

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](#).

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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

Datasets from our manuscript have been generated for the purpose of the study, we did not collect additional datasets.

Data analysis

scChIP-seq and scRNA-seq sequencing data were analyzed using Python (v2.7.12) and R (v3.3.3). Specific R Bioconductor packages include ConsensusClusterPlus, Sushi, Scater, ccRemover and HMMcopy. Codes are available upon request. We also used Cushaw3, bowtie 1.2.2, bedtools 2.17, bamcmp, Picard tools and 10x Cell Ranger Single Cell Software Suite 1.3.1.

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/reviewers. 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 GEO accession number of the data produced in this manuscript is GSE117309. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117309> with the token wnliuuuntelzj

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	No sample-size calculation was performed; the aim of our manuscript was to develop and validate a droplet-based scChIP-seq approach.
Data exclusions	We excluded cells from analyses based on pre-established Quality Control metrics, detailed in Methodology questions (based on minimal and maximum coverage, and correlation score to other cells).
Replication	We have validated the reproducibility of our single-cell chromatin profiling method by analyzing n=3 technical replicates for H3K4me3 and n=2 biological replicates, ie independent cell suspensions processed independently on our micro-fluidics system, for H3K27me3 (All attempts at replication were successful). We also confirmed that the clustering of scChIP-seq data is not driven by technical artifact (batch effect) but cell type-specific biological similarities.
Randomization	Randomization is not relevant to our study as it aims at comparing chromatin and transcriptional states between a sensitive and a resistant tumor specimen.
Blinding	No blinding was performed as the study aimed at comparing chromatin and transcriptional states between a sensitive and a resistant tumor specimen.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	anti-H3K4me3 [Millipore, # 07-473 , lot # 2664283, dilution 2/1000] anti-H3K27me3 (C36B11) [Cell Signaling Technology, # 9733, lot # 008, dilution 2.5/1000]
Validation	- Anti-trimethyl-Histone H3 (Lys4) Antibody is a rabbit polyclonal antibody for detection of Histone H3 trimethylated at lysine 4. This highly specific and well published antibody has been validated in ChIP, DB, WB, PIA, ChIP-seq (Merck Millipore's website) - Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb detects endogenous levels of histone H3 only when tri-methylated on Lys27. The antibody does not cross-react with non-methylated, mono-methylated or di-methylated Lys27. In addition, the antibody does not cross-react with mono-methylated, di-methylated or tri-methylated histone H3 at Lys4, Lys9, Lys36 or Histone H4 at Lys20. (Cell Signaling Technology's website)

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	In the study, cell lines were used to demonstrate and benchmark the performance of the single-cell ChIP-seq technology. For this purpose, we used: - Human Jurkat cells (ATCC, T18-125) - Human Ramos cells (ATCC, CRL-1596) - Mouse M300.19 cells was a gift from B. Moser
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Authentication	Human cell lines were obtained from ATCC and not authenticated. Mouse M300.19 cells were not authenticated.
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	8- to 12-week-old female Swiss nude mice purchased from Charles River and maintained under specific pathogen-free conditions.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The care and housing of mice used in this study were in accordance with institutional guidelines and the rules of the French Ethics Committee (project authorization no. 02163.02).

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

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Not applicable, study focuses on two patient samples which have been used to generate two patient-derived xenograft models.
Recruitment	The two PDX models come from two patients of the Institut Curie hospital.
Ethics oversight	We have obtained consent from all participants of the study

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 <i>May remain private before publication.</i>	The GEO accession number of the data produced in this manuscript is GSE117309. The following secure token has been created to allow review of record GSE117309 while it remains in private status: wnliuuuntelzcyj
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Files in database submission	- Raw sequencing files Jurkat-Ramos_scChIP_K27me3_R1.fastq.gz Jurkat-Ramos_scChIP_K27me3_R2.fastq.gz Jurkat-Ramos_scChIP_K4me3_R1.fastq.gz Jurkat-Ramos_scChIP_K4me3_R2.fastq.gz HBCx-95_scChIP_H3K27me3_R1.fastq.gz HBCx-95_scChIP_H3K27me3_R2.fastq.gz HBCx-95-CapaR_scChIP_H3K27me3_R1.fastq.gz HBCx-95-CapaR_scChIP_H3K27me3_R2.fastq.gz HBCx-22_scChIP_H3K27me3_R1.fastq.gz HBCx-22_scChIP_H3K27me3_R2.fastq.gz HBCx-22-TamR_scChIP_H3K27me3_R1.fastq.gz HBCx-22-TamR_scChIP_H3K27me3_R2.fastq.gz HBCx-95_scRNA.bam HBCx-95-CapaR_scRNA.bam HBCx-22_scRNA.bam HBCx-22-TamR_scRNA.bam - Processed data files CountTable_Jurkat_scChIP_K4me3.txt.gz CountTable_Ramos_scChIP_K4me3.txt.gz CountTable_Jurkat_scChIP_K27me3.txt.gz CountTable_Ramos_scChIP_K27me3.txt.gz CountTable_HBCx-95_scChIP_H3K27me3_hg38.txt.gz CountTable_HBCx-95-CapaR_scChIP_H3K27me3_hg38.txt.gz
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CountTable_HBCx-95_scChIP_H3K27me3_mm10.txt.gz
 CountTable_HBCx-95-CapaR_scChIP_H3K27me3_mm10.txt.gz
 CountTable_HBCx-22_scChIP_H3K27me3_hg38.txt.gz
 CountTable_HBCx-22-TamR_scChIP_H3K27me3_hg38.txt.gz
 CountTable_HBCx-22_scChIP_H3K27me3_mm10.txt.gz
 CountTable_HBCx-22-TamR_scChIP_H3K27me3_mm10.txt.gz
 CountTable_HBCx-95_scRNA.tar
 CountTable_HBCx-95-CapaR_scRNA.tar
 CountTable_HBCx-22_scRNA.tar
 CountTable_HBCx-22-TamR_scRNA.tar

