines were tested to be mycoplasma-negative monthly.
Commonly misidentified lines (See ICLAC register)	We have checked the list of known misidentified cell lines maintained by the International Cell Line Authentication Committee and found that a potential issue of U-937 was documented. "Some ATCC stocks of U937 were cross-contaminated by K-562 initially (Reid et al, 1995; PMID:7759961). The problem was corrected and subsequent stocks have been confirmed to carry only U-937. The problem referred to by Drexler et al (1999) may be separate." Other cells used in this study were not in the misidentified cell line list. It should be mentioned that all of the cell lines (including U-937) used in this study have been authenticated through STR analyses by us.

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	C57BL/6, 7- to 9-week old; C57BL/6 NAT10fl/fl, strain No: T007971, 7- to 19-week old,; C57BL/6 R26-CreERT2, Cat. No: NM-KI-220020, 7- to 19-week old; B6.SJL (CD45.1), strain No: 002014, 5- to 9-week old;
Wild animals	The study does not involve wild animals.
Reporting on sex	Our findings do not apply to only one sex.
Field-collected samples	The study does not involve samples collected from the field.
Ethics oversight	All procedures involving mice and experimental protocols were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University Cancer Center (L025504202111015), Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences (2020066), and Ruiye Bio-tech Guangzhou Co.Ltd (RYEth-20221010411).

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

Plants

Seed stocks	We do not have related information in our work.
Novel plant genotypes	We do not have related information in our work.
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	MOLM13 cells and MonoMac6 cells with different treatments were harvested and washed with cold PBS, followed by staining with FITC-Annexin V and propidium iodide (PI) to assess cell apoptosis. EdU incorporation assays were performed using the Click-iT™ Plus EdU Alexa Fluor™ 488 Kit (C10632, ThermoFisher Scientific) followed by PI staining. Engraftment cells in PB of recipient mice were washed with cold PBS, followed by staining with PE-CD45.2.
Instrument	Apoptosis and EdU incorporation assays were examined by flow cytometry on a CytoFLEX LX Flow Cytometer (Beckman). Engraftment cells were analyzed by BD LSRFortessa™X-20 (BD Biosciences).
Software	CytExpert version 2.4 and FlowJo version 10 were used to analyze the flow cytometry data according to manufacturer's instructions.
Cell population abundance	At least 10,000 viable cells were analyzed for each sample.
Gating strategy	For all experiments, cells were first gated by FSC/SSC to exclude debris. Then, target cell population for further analysis were gated by indicated cell surface marker or specific dyes. The gating strategy of each assay has been provided in Supplementary Figure 1.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.