

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	Imaging: Leica Stellaris 8 (Leica Application Suite X (LAS X software), THUNDER imager, Leica DMI8 Live cell imaging: Leica DMI8 widefield Laser microdissection: Leica LMD7 and LMD7000 Immunoblots: Odyssey imaging System (LICOR) or ChemiDoc (Bio-Rad) Sequencing: NovaSeq X Plus sequencer (Illumina)
Data analysis	For image analysis: FIJI (ImageJ2 v.1.53t), Huygens software (deconvolution, v24.10), FIJI (tile stitching, v2.14), Imaris (colocalization analysis, v10.2) Analysis: R (v. 4.4.2), R (v. 3.6), Python 3.7 Amplicon Reconstruction: Amplicons were reconstructed using Decoil 1.1.2. One amplicon (CDK4) was assembled using Shasta (v0.6.0) and aligned to hg38 using minimap2 with parameter -x asm10. Micronucleus sequencing: Reads were quality and adapter-trimmed using Trim Galore (v0.6.10) and aligned to the human reference assembly GRCh38 with added EBV contig (NC_007605.1) using BWA-MEM (v 0.7.18) with default parameters. Duplicate reads were removed using Picard (v3.3.0) MarkDuplicates. A detailed description of the sequence identification process is provided in the Supplementary Information Methods section.

ChIPseq:

Raw reads were quality and adapter-trimmed using Trim Galore (v.0.6.10) and aligned to the human reference assembly GRCh38 with an added EBV contig (NC_007605.1) using BWA-MEM with default parameters. Duplicate reads were removed using Picard MarkDuplicates. Library quality was assessed using strand cross-correlation metrics (RSC and NSC) from Phantompeakqualtools (v1.2.2). Using the deduplicated BAM file, read densities per 10 bp window were generated using DeepTools2 bamCoverage. Genomic bins overlapping the ENCODE DAC blacklist regions were filtered out using the parameters: --ignoreForNormalization chrX chrM NC_007605.1 --scaleFactor 10 --effectiveGenomeSize 2805636231 --exactScaling --extendReads 200 --normalizeUsing CPM and input-subtracted using the bigwigCompare function.

RNAseq:

Reads were quality and adapter-trimmed using Trim Galore (v0.6.10) and aligned to the human reference assembly GRCh38 with an added EBV contig (NC_007605.1) using STAR (v2.7.11b) with parameters --twopassMode Basic --outSAMtype BAM SortedByCoordinate. Reads were counted per gene against GENCODE gene annotation (Release 47) using htseq (v2.0.5) htseq-count with parameters -r pos -s no. Counts were normalized for library size and composition using sizefactors from DESeq2 (v1.38.3). Counts were z-score normalized for visualization using the scale function in R-base (v4.2.2). Differentially expressed genes between the untreated and treated samples were identified using DESeq2 and p-values were corrected for multiple testing using the Benjamini-Hochberg procedure. Gene set enrichment analysis was against MSigDB hallmark gene sets using the GSEA function in Clusterprofiler (v4.6.2) and visualized with DOSE (v3.24.2).

Nanopore:

Reads were basecalled using Guppy (v6.4.6, dna_r9.4.1_450bps_hac model, ONT) and aligned to hg38 using minimap (v2.30). We used DNAscent (v3.1.2) to investigate replication fork dynamics. We applied the DNAscent detect function to detect EdU and BrdU incorporation on nanopore reads in batches of around 4000 reads. The forkSense function was then used to classify replication forks based on the order EdU and BrdU incorporation. To determine replication fork speeds, we only retained complete forks (forks running off the end of a read were excluded) from the forkSense output. We then overlapped the remaining forks with the Decoal amplicon reconstruction for CHP212 and classified them as either circular (overlapping with ecDNA regions) or linear (overlapping with any other region). To compare ecDNA forks with linear forks of similar chromatin context, we further filtered forks which overlapped with active regions, by H3K27ac ChIPseq peaks. Bed files for peak calls were taken from and downloaded from GEO (GSE90683) and converted from hg19 to hg38 using the UCSC liftover tool (<https://github.com/ucscGenomeBrowser/kent>). Fork speeds were calculated by dividing the length of each detected fork by the treatment time beginning with the start EdU treatment until the start of the thymidine chase out (Untreated: 15 min; HU: 16.4 min).

