

Micronuclear ecDNA constrains tumor cell fitness and is associated with favorable patient survival

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Extrachromosomal DNA (ecDNA) contributes to cancer genome instability by enabling high-copy oncogene amplification and genetic intratumoral heterogeneity. Micronuclei (MNs) are common in chromosomally unstable cancers, yet their role in ecDNA-driven tumors remains unclear. Here we investigate how ecDNA damage, mitotic segregation and MN formation are linked. We find that ecDNAs are a major source of MNs following their damage, particularly under replication stress. Replication stress promotes mitotic ecDNA clustering, asymmetric inheritance, mis-segregation into MNs and reduced transcriptional output. Single-MN sequencing reveals that multiple distinct ecDNA species coalesce within individual MNs. Cells inheriting ecDNA⁺ MNs display impaired proliferative capacity and are more likely to undergo cell death. In patients with *MYCN*-amplified neuroblastoma, higher frequencies of ecDNA⁺ MNs at diagnosis are associated with improved overall survival. Together, these findings identify ecDNA mis-segregation as a mechanism that limits tumor fitness, highlighting mitotic ecDNA retention as a potential therapeutic vulnerability.

Extrachromosomal DNA (ecDNA) has emerged as a major form of cancer genome instability, driving high-copy oncogene amplification, intratumoral heterogeneity, rapid tumor evolution and therapy resistance^{1,2}. Across diverse cancer types, the presence of ecDNA (ecDNA⁺) correlates with poor outcomes compared to tumors with linear chromosomal amplifications or other types of genome instability³. ecDNAs are relatively large (approximately 1–2 Mb)⁴, chromatinized, circular DNA structures that are enriched for oncogenes as well as regulatory and immunomodulatory elements^{5–7}. They are observed predominantly in cancer cells and are distinct from extrachromosomal circular DNA, which comprises smaller, typically non-clonal circular DNA elements that rarely encompass full genes and can also be detected in normal cells^{8–10}. The probable absence of centromeres on ecDNA^{11,12} permits uneven segregation during mitosis, fueling heterogeneity and accelerating tumor evolution through non-Mendelian inheritance¹¹. Chromosomal tethering mechanisms are thought to promote ecDNA transmission during cell division^{13,14}.

MNs are small, extranuclear bodies initially enclosed by nuclear envelopes that arise at the end of mitosis and contain mis-segregated chromosomes or chromatin fragments^{15,16}, distinguishing them from other extranuclear structures such as exclusomes¹⁷. They are a

hallmark of chromosomal instability. Chromatin in MNs can undergo chromothripsis^{18–20} and epigenetic remodeling^{21–23}, resulting in long-term genomic and transcriptional alterations. MNs can serve as intermediates for ecDNA generation²⁰, suggesting reciprocal links between ecDNA and MN biology. Treatment of ecDNA-bearing cells with hydroxyurea (HU) or other DNA-damaging agents increases MN frequency and decreases oncogene levels, implying that damaged ecDNA may seed MN formation^{24–26}. However, the mechanisms of MN generation in ecDNA⁺ tumors, their molecular composition and functional consequences remain unknown.

Using cell lines, engineered mouse models, patient tumor samples, single-MN sequencing and live-cell imaging, we set out to define how micronucleation arises from ecDNA and how this process shapes cancer cell fitness and patient outcome.

Results

MNs are enriched for ecDNA in ecDNA⁺ cancers

To assess how frequently ecDNA is found outside primary nuclei and incorporated into extranuclear envelopes—which we refer to as MNs even though a subset might represent exclusomes¹⁷—we compared near-isogenic cancer cell lines carrying oncogene amplifications either

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on ecDNA or re-integrated as homogeneously staining regions (HSRs). (Fig. 1a and Supplementary Fig. 1). Even though additional genes are also co-amplified on the same amplicon^{27–29}, we focused our analysis on the most prominent, tumor-relevant oncogenes, for which fluorescence in situ hybridization (FISH) probes are well validated and accessible. Across three such pairs, COLO 320DM/HSR (*MYC*), GBM39EC/HSR (*EGFRvIII*) and PC3DM/HSR (*MYC*), ecDNA⁺ cells displayed significantly higher frequencies of oncogene-positive MNs than their HSR counterparts (Fig. 1b). By contrast, the frequency of oncogene-negative MNs was similar, indicating that ecDNA mis-segregation, and not chromosomal instability, contributes to elevated MN frequency (Fig. 1b–c). Lamin B1 staining intensity was higher in oncogene-positive MNs than in oncogene-negative MNs in ecDNA⁺ cell lines, suggesting that oncogene-positive MNs have an intact inner nuclear membrane (Supplementary Fig. 2a–c). Analysis of 275 patient tumor biopsies from diverse cancer types with FISH-defined amplification status of distinct oncogenes (Fig. 1d and Supplementary Table 1) confirmed this pattern: ecDNA⁺ tumors contained a significantly greater fraction of cells with MNs than non-amplified tumors or tumors with chromosomal amplifications, and the proportion of oncogene-positive MNs was correspondingly higher (Fig. 1e, f and Supplementary Fig. 2d). MNs in ecDNA⁺ cells were smaller than those in HSR cells, consistent with their predominant composition of ecDNA rather than larger chromosomal fragments (Supplementary Fig. 2e). MN abundance positively scaled with overall ecDNA copy number in most tumor types (Fig. 1g–j and Supplementary Fig. 2f). A binomial segregation model estimated an ecDNA mis-segregation rate of 1.7% (range, 0–7.8%), indicating overall efficient, but occasionally faulty, retention during mitosis (Supplementary Fig. 2g).

To rule out high oncogene expression as the cause of increased MN formation, we analyzed adult neural stem cells (aNS cells) from *Trp53^{fl/fl};Myc^{ec/+}* mice following transduction with Cre recombinase, which results in engineered *Myc*-containing ecDNAs, and compared them with *Trp53^{fl/fl};Myc*-overexpressing control cells with chromosomally integrated *Myc* that express comparably high levels of MYC protein (Fig. 1k). Only ecDNA⁺ cells exhibited elevated MN abundance, and these MNs were enriched for *Myc* sequences (Fig. 1l). Therefore, the presence of ecDNA is linked to elevated MN frequencies in cancer cells.

Replication stress promotes ecDNA micronucleation

Given that ecDNAs lack centromeres, they can rely on chromosomal tethering to ensure inheritance during mitosis^{11,14,30}. MN formation can occur when ecDNAs fail to tether to chromosomes during cell division (Fig. 2a), as also evidenced by recent reports^{14,31}. If ecDNAs detached, mis-segregated stochastically and remained independent, individual ecDNA molecules would be expected to segregate into separate MNs and be inherited by both daughter cells. Instead, live-cell imaging of unperturbed COLO 320DM cells expressing fluorescently labeled *MYC* ecDNA (tetR–mNeonGreen) and chromatin (H2B–emRFP670)³⁰ revealed that ecDNA frequently detach from chromatids as clusters during mitosis and are subsequently encapsulated on average into single MNs in daughter cells at the end of mitosis (Fig. 2a, b and Supplementary Video 1). The frequency of detached clusters positively scaled with the abundance of ecDNA in cells (Fig. 2c–d), suggesting that although individual detachment events may occur stochastically, ecDNA coalescence and subsequent mis-segregation are governed by non-random processes.