- Supplementary files (barcode sequences used in scChIP-seq experiments)
 GSE117309_BarcodeLibrary_scChIPseq.fasta.gz

Genome browser session
 (e.g. [UCSC](https://genome.ucsc.edu))

- Genome browser session H3K4me3 and H3K27me3 Jurkat Ramos
https://genome.ucsc.edu/s/kevin%20grosselin/Grosselin_Jurkat_Ramos_hg38

- Genome browser session H3K27me3 HBCx-95 model stromal cells
https://genome.ucsc.edu/s/kevin%20grosselin/Grosselin_HBCx%2D95_mm10

- Genome browser session H3K27me3 HBCx-95 model tumor cells
https://genome.ucsc.edu/s/kevin%20grosselin/Grosselin_HBCx%2D95_hg38

- Genome browser session H3K27me3 HBCx-22 model tumor cells
https://genome.ucsc.edu/s/kevin%20grosselin/Grosselin_HBCx%2D22_hg38

Methodology

Replicates

We have validated the reproducibility of our single-cell chromatin profiling method by analyzing n=3 technical replicates for H3K4me3 and n=2 biological replicates for H3K27me3 ($r = 0.96$ and 0.98 with $p < 10e-15$ respectively). We also confirmed that the clustering of scChIP-seq data is not driven by technical artifact (batch effect) but cell type-specific biological similarities.

Sequencing depth

scChIP-seq libraries were sequenced on an Illumina NextSeq 500 MidOutput 150 cycles. Cycles were distributed as follows: 50 bp (Read #1) were assigned for the genomic sequence and 100 bp (Read #2) were assigned to read the single-cell barcode sequence.

Number of mapped reads refers to the number of reads aligned to the reference genome by keeping only reads having no more than one reportable alignments and 2 mismatches.

On the other hand, number of uniquely mapped reads refers to the number of reads obtained after de-multiplexing of the single-cell barcodes and removal of duplicated reads (see Methods).

- scChIP-seq H3K4me3 Jurkat Ramos
 Number of raw reads: 64,396,048
 Number of mapped reads (hg38): 42,659,092
 Number of uniquely mapped reads: 10,877,052

- scChIP-seq H3K27me3 Jurkat Ramos
 Number of raw reads: 72,069,685
 Number of mapped reads (hg38): 47,123,836
 Number of uniquely mapped reads: 8,121,495

- scChIP-seq H3K27me3 HBCx-95
 Number of raw reads: 151,963,813
 Number of mapped reads (mm10): 34,586,767
 Number of uniquely mapped reads (mm10): 5,187,802
 Number of mapped reads (hg38): 50,472,984
 Number of uniquely mapped reads (hg38): 8,715,197

- scChIP-seq H3K27me3 HBCx-95-CapaR
 Number of raw reads: 204,907,172
 Number of mapped reads (mm10): 23,862,992
 Number of uniquely mapped reads (mm10): 3,739,207
 Number of mapped reads (hg38): 92,698,620
 Number of uniquely mapped reads (hg38): 15,333,344

- scChIP-seq H3K27me3 HBCx-22
 Number of raw reads: 114,823,151
 Number of mapped reads (hg38): 45,798,204
 Number of uniquely mapped reads (hg38): 5,990,857

- scChIP-seq H3K27me3 HBCx-22-TamR
 Number of raw reads: 109,501,758
 Number of mapped reads (hg38): 33,037,774
 Number of uniquely mapped reads (hg38): 2,844,194

Antibodies

anti-H3K4me3 [Millipore, # 07-473 , lot # 2664283, dilution 2/1000]
anti-H3K27me3 (C36B11) [Cell Signaling Technology, # 9733, lot # 008, dilution 2.5/1000]

Peak calling parameters

For single-cell ChIP-seq experiments, we do not perform peak calling, as there is no input sample. For control bulk specimen, we performed peak calling using MACS for H3K4me3 and Zerone for H3K27me3 with default parameters.

Data quality

Data quality for scChIP-seq was assessed with QC metrics detailed in the Methods section: average coverage per cell with standard deviation, correlation with bulk profiles and ability to detect cell populations with clustering algorithms.

Software

scChIP-seq datasets were analyzed with Python (v2.7.12) and R (v3.3.3). All thresholds and steps are detailed in the Method section of the manuscript. Codes available upon request.