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](#)

Sequencing data generated for this study has been deposited to ArrayExpress under accessions E-MTAB-16933 (RNAseq), E-MTAB-16944 (ChIPseq) and ENA under accessions PRJEB112294 (single micronucleus sequencing) and PRJEB111610 (long read sequencing). Peak calls for CHP212 H3K27ac ChIPseq data were downloaded from GEO (GSE90683). The data that support the findings of this study are available from the corresponding author upon request. Scripts for data analysis within the scope of this publication are available under https://github.com/henssen-lab/ecDNA_micronucleus/ and Zenodo (<https://doi.org/10.5281/zenodo.21207200>).

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	Research findings do not apply to one sex only. Sex was not taken into consideration and was not reported for the majority of the patient images analyzed in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Data on race and ethnicity were not collected.
Population characteristics	This study comprised the analyses of tumour and blood samples of patients diagnosed between 1991 and 2022.
Recruitment	Patients were registered and treated according to the trial protocols of the German Society of Pediatric Oncology and Hematology (GPOH).
Ethics oversight	The collection and use of patient specimens was approved by the institutional review boards of Charité-Universitätsmedizin Berlin and the Medical Faculty, University of Cologne. Specimens and clinical data were archived and made available by

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	Sample size represents the number of cell lines, patient images or animals used in this study. The sample size was not predetermined using a statistical method, but was based on previous publications and cell line availability. Sample size for each experiment are provided in the figure legends.
Data exclusions	No data excluded
Replication	The number of replicates is indicated in the figure legend.
Randomization	Randomization was not relevant for this study.
Blinding	The data was not analysed blinded. Blinding was not feasible because the investigator performing the experiment had to know the treatment assignments to correctly prepare, administer, and monitor the different cell culture/ treatment conditions.

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-vinculin (1:5,000; Millipore, MAB3574),
 anti-c-MYC (1:1,000; Cell Signaling, D84C12),
 IRDye 800 anti-rabbit (LICOR, 926-32213) ,
 IRDye 680 anti-mouse (LICOR, 926-68072),
 anti-pan-histone H11-4 (1:500; Merck, MAB3422)
 Alexa488 anti-mouse secondary antibody (1:1000; Invitrogen, A-11001)
 Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 555 (1:1000; Invitrogen, A-31570)
 Anti-H3K27ac (1:500, Active Motif, 39133)
 Anti-H3K27me3 (1:500, Thermo Fisher, G.299.10)
 Alexa Fluor 647, Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (1:1000, Invitrogen, A21244)
 Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (1:1000, Invitrogen, A21235)
 Anti-CIP2A (1:500, Santa Cruz, sc-80659)
 Anti-TOPBP1 (1:500, Sigma-Aldrich, ABE1463)
 Anti-c-MYC (1:5000, proteintech, 10828-1-AP)
 goat anti-rabbit IgG (H+L), horseradish peroxidase conjugates (1:5000, Invitrogen, G21234)
 anti-beta actin (1:10000, Cell Signalling, 3700S)
 Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP (1:10000, Thermo Fisher, 62-6520)
 Anti-H2AX (1:500/ 1:250, Sigma Aldrich, 05-636)
 Anti-H3K27Ac (1:600, Diagonode, C15410196)

Anti-H3K27me3 (1:600, Diagonode, C15410195)
 anti- pRPA2-S33 (1:1000, Novus Biological, NB100-544)
 anti-Lamin B1 Monoclonal antibody (proteintech, 66095-1-Ig, IF - 1:500)
 anti-MDC1-antibody (abcam, ab11171, 1:5000)