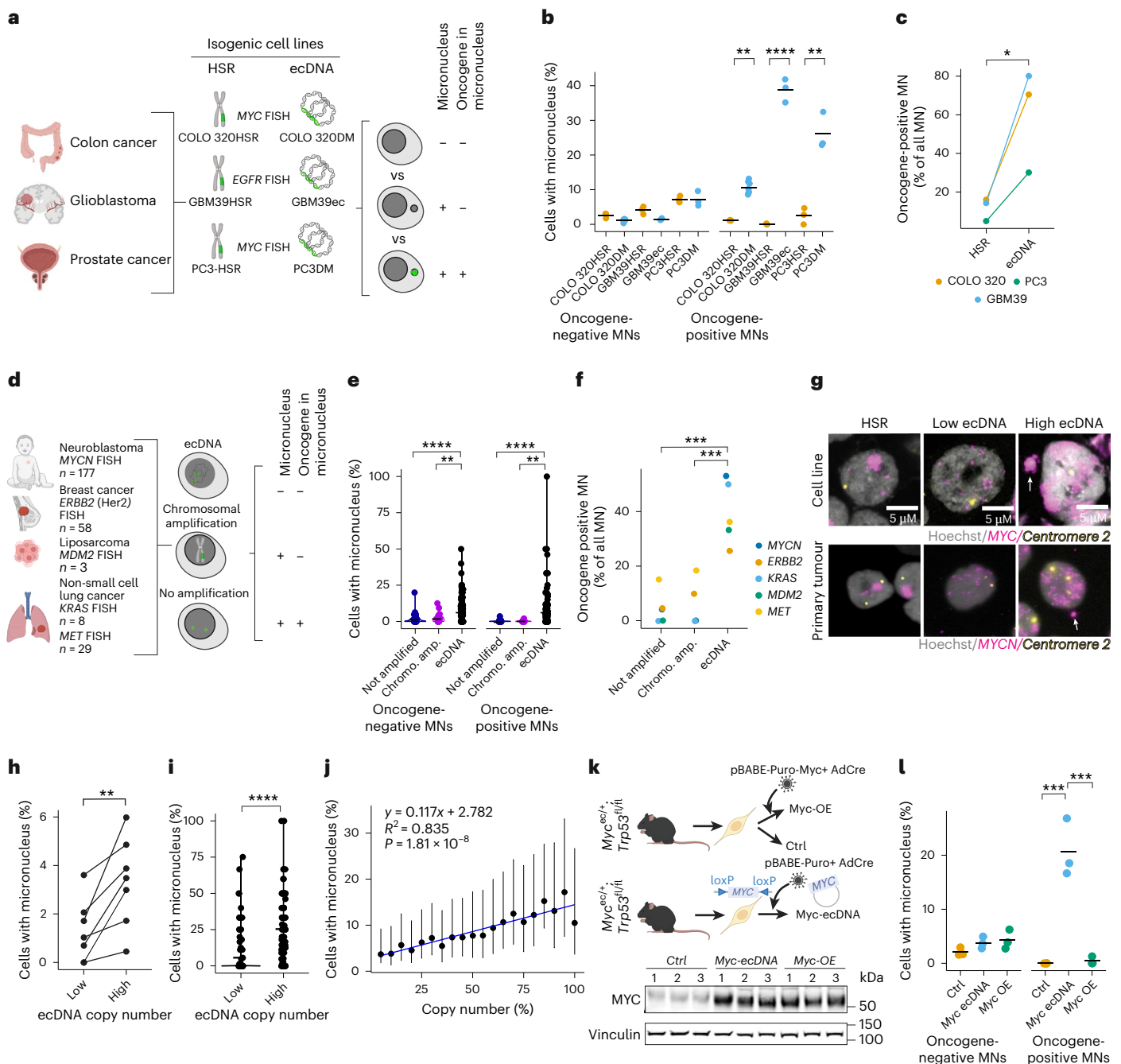
ecDNAs are frequently damaged and prone to replication stress^{29,32}, prompting us to test whether DNA damage can promote clustered detachment and micronucleation. Consistent with these prior findings, significantly higher signs of replication stress and DNA damage were observed on ecDNA compared to HSRs (Supplementary Fig. 3a–f). Treatment with HU, which induces replication stress and causes DNA damage by depleting nucleotides, increased signs of replication stress, especially on ecDNA (Supplementary Fig. 3a–f). Nanopore-based fork progression analysis confirmed reduced nucleotide incorporation and disproportional replication fork slowing on ecDNA, both in untreated conditions and upon HU treatment (Supplementary Fig. 4). This resulted in substantially more untethered, yH2AX⁺ ecDNA⁺ clusters during anaphase in COLO 320DM cells compared to their isogenic HSR counterparts as well as in non-isogenic cell line pairs (Fig. 2e–g and Supplementary Fig. 5). As a result, ecDNA⁺ cells exhibited increased MN frequency, and MNs were significantly enriched for ecDNA oncogene sequences (oncogene-positive MNs) to an extent exceeding expectations from random segregation (Supplementary Fig. 6a, b). The increase in MN abundance was rescued by transfection of nucleotides, confirming that HU-induced nucleotide depletion promoted MN formation (Supplementary Fig. 6c, d). No enrichment of MNs for oncogene

Fig. 1 | Cancer cells with ecDNA more frequently have MNs than cells with chromosomal amplifications. **a**, Schematic of the experimental design to quantify MNs in near-isogenic cancer cell lines. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **b**, Fraction of cells with oncogene-negative and oncogene-positive MNs in three near-isogenic cell line pairs ($n = 6$ cell lines), as determined using oncogene-specific FISH. Each dot represents one experimental replicate ($n = 7$ COLO 320DM, $n = 3$ other cell lines; experiments per cell line, $n = 37$ –837 cells per replicate; exact numbers in Supplementary Table 3). Two-sided Student's *t*-test; exact *P* values in Supplementary Table 3; ***P* < 0.01; *****P* < 0.0001. **c**, Fraction of MNs containing the amplified oncogene in near-isogenic cell lines ($n = 3$ isogenic cell line pairs, $n = 25$ –522 MNs per cell line; exact numbers in Supplementary Table 2). Each dot represents one cell line. **d**, Schematic of the experimental design to quantify MNs in patient biopsies. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **e**, Fraction of cells with oncogene-negative and oncogene-positive MNs in primary tumors with ecDNA, chromosomal amplification or without amplification ($n = 236$ tumors, $n = 5$ –574 cells per tumor sample; Kruskal–Wallis test; exact *P* values in Supplementary Table 3; ***P* < 0.01; *****P* < 0.0001, as determined using oncogene-specific FISH). Each dot represents one patient sample. **f**, Fraction of MNs containing the amplified oncogene ($n = 5$ for ecDNA and not amplified, $n = 4$ for chromosomally amplified) across patient cohorts stratified by oncogene (one-way ANOVA; exact *P* values in Supplementary Table 3; ****P* < 0.001). **g**, Exemplary micrographs of cell line and *MYCN*-amplified neuroblastoma tumor sample with an HSR (cell line: COLO 320HSR), low ecDNA content or high ecDNA content (cell lines: both COLO 320DM) and MNs (arrowheads) stained with Hoechst

(gray), *MYCN*-specific FISH (magenta) and chromosome 2 centromeric FISH (yellow). **h, i**, Fraction of micronucleated cells in the cells with low (bottom 30%, $n = 62$ –312 cells) and high (top 30%, $n = 67$ –323 cells; exact numbers in Supplementary Table 2) ecDNA content in cell lines ($n = 7$ cell lines, COLO 320DM, CHP-212, STA-NB10DM, Tr-14, UKF-NB6, Lan5, SMS-KAN; two-sided paired Student's *t*-test; exact *P* values in Supplementary Table 3; ***P* < 0.01) (**h**) and in primary tumors ($n = 148$, two-sided Student's *t*-test; exact *P* values in Supplementary Table 3; *****P* < 0.0001) (**i**). Each dot corresponds to one cell line (**h**) or one patient sample (**i**). **j**, Combined cell line and tumor cell fraction from **h** and **i** with MNs and their ecDNA copy number. For each dataset, overall ecDNA copy number as determined by FISH was partitioned into deciles, and micronucleation frequency was calculated within each bin ($n = 148$ tumors, $n = 7$ cell lines). Significance was tested using the two-sided one-sample proportion test. The blue line represents the linear regression fit; error bars represent the mean of the 95% confidence interval bounds calculated separately for each sample type. **k**, Experimental design to induce ecDNA in mouse aNS cells (top), and *MYC* immunoblot (bottom) in three independent *Myc* ecDNA-containing cells (*Myc*-ecDNA) and *Myc*-overexpressing (*Myc*-OE) cells compared to control cells (Ctrl) without ecDNA and not expressing *Myc*, respectively. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **l**, Fraction of cells with *Myc*-negative (oncogene-negative) and *Myc*-positive (oncogene-positive) MNs in *Myc*-ecDNA cells, *Myc*-OE or control aNS cells, as determined by *Myc*-specific FISH and Hoechst staining. Each dot represents one independent experiment ($n = 3$; one-way ANOVA; exact *P* values in Supplementary Table 3; ****P* < 0.001).