Validation

All antibodies are commercially available and were tested for species and application as described in each manufacturer's protocol:
 anti-vinculin – Millipore: https://www.merckmillipore.com/DE/de/product/Anti-Vinculin-Antibody-clone-VIIIF9-7F9,MM_NF-MAB3574
 anti-c-MYC- Cell Signalling: https://www.cellsignal.com/products/primary-antibodies/c-myc-d84c12-rabbit-mab/5605?srsltid=AfmBOoqiUuqHBakEtXfACpDe_PTNvlo3am4WWkaGC_r_eW6cof5A8XC
 IRDye 800 anti-rabbit- LICOR: <https://shop.licor.com/bio/reagents/irdye-800cw-donkey-anti-rabbit-igg-secondary-antibody>
 IRDye 680 anti-mouse- LICOR: <https://shop.licor.com/bio/reagents/irdye-680rd-donkey-anti-mouse-igg-secondary-antibody>
 anti-pan-histone H11-4- Merck: https://www.merckmillipore.com/DE/de/product/Anti-Histone-Antibody-clone-H11-4,MM_NF-MAB3422
 Alexa488 anti-mouse secondary antibody- Invitrogen: <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001>
 Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 555 – Invitrogen: <https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31570>
 Anti-H3K27ac- Active Motif: <https://www.activemotif.com/catalog/details/39133/histone-h3-acetyl-lys27-antibody-pab>
 Anti-H3K27me3- Thermo Fisher: <https://www.thermofisher.com/antibody/product/H3K27me3-Antibody-clone-G-299-10-Monoclonal/MA5-11198>
 anti-Lamin B1 Monoclonal antibody- Alexa Fluor 647, Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody- Invitrogen: <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21244>
 Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647- Invitrogen: <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21235>
 Anti-CIP2A - Santa Cruz: <https://www.scbt.com/de/p/cip2a-antibody-2g10-3b5?srsltid=AfmBOooiRXNwldcd4pnvAD9rLZEBQl0zjf4cNvdJS6Q9CS6VHjesdbH>
 Anti-TOPBP1- Sigma-Aldrich: https://www.sigmaaldrich.com/DE/de/product/mm/abe1463?srsltid=AfmBOooBPd8V6m8to58lV58EzajQekfFijo3wR6J-F_Zl8_-lcs1rXdM
 Anti-c-MYC- Proteintech: <https://www.ptglab.com/de/products/MYC-Antibody-10828-1-AP.htm?srsltid=AfmBOoo59lDbQqEwidRTVSpt5koU1k2WE-u0KIYcKQHiP2a2zCuOo53>
 goat anti-rabbit IgG (H+L), horseradish peroxidase – Invitrogen: <https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/G-21234>
 anti-beta actin-Cell Signalling: https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700?srsltid=AfmBOooHEP--vNw_ThTJju4sgnDe7qjWH6SPyuYS34pQbDc2yGITQzh
 Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP- Thermo Fisher: <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Secondary-Antibody-Polyclonal/62-6520>
 Anti-H2AX- Sigma-Aldrich: https://www.sigmaaldrich.com/DE/de/substance/antiphosphohistoneh2axser139antibodyclonejwb3011234598765?utm_source=google&utm_medium=cpc&utm_id=12481160316&utm_campaign={campaignname}&utm_content=119127149456&utm_term=&gad_source=1&gbraid=OAAAAAD8kLQRUJjcPWDAzAD7SCchfUn8cj&gclid=Cj0KCQjwhr6_BhD4ARIsAH1YdjAbNmUO0qRhFetWG6y74spnPafZlhvyEl3nlegkthAb397-Ut5E8laJHmEALw_wcB
 Anti-H3K27Ac- Diagonode: <https://www.diagonode.com/en/p/h3k27ac-polyclonal-antibody-premium-50-mg-18-ml>
 Anti-H3K27me3- Diagonode: <https://www.diagonode.com/en/p/h3k27me3-polyclonal-antibody-premium-50-mg-27-ml>
 anti- pRPA2-S33 -Novus Biological: https://www.novusbio.com/products/rpa2-antibody_nb100-544?srsltid=AfmBOorJKwPfCtHvB6uH6LRi3WEyR5d586oamFVjLZMADyaT-HcVN5ck
 anti-MDC1-antibody- <https://www.abcam.com/en-us/products/primary-antibodies/mdc1-antibody-ab11171>
https://www.ptglab.com/products/LMNB1-Antibody-66095-1-Ig.htm?srsltid=AfmBOopGd2J5xuEROCQOZgacU3Z5QrgGZ7av_V-iojHWu7Ek6HrV0bpK

Eukaryotic cell lines

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

Cell line source(s)

Human cell lines were obtained from the American Type Culture Collection. Tr-14 was provided by J. J. Molenaar (Princess Máxima Center for Pediatric Oncology), STA-NB10DM/HSR were provided by Sabine Taschner (St. Anna Kinderkrebsforschungsinstitute).

Authentication

All cell lines were authenticated by STR profiling.

Mycoplasma contamination

The absence of Mycoplasma sp. contamination was determined using a Lonza MycoAlert system (Lonza).

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

The cell lines in this study were not found in the ICLAC register.

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/6J, p53fl/fl)

Wild animals	n/a
Reporting on sex	Findings apply to both sexes.
Field-collected samples	n/a
Ethics oversight	All animal experiments were approved by MSKCC's Institutional Animal Care and Use Committee.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NB90, NB97, NB2004, NB2016 (German Society of Pediatric Oncology and Hematology (GPOH))
Study protocol	NB90: Simon, T. et al. Long term outcome of high-risk neuroblastoma patients after immunotherapy with antibody ch14.18 or oral metronomic chemotherapy. BMC Cancer 11, 21 (2011). https://doi.org/10.1186/1471-2407-11-21 NB97: Berthold, F. et al. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. Lancet Oncol 6, 649-658 (2005). https://doi.org/10.1016/S1470-2045(05)70291-6 NB2004: NCT00410631 NB-2016 registry- NA
Data collection	In study design protocols, survival analysis was done blinded
Outcomes	Overall and event free survival

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>

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>	Raw data and BigWig files have been deposited to ArrayExpress under E-MTAB-16944.
Files in database submission	COLO320DM_H3K27ac_HU_vs_UT.bw, COLO320DM_HU_H3K27ac_10bp_10Mb.bw, COLO320DM_INPUT_10bp_10Mb.bw, COLO320DM_UT_H3K27ac_10bp_10Mb.bw
Genome browser session (e.g. UCSC)	<i>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.</i>

Methodology

Replicates	1 biological replicate, 1 technical replicate
Sequencing depth	Libraries were sequenced on a NovaSeq X Pluse sequencer in 100 nt - single end mode. Sample, Total Reads, Reads after QC (samtools view -F 1804) COLO320DM_INPUT, 91203041, 61718855 COLO320DM_HU_H3K27ac, 125995829, 80224701 COLO320DM_UT_H3K27ac, 133482556, 82531329

	COLO320DM_HU_H3K27me3, 109318786, 68926618 COLO320DM_UT_H3K27me3, 129108392, 79958856
Antibodies	Diagenode H3K27me3 Antibody cat. C15410195 lot. A0824D Diagenode H3K27ac Antibody cat. C15410196 lot. A1723-0041D
Peak calling parameters	<i>Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.</i>
Data quality	Library quality was assessed using strand cross-correlation metrics (RSC and NSC) from Phamtompeakqualtools (v1.2.2) For H3K27ac with FDR at 0.05% and H3K27me3 with FDR at 0.1% Sample, Total Peaks, Peaks >=5FC COLO320DM_HU_H3K27ac, 116896, 48742 COLO320DM_UT_H3K27ac, 91816, 39081 COLO320DM_HU_H3K27me3, 113380, 717 COLO320DM_UT_H3K27me3, 162557, 27664
Software	TrimGalore v0.6.10, Phantompeakqualtools v1.2.2, BWA-MEM v0.7.18, Picard v3.3.0, Deeptools2 v3.5.5, Bedtools v3.31.1, Samtools v.1.21