sequences was observed in cells with chromosomal amplifications, in which the overall MN rate increased following HU, but those MNs did not contain oncogene sequences (oncogene-negative MNs; Fig. 2h–j and Supplementary Fig. 6e,f). The frequency of MNs after HU treatment scaled with the abundance of ecDNA in cells (Supplementary Fig. 6g,h). As in untreated cells, despite cells harboring high numbers of the same ecDNA species, each daughter cell typically contained only a single MN (mean of 1.03 and 1.19 per cell for CHP-212 and COLO 320DM, respectively), suggesting that detached ecDNAs coalesce into a single cluster before segregation and micronucleation in one daughter cell. Targeted CRISPR–Cas9 cleavage of ecDNA reproduced this effect, producing untethered ecDNA clusters that segregated into MNs (Fig. 2k,l and Supplementary Fig. 7), suggesting that damage on ecDNA promotes clustered detachment and micronucleation. Thus, ecDNA is more prone than chromosomal amplifications to respond to replication stress with clustered detachment in mitosis, resulting in MN formation.

The MDC1–CIP2A–TOPBP1 complex stabilizes damaged chromosomal fragments during mitosis^{33,34}, raising the possibility that it mediates ecDNA clustering following their damage. Immunofluorescence staining revealed that a subset of detached clusters colocalized with CIP2A in unperturbed cells, which was increased following HU treatment (Fig. 2m and Supplementary Fig. 8a,b). Pharmacological reduction of CIP2A expression using TD19 dispersed Hoechst-stained clusters into individual foci in ecDNA⁺ cells and increased the number of small MNs per cell (Supplementary Fig. 8c–f). Similarly, short hairpin RNA (shRNA)-mediated knockdown of CIP2A, TOPBP1 or MDC1 reduces chromatin clustering during anaphase in the presence, but not in the absence, of HU, while not changing overall detachment frequency (Fig. 2n,o and Supplementary Fig. 8g,h). This indicates that following replication stress, the MDC1–CIP2A–TOPBP1 complex is at least in part required for the coalescence of damaged ecDNAs into clusters, similar to what was described for pulverized DNA during chromothripsis^{33,34},



which are then inherited as a single MN in daughter cells. Thus, replication stress promotes ecDNA detachment as clusters, which is in part enabled by MDC1–CIP2A–TOPBP1.

Distinct ecDNAs co-segregate into single MNs

Up to 25% of ecDNA⁺ tumors harbor more than one ecDNA species³⁰. Given that damaged ecDNAs cluster before the end of mitosis, individual MNs might contain oncogenes from distinct ecDNA species. At the same time, DNA damage could, in principle, generate chromosomal fragments that co-segregate into MNs together with attached ecDNA. To determine the genomic content of MNs in cancer cell lines with multiple ecDNA species, we developed a laser microdissection method to isolate single MNs and their corresponding primary nuclei, followed by paired-end sequencing (Fig. 3a). This approach was applied to TR-14 cells, which harbor multiple ecDNA species, each carrying a distinct oncogene (for example, *MYCN*, *MDM2* or *CDK4*) alongside additional passenger genes. The circular structure of these ecDNAs was previously assessed by sequence reconstruction²⁷, and their extrachromosomal presence, oncogene content and copy number per cell were confirmed by metaphase-FISH (Fig. 3b). After HU treatment to induce replication stress, 70% of MNs were strongly enriched in all ecDNA species (Fig. 3c and Supplementary Fig. 9a,b), consistent with collective micronucleation of separate ecDNAs. Notably, the majority of ecDNA⁺ MNs (92.3%) lacked detectable linear chromatin fragments, arguing against frequent co-segregation of chromosomal fragments together with ecDNA and indicating that ecDNA mis-segregates more readily than chromosomal DNA under replication stress (Fig. 3d,e and Supplementary Fig. 9c). Importantly, ecDNA was markedly enriched relative to linear chromosomal DNA in MNs compared with the corresponding primary nuclei, a pattern that would not be expected if ecDNA mis-segregated stochastically, in which case similar relative enrichments would be anticipated in

both compartments. Copy number proportions among ecDNA species within individual MNs mirrored those in the primary nucleus (Fig. 3f and Supplementary Fig. 9d), and enrichment of different ecDNA species correlated positively in MNs (Fig. 3g–j), in line with the collective micronucleation of structurally distinct ecDNAs.

To test whether damage to one ecDNA species can result in collective micronucleation of other ecDNA species, we introduced CRISPR–Cas9-mediated double-strand breaks on *MYC* ecDNA of DMS273 cells, which also harbor a separate ecDNA species encoding *MYCL* (Supplementary Fig. 10a). Targeted cleavage increased γ H2AX foci on *MYC* and promoted its presence in MNs, while concurrently enriching *MYCL* ecDNA in the same MN (Fig. 3k and Supplementary Fig. 10b–f).

Taken together, these findings suggest that ecDNAs mis-segregate into MNs en masse in cells with elevated ecDNA damage and are largely independent of linear chromatin fragments, raising the possibility that this impacts ecDNA inheritance patterns (Supplementary Fig. 11).

Micronucleation constrains ecDNA function

Live-cell imaging revealed that ecDNA clusters, both under basal conditions and following HU treatment, segregated on average into a single MN and one of the two daughter cells, suggesting that micronucleation introduces asymmetry into ecDNA inheritance (Supplementary Fig. 12a,b and Supplementary Videos 2 and 3). This suggests that ecDNA micronucleation skews previously observed random inheritance patterns¹¹, which would have implications for tumor evolution. To quantify this effect, we measured ecDNA copy number in paired daughter cells at cytokinesis using DNA FISH after HU treatment. Compared to untreated controls, HU treatment markedly increased the degree of ecDNA imbalance between daughter cells (Supplementary Fig. 12b), confirming that replication stress promotes asymmetric segregation through micronucleation.

Fig. 2 | Replication stress induces MDC1–CIP2A–TOPBP1-associated clustered detachment and micronucleation. **a**, Schematic of experimental design. Live-cell imaging of COLO 320DM-Tg19 cells with fluorescently tagged ecDNA was used to evaluate detachment and micronucleation of ecDNA. FISH combined with immunofluorescence (ImmunofISH) in fixed interphase and anaphase cells was used to measure replication stress, DNA damage and MDC1–CIP2A–TOPBP1 on ecDNA clusters in mitosis following genetic and pharmacologic perturbations. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **b**, Exemplary photomicrographs taken during live-cell imaging of a COLO 320DM-Tg19 cell engineered to visualize *MYC* ecDNA (TetO–TetR–mNeonGreen) during mitotic transition. Cell with ecDNA (green) within its primary nucleus (magenta) before mitosis (left), during anaphase (middle) and after mitosis (right). Dotted lines indicate MNs, which were confirmed to be outside the primary nucleus ($n = 3$). **c**, Exemplary micrographs of fixed, untreated CHP-212 cells that contain *MYCN* ecDNA during anaphase with (bottom) or without (top) detached ecDNA cluster, stained with Hoechst (gray) and *MYCN* DNA FISH (magenta). **d**, Number of ecDNA copies per cell in CHP-212 cells with detached ecDNA clusters during anaphase in untreated cells compared to those cells without such detached clusters. Each dot denotes one anaphase ($n = 52$; two-sided Student's *t*-test, exact *P* values in Supplementary Table 3; *****P* < 0.0001). Black line indicates the average in all plots. **e**, Exemplary photomicrographs of representative fixed COLO 320DM (contains *MYC* as ecDNA, bottom) and COLO 320HSR (contains *MYC* as HSR, top) cells in anaphase that were incubated in the presence or absence of HU (80 μ M) for 3 days and stained with Hoechst and for γ H2AX. **f**, Frequency of DNA clusters during anaphase as measured using Hoechst staining of fixed cells in cell lines with ecDNA ($n = 4$ independent cell lines) compared to cell lines with HSRs ($n = 2$ independent cell lines) in the presence (HU) or absence (UT) of hydroxyurea (80 μ M) ($n = 10$ –12 anaphases per cell line and treatment group; exact numbers in Supplementary Table 2). Each dot represents the average fraction of cells with a DNA cluster per cell line. **g**, Frequency of detached chromatin clusters positively staining for γ H2AX in cell lines containing ecDNA ($n = 4$ independent cell lines) compared to cells with HSRs ($n = 2$ independent cell lines) incubated in the presence or absence of hydroxyurea (80 μ M, 24 h) ($n = 10$ –12 anaphases per cell line and treatment group were assessed; exact numbers in Supplementary Table 2). Each dot represents

the average per cell line. **h**, Exemplary photomicrographs of representative fixed COLO 320DM containing *MYC* ecDNA and COLO 320HSR containing *MYC* HSR, stained with *MYC* FISH after 3 d of HU treatment (80 μ M). White arrows indicate MNs. **i**, Fraction of COLO 320DM and COLO 320HSR cells with oncogene-negative versus oncogene-positive MNs based on oncogene-specific FISH in the presence or absence of HU (50 μ M; $n = 20$ –26 MNs; two-way ANOVA; exact *P* values in Supplementary Table 3; *****P* < 0.0001). Each dot represents one image. **j**, Quantification of *MYC* FISH signal in MNs in the presence of HU (3 days, 50 μ M) in COLO 320DM cells compared to COLO 320HSR cells ($n = 100$ –923 MNs; two-sided Wilcoxon test; exact *P* values in Supplementary Table 3; *****P* < 0.0001). Each dot denotes one MN. A.U., arbitrary units. **k**, Exemplary photomicrographs of COLO 320DM cells after CRISPR–Cas9-mediated cutting of *MYC*, which is present on ecDNA in these cells, and stained with DAPI (gray), *MYC* FISH (green), γ H2AX immunofluorescence (red) as well as a merged image. **l**, Fraction of detached ecDNA in anaphase, measured using *MYC* FISH in COLO 320DM cells 24 h after nucleofection of Cas9 and a guide RNA targeting *MYC* (sg*MYC*, $n = 15$) compared to cells expressing a scrambled sgRNA control (NT, $n = 27$) (two-sided Student's *t*-test; exact *P* values in Supplementary Table 3; **P* < 0.05). **m**, Exemplary photomicrographs of a COLO 320DM cell with ecDNA in anaphase (top) as well as an enlarged view of the DNA cluster (bottom) stained with Hoechst (gray) and immunofluorescence for TOPBP1 (yellow) or CIP2A (magenta). **n**, Exemplary photomicrographs of COLO 320DM cells transduced to inducibly express an shRNA targeting CIP2A in the presence (bottom) or absence (top) of doxycycline (2 μ g ml^{−1}, 2 days) and in the presence (right) or absence (left) of HU (80 μ M, 24 h) in anaphase ($n = 1$, 12 anaphases imaged). Dotted circles represent outlines of small chromatin bodies representing ecDNA. **o**, Fraction of clustered chromatin as measured using Hoechst staining in cells harboring ecDNA and inducibly expressing shRNAs targeting *MDC1* (two independent sgRNAs, green), *TOPBP1* (one shRNA, blue) or *CIP2A* (three independent shRNAs, orange) in the presence or absence of either doxycycline (2 μ g ml^{−1}, 2 days) and/or HU (80 μ M, 24 h) in COLO 320DM cells ($n = 10$ –12 anaphases per condition; exact numbers in Supplementary Table 3; one-way ANOVA; exact *P* values in Supplementary Table 3; ***P* < 0.001). Each dot denotes the mean per anaphase; horizontal lines indicate the mean.

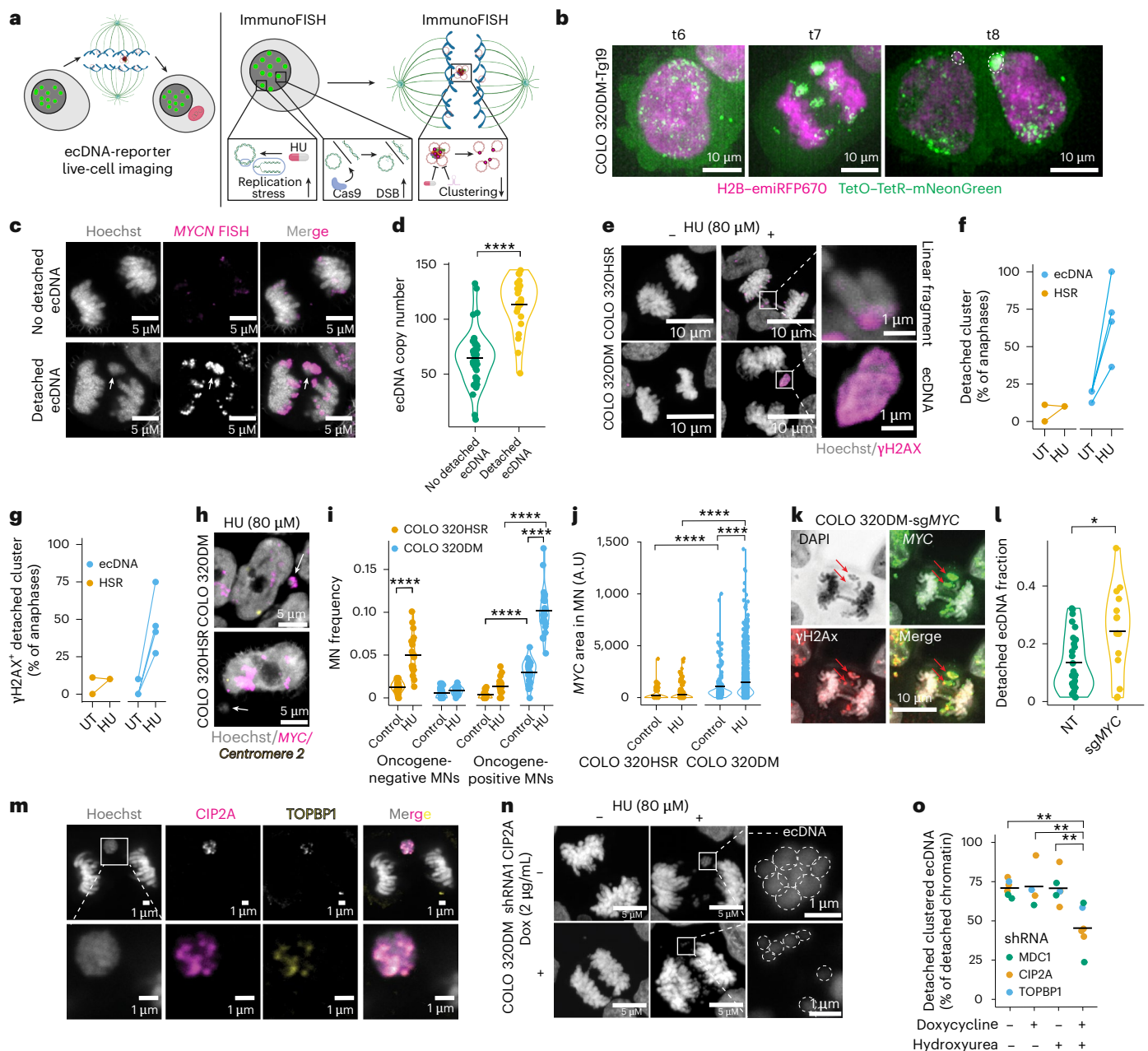
To further investigate the consequence of asymmetric segregation for the inheritance of ecDNA, we developed a computational model (Supplementary Fig. 12c). Assuming a probability q of asymmetric segregation and probability $1 - q$ of random segregation, the resulting ecDNA distribution in daughter cells shifted from unimodal (random segregation) to bimodal or trimodal patterns as q and p_1 varied (Supplementary Fig. 12d). Using an approximate Bayesian computation framework, we inferred optimal parameter estimates for p_1 and q (Supplementary Fig. 12e–i). Applying this procedure to our experimental data from pairs of daughter cells with and without HU treatment, we observed increased biased segregation post-treatment (Supplementary Fig. 12j–l). Therefore, replication stress enhances ecDNA segregation asymmetry (Supplementary Fig. 12).

Linear chromatin fragments incorporated in MNs remain epigenetically and transcriptionally silenced^{21–23}. We set out to test whether ecDNA is also repressed in MNs following replication stress (Fig. 4a). During mitosis, ecDNA retained high H3K27Ac and low H3K27me3, unlike chromosomal DNA, which showed the expected inactive chromatin patterns (Fig. 4b,c). However, ecDNA in MNs had significantly

reduced H3K27Ac and stably low H3K27me3 compared to ecDNA in mitosis and the primary nuclei following HU treatment (Fig. 4d,e). Chromatin immunoprecipitation with sequencing confirmed reduced H3K27ac at ecDNA loci, including *MYC* (Fig. 4f). MNs also exhibited significantly lower levels of pRNAPII-S5, indicating reduced transcriptional activity in MNs (Fig. 4g,h). Indeed, intron RNA FISH further confirmed that most MNs in ecDNA-containing cell lines lacked detectable oncogene expression, independent of the oncogene encoded (Fig. 4i). RNA sequencing confirmed the decreased expression of ecDNA-encoded genes, including *MYC*, alongside reduced *MYC*-driven transcriptional programs (Fig. 4j–l). Collectively, these results show that unlike its high expression in the nucleus and throughout mitosis^{30,31}, ecDNA within MNs is transcriptionally silenced, suggesting that micronucleation restrains the oncogenic potential of ecDNA.

ecDNA micronucleation constrains tumor cell fitness

To investigate the functional consequences of ecDNA⁺ MNs, we performed live-cell imaging under both untreated conditions and



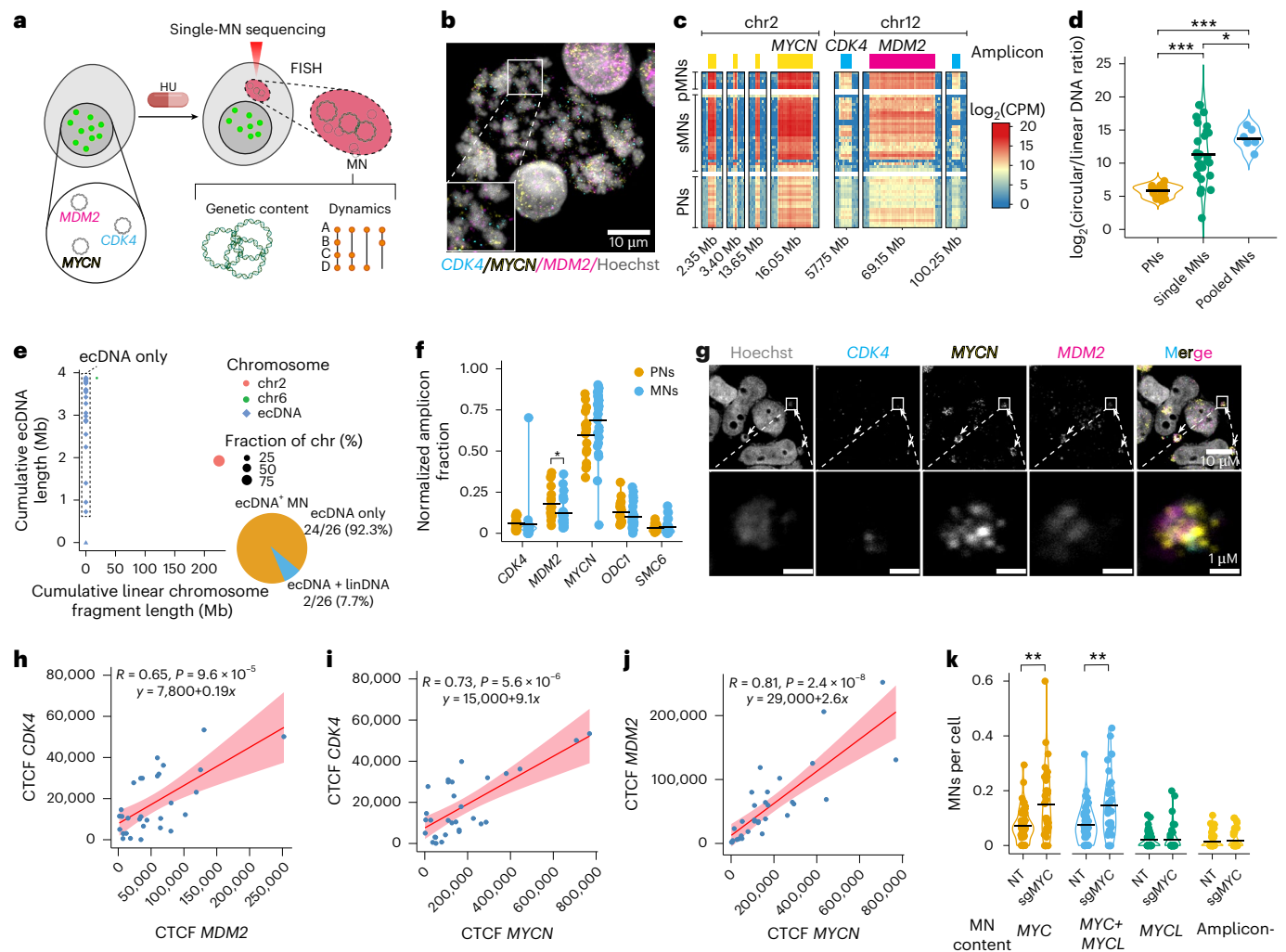


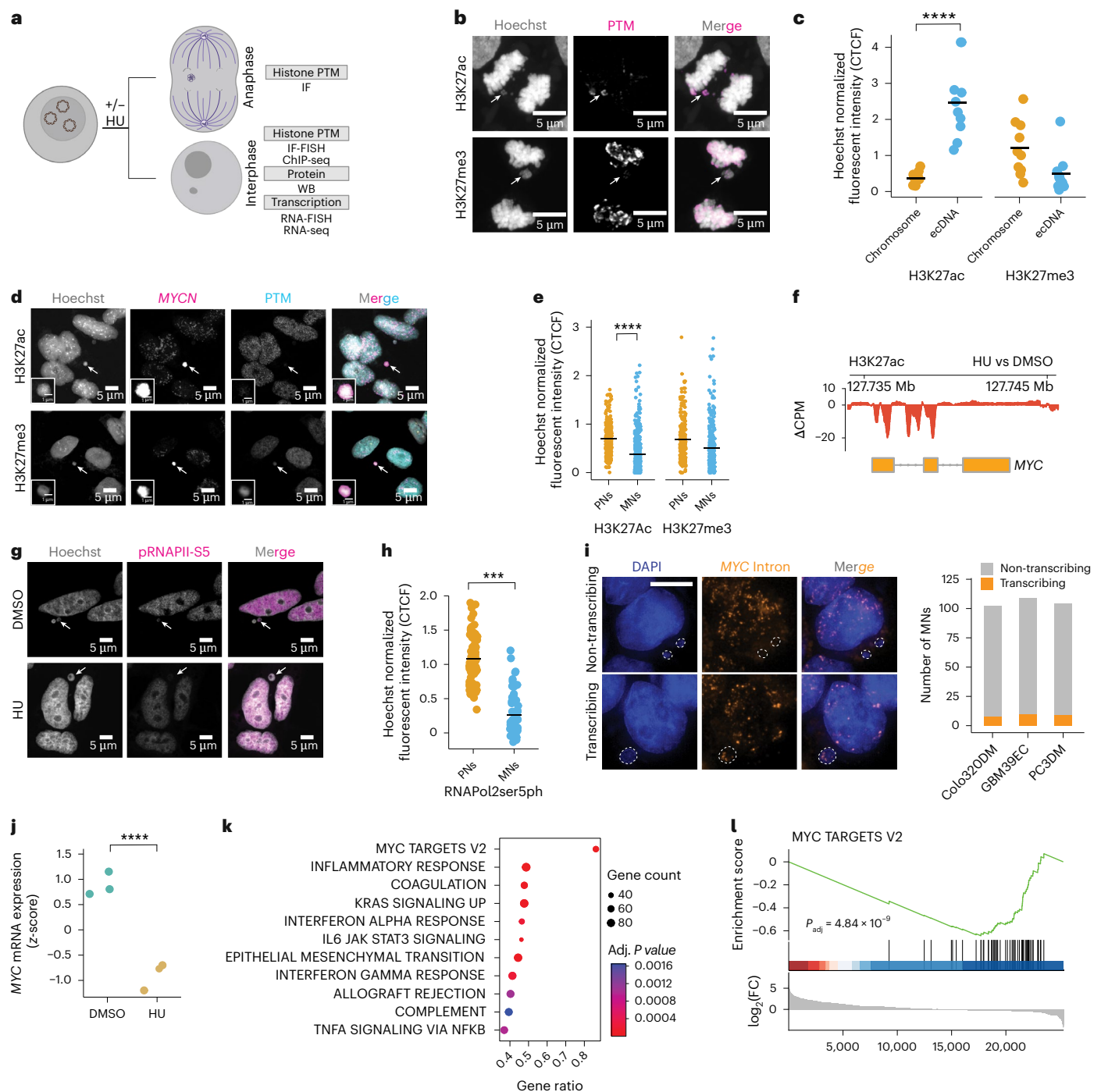
Fig. 3 | Collective entrapment of distinct ecDNAs in the same MN.

a, Experimental design. Laser microdissection (red triangle) was used to isolate MNs or primary nuclei, followed by sequencing. FISH was used to validate findings. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **b**, Exemplary photomicrograph of a metaphase spread of a TR-14 cell stained with Hoechst (gray) and FISH for oncogenes (*CDK4*, blue; *MYCN*, yellow; *MDM2*, red) present on distinct ecDNA species ($n = 1$, $n = 52$ cells with MN imaged). **c**, Laser microdissection-directed sequencing of primary nuclei (PNs) and MNs after incubation of TR-14 cells in the presence of HU (80 μ M). Counts per million (CPM), normalized and $\log_2$ -transformed read coverage (25 kb bins) across ecDNA (*CDK4*, blue, chr12:57,660,304–57,847,624, chr12:110,180,018–110,180,076; *MYCN*, yellow, chr2:2,292,204–2,403,180, chr2:3,389,951–3,420,333, chr2:13,647,219–13,712,870, chr2:15,812,368–16,321,546; *MDM2*, red, chr12:68,647,575–696,577,46) and their flanking regions (100 kb; PNs, $n = 18$; single MNs (sMNs), $n = 27$; pooled MNs (pMNs), $n = 6$). **d**, $\log_2$ -transformed mean coverage ratio of circular reads over the winsorized mean of all linear reads in PNs, sMNs and pMNs (two-sided Welch's t-test; exact P values in Supplementary Table 3; $*P < 0.05$; $***P < 0.001$). **e**, Cumulative ecDNA and linear chromosome length of enriched fragments in a single MN. Size of

linear chromosomal fragments (dots) denotes the fraction of the incorporated whole chromosome or chromosomal fragment. Bottom right: fraction of ecDNA-positive MNs containing either only ecDNA or ecDNA and linear chromatin. **f**, Amplicon-size normalized fraction of circular reads mapping to specific ecDNA species in primary nuclei ($n = 18$) and MNs ($n = 27$) (permutation test, 10,000 permutations; two-sided; *MDM2*- $P = 0.0326$; $*P < 0.05$). **g**, Exemplary photomicrograph of an MN in the TR-14 cell line harboring multiple ecDNAs, stained for *CDK4*, *MYCN* and *MDM2* using FISH. **h–j**, Correlation between normalized corrected total cell fluorescence (CTCF) from FISH in MNs for *CDK4* and *MDM2* (**h**), *CDK4* and *MYCN* (**i**) and *MDM2* and *MYCN* (**j**). Significance was tested using the two-sided one-sample proportion test. The line represents the linear regression fit, and the shaded region represents the 95% confidence interval around the fitted mean. **k**, Frequency of MNs per cell in DMS273 cells 24 h after expression of Cas9 and sgRNA targeting *MYC* (sg*MYC*) or a scrambled sgRNA (NT) and stained with DAPI and oncogene-specific FISH for *MYC* or *MYCL* to classify MNs according to their ecDNA content ($n = 38–48$; two-sided Wilcoxon test; exact P values in Supplementary Table 3; $**P < 0.01$). Each dot represents one image.

following HU exposure to track daughter cells inheriting an MN after mitosis (Fig. 5a). Cells that inherited large, ecDNA-containing MNs ($>2 \mu$ m in diameter) showed delayed progression to the next division and higher death rates than the daughter cell lacking an MN (Fig. 5b–c, Supplementary Fig. 13a,b and Supplementary Videos 4–6). These effects were independent of ecDNA copy number in the primary nucleus (Supplementary Fig. 13c,d) but were strongest when MNs contained high ecDNA content, indicating that ecDNAs in MNs impose a direct fitness penalty.

If this penalty constrains tumor growth, patients whose tumors contain abundant ecDNA⁺ MNs should have less aggressive disease. We examined diagnostic biopsies from 387 patients and selected 86 children with *MYCN*-amplified neuroblastoma in which ecDNA was identified by FISH before treatment (Fig. 5d and Supplementary Fig. 14a). For each case, we quantified the proportion and ecDNA content of MNs at baseline and compared these with clinical outcome after standard-of-care treatment. Neither total ecDNA copy number in the primary nucleus nor overall MN frequency correlated with survival



(Supplementary Fig. 14b–g). By contrast, tumors with a high fraction of ecDNA⁺ MNs exhibited significantly longer event-free and overall survival than tumors with few or no ecDNA⁺ MNs (Fig. 5e and Supplementary Fig. 14h). Modeled as a continuous variable and assuming linearity, the frequency of ecDNA⁺ MNs remained independently prognostic (overall survival: hazard ratio, 0.951; 95% CI, 0.914–0.990; P = 0.015; event-free survival: hazard ratio, 0.956; 95% CI, 0.923–0.990; P = 0.012), corresponding to a 4.9% reduction in risk of death for every 1% increase in ecDNA⁺ MNs. These findings indicate that an increase in MNs containing ecDNA is associated with impaired proliferative capacity of cancer cells and reduced tumor fitness (Fig. 5f).

Discussion

This study elucidates the role of ecDNA mis-segregation into MNs and its implications for oncogene regulation. Our results support the view

that faithful inheritance of ecDNA depends on chromosomal tethering during mitosis^{13,14,35}. When this process fails, oncogene-bearing ecDNAs can coalesce into clusters that segregate asymmetrically and are sequestered into MNs, a process that is potentiated by replication stress-induced damage. This asymmetric accumulation of ecDNA within MNs is associated with decreased oncogene output and impaired daughter cell fitness, and it corresponds to improved survival in children with *MYCN*-amplified neuroblastoma. Together, these findings link ecDNA⁺ MN formation to reduced tumor fitness, including at the patient level.

We observed that ecDNA content correlates with detachment and micronucleation (Fig. 1). This observation suggests that its acentric structure stochastically predisposes ecDNA to mis-segregation. This conclusion is in line with recent reports that identified molecular mechanisms controlling ecDNA segregation in mitosis, including

Fig. 4 | Reduced oncogenic transcription of ecDNA in MNs. **a**, Schematic of experimental setup. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). IF, immunofluorescence; ChIP-seq, chromatin immunoprecipitation with sequencing; WB, western blot. **b**, Exemplary photomicrographs of COLO 320DM cells with *MYC* ecDNA in anaphase stained using immunofluorescence against H3K27Ac or H3K27me3 (ecDNA is indicated with white arrowheads). **c**, Ratio of H3K27Ac and H3K27me3 intensity on COLO 320DM *MYC* ecDNA versus linear chromosomes ($n = 9$; two-way ANOVA; exact P values in Supplementary Table 3; **** $P < 0.0001$). Each dot represents one detached ecDNA or linear cluster in anaphase. **d**, Exemplary photomicrographs of Tr-14 cells with *MYCN* ecDNA stained using immunofluorescence against H3K27Ac or H3K27me3 (MNs are indicated with arrowheads). **e**, Hoechst-normalized H3K27Ac and H3K27me3 fluorescence intensity in MNs versus PNs, quantified by immunofluorescent staining in Tr-14 and CHP-212 ($n = 271$ –367, exact numbers in Supplementary Table 2; two-sided Student's t -test, exact P values in Supplementary Table 3; **** $P < 0.0001$). Each dot represents one PN or MN. **f**, Chromatin immunoprecipitation sequencing of H3K27ac in COLO 320DM cells. DMSO-subtracted read density (chr8:127,734,090–127,745,607 (hg38) shown; *MYC* gene annotated) as counts per million. **g**, Exemplary

photomicrographs of Tr-14 cells with *MYCN* ecDNA incubated with hydroxyurea (80 μ M) or DMSO (vehicle control) and stained using immunofluorescence against RNA polymerase II phosphorylation at serine 5 (MNs are indicated with arrowheads). **h**, Hoechst-normalized intensity in MNs versus PNs from Tr-14 cells stained using immunofluorescence against RNA polymerase II phosphorylation at serine 5 ($n = 66$). Each dot represents one PN or MN. **i**, Exemplary photomicrographs of COLO 320DM cells and their MNs (white dashed line) labeled with intron *MYC* RNA FISH signal (left), showing a non-transcribing MN (top) and an actively transcribing MN (bottom). Quantification of the number of transcribing and non-transcribing MNs in COLO 320DM ($n = 102$), GBM39EC ($n = 109$) and PC3DM ($n = 104$) cells (right). Scale bar, 10 μ m. **j**, z-score-normalized transcript counts of the circularly amplified *MYC* oncogene in COLO 320DM cells. Three replicates per treatment condition. DESeq2-derived Benjamini–Hochberg-corrected P value is shown (Wald test); $P = 1.08 \times 10^{-15}$; **** $P < 0.0001$. **k**, Significantly enriched MSigDB hallmark gene sets between HU-treated and DMSO-treated cells. Benjamini–Hochberg-corrected P values (permutation test) and gene counts are shown. **l**, Gene set enrichment analysis of *MYC* target genes. Benjamini–Hochberg-corrected P value is shown (permutation test). FC, fold change.

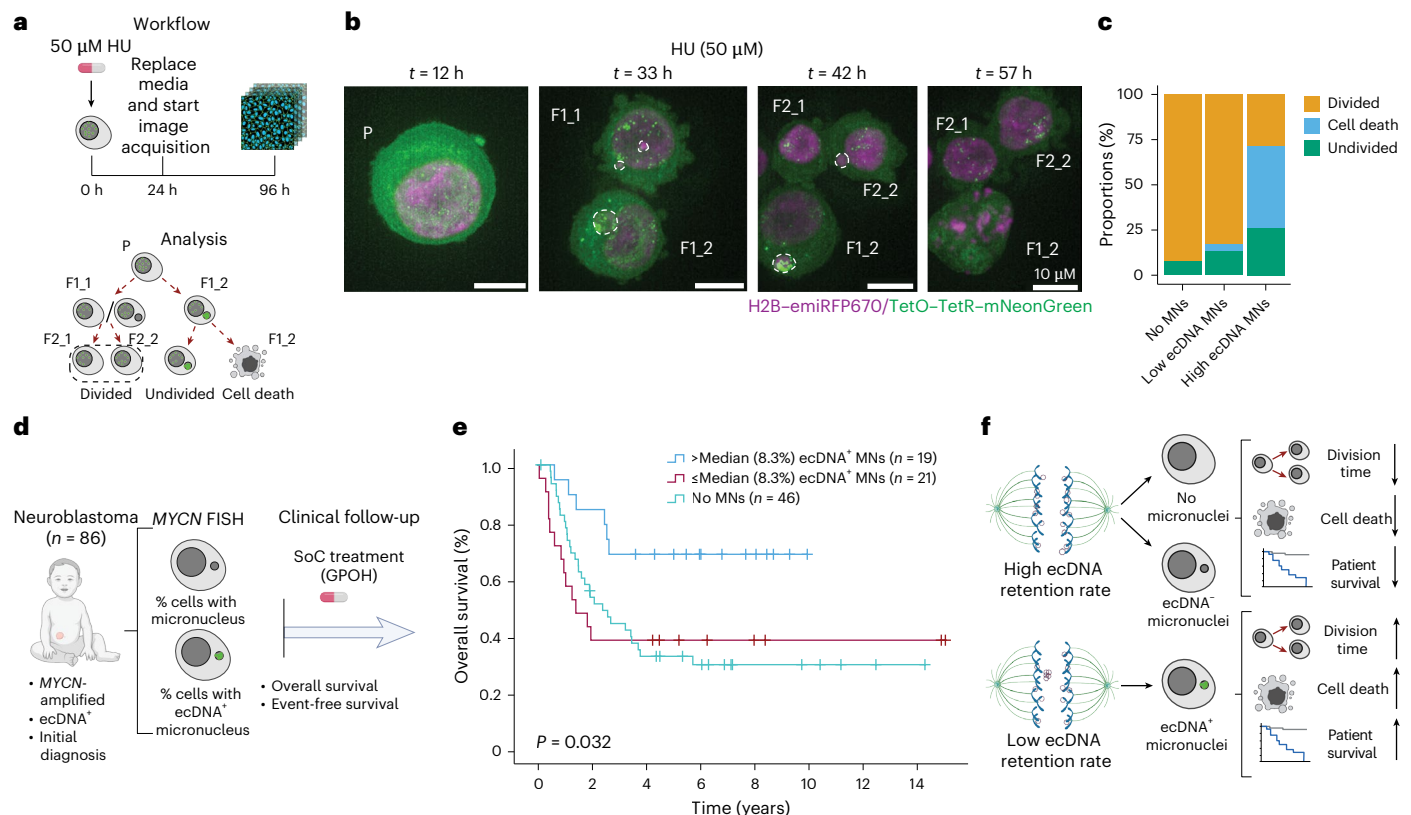


Fig. 5 | The presence of ecDNA in MNs is associated with reduced cancer cell fitness and improved patient outcome. **a**, Experimental design for live-cell imaging and analysis. Cells were tracked for two generations, from the parental cell (P) to generation F_1 (daughter cells $F_{1,1}$ and $F_{1,2}$) to generation F_2 (granddaughter cells $F_{2,1}$ and $F_{2,2}$). Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **b**, Representative frames from time-lapse imaging of a COLO 320DM-Tg19 parental cell (left) undergoing cell division twice (F_1 and F_2 generations) in the presence of HU (50 μ M), in which *MYC* ecDNA (green) and chromatin (magenta) are fluorescently labeled. MNs are marked with dotted lines and were confirmed in 3D reconstructions to be outside the nucleus. A cell with ecDNA-containing MNs ($F_{1,2}$) exhibits nuclear fragmentation at $t = 57$ h, indicative of cell death. **c**, Quantification of COLO 320DM-Tg19 cell fates as determined using live-cell imaging in the presence of HU (50 μ M) for cells without MNs, with MNs containing low ecDNA content

(below median; low ecDNA MNs) or with MNs containing high ecDNA content (above median; high ecDNA MNs). Cell fates include cell division, cell death or remaining undivided ($n = 143$ individual cells at the end of the 72 h imaging period). **d**, Schematic overview of the analysis of 86 *MYCN* ecDNA⁺ neuroblastoma biopsies, in which the presence of MNs and their content was determined using *MYCN* FISH and DAPI staining (registered in the trials NB97, $n = 2$; NB04, $n = 67$; and NB2016, $n = 17$). Median follow-up was 6.8 years (range, 0.14–15.9 years) for 36 survivors. SoC, standard of care. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026). **e**, Kaplan–Meier survival curves for patients with more than 8.3% cells (median) in their biopsy with ecDNA-containing MNs (ecDNA⁺ MNs), patients with less than 8.3% ecDNA⁺ MNs or those patients without MNs (no MNs; log-rank test). **f**, Summary schematic of the main findings. Figure partially created in BioRender; Xu, R. <https://BioRender.com/8k2z6r7> (2026).

chromatin acetylation readers³⁶, non-coding RNA-mediated tethering³¹ and genetically encoded retention elements¹⁴. Our findings further demonstrate that when ecDNA is damaged, its mis-segregation occurs en masse into the same MN and same daughter cell. This is enhanced after replication stress and, at least in part, mediated by the MDC1–CIP2A–TOPBP1 complex (Figs. 2 and 3). Therefore, our current understanding of the mechanisms governing mitotic ecDNA segregation points to DNA damage as one among other determinants of ecDNA fate.

Our study also uncovers a previously unrecognized link between ecDNA micronucleation and cancer cell fate, with direct implications for patient outcomes. We demonstrate that the ability of cancer cells to faithfully retain ecDNA during mitosis is a critical determinant of cellular fitness. Elevated rates of ecDNA micronucleation impair proliferative capacity and increase cell death, thereby attenuating cancer cell aggressiveness (Fig. 5). The observation that sequestration of oncogene-containing ecDNA into MNs is associated with improved event-free and overall survival in children with neuroblastoma highlights the functional relevance of ecDNA-containing MNs and suggests their potential utility as biomarkers for patient stratification and for therapeutic strategies aimed at selectively damaging ecDNA.

Even though the exact mechanism through which MNs affect cancer cell fates remains to be determined, we provide evidence for at least one potential contributor. We show that although ecDNA retains marks of active chromatin in mitosis³⁰, micronucleation triggers epigenetic remodeling, similar to what has been shown for linear chromosome fragments in MNs^{21,22}. ecDNA primarily loses H3K27ac in MNs while retaining mitotic H3K27me3 levels, potentially distinguishing ecDNA from linear acentric fragments. This process is accompanied by reduced oncogene transcription, effectively reducing the oncogenic output of ecDNAs (Fig. 4). As cells are addicted to high oncogene levels, such reduced oncogene output could explain the decreased proliferative capacity of cells with ecDNA in MNs. Even though ecDNA silencing may contribute to altered cancer cell fitness, it probably does not represent the only mechanism through which ecDNA⁺ MNs impact cell physiology.

In summary, our findings provide functional and clinical evidence that ecDNA segregation during mitosis is more than a curious cellular phenomenon but rather represents a biologically and clinically relevant process. By establishing ecDNA micronucleation as both a hallmark of ecDNA-driven cancers and a potential biomarker of patient outcome, our work opens avenues for its clinical translation. More broadly, the discovery that ecDNA micronucleation can shape tumor fitness highlights a vulnerability of ecDNA-bearing cancer cells and suggests that targeting ecDNA segregation could represent a tractable therapeutic strategy.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41588-026-02782-w>.

References

- Kim, H. et al. Mapping extrachromosomal DNA amplifications during cancer progression. *Nat. Genet.* **56**, 2447–2454 <https://doi.org/10.1038/s41588-024-01949-7> (2024).
- Yi, E., Chamorro Gonzalez, R., Henssen, A. G. & Verhaak, R. G. W. Extrachromosomal DNA amplifications in cancer. *Nat. Rev. Genet.* **23**, 760–771 <https://doi.org/10.1038/s41576-022-00521-5> (2022).
- Kim, H. et al. Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. *Nat. Genet.* **52**, 891–897 <https://doi.org/10.1038/s41588-020-0678-2> (2020).
- Hung, K. L. et al. Targeted profiling of human extrachromosomal DNA by CRISPR–CATCH. *Nat. Genet.* **54**, 1746–1754 <https://doi.org/10.1038/s41588-022-01190-0> (2022).
- Morton, A. R. et al. Functional enhancers shape extrachromosomal oncogene amplifications. *Cell* **179**, 1330–1341.e13 <https://doi.org/10.1016/j.cell.2019.10.039> (2019).
- Helmsauer, K. et al. Enhancer hijacking determines extrachromosomal circular MYCN amplicon architecture in neuroblastoma. *Nat. Commun.* **11**, 5823 <https://doi.org/10.1038/s41467-020-19452-y> (2020).
- Bailey, C. et al. Origins and impact of extrachromosomal DNA. *Nature* **635**, 193–200 <https://doi.org/10.1038/s41586-024-08107-3> (2024).
- Chamorro González, R. et al. Parallel sequencing of extrachromosomal circular DNAs and transcriptomes in single cancer cells. *Nat. Genet.* **55**, 880–890 <https://doi.org/10.1038/s41588-023-01386-y> (2023).
- Koche, R. P. et al. Extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma. *Nat. Genet.* **52**, 29–34 <https://doi.org/10.1038/s41588-019-0547-z> (2020).
- Wang, Y. et al. eccDNAs are apoptotic products with high innate immunostimulatory activity. *Nature* **599**, 308–314 <https://doi.org/10.1038/s41586-021-04009-w> (2021).
- Lange, J. T. et al. The evolutionary dynamics of extrachromosomal DNA in human cancers. *Nat. Genet.* **54**, 1527–1533 <https://doi.org/10.1038/s41588-022-01177-x> (2022).
- Levan, A. & Levan, G. Have double minutes functioning centromeres? *Hereditas* **88**, 81–92 <https://doi.org/10.1111/j.1601-5223.1978.tb01606.x> (1978).
- Kanda, T., Otter, M. & Wahl, G. M. Mitotic segregation of viral and cellular acentric extrachromosomal molecules by chromosome tethering. *J. Cell Sci.* **114**, 49–58 <https://doi.org/10.1242/jcs.114.1.49> (2001).
- Sankar, V. et al. Genetic elements promote retention of extrachromosomal DNA in cancer cells. *Nature* **649**, 152–160 <https://doi.org/10.1038/s41586-025-09764-8> (2026).
- Di Bona, M. & Bakhoum, S. F. Micronuclei and cancer. *Cancer Discov.* **14**, 214–226 <https://doi.org/10.1158/2159-8290.CD-23-1073> (2024).
- Terradas, M., Martin, M., Tusell, L. & Genesca, A. Genetic activities in micronuclei: is the DNA entrapped in micronuclei lost for the cell? *Mutat. Res.* **705**, 60–67 <https://doi.org/10.1016/j.mrrev.2010.03.004> (2010).
- Schenkel, L., Wang, X., Le, N., Burger, M. & Kroschewski, R. A dedicated cytoplasmic container collects extrachromosomal DNA away from the mammalian nucleus. *Mol. Biol. Cell* **34**, 105 <https://doi.org/10.1091/mbc.E23-04-0118> (2023).
- Ly, P. et al. Chromosome segregation errors generate a diverse spectrum of simple and complex genomic rearrangements. *Nat. Genet.* **51**, 705–715 <https://doi.org/10.1038/s41588-019-0360-8> (2019).
- Umbreit, N. T. et al. Mechanisms generating cancer genome complexity from a single cell division error. *Science* **368**, eaba0712 <https://doi.org/10.1126/science.aba0712> (2020).
- Zhang, C. Z. et al. Chromothripsis from DNA damage in micronuclei. *Nature* **522**, 179–184 <https://doi.org/10.1038/nature14493> (2015).
- Agustinus, A. S. et al. Epigenetic dysregulation from chromosomal transit in micronuclei. *Nature* **619**, 176–183 <https://doi.org/10.1038/s41586-023-06084-7> (2023).
- Papathanasiou, S. et al. Heritable transcriptional defects from aberrations of nuclear architecture. *Nature* **619**, 184–192 <https://doi.org/10.1038/s41586-023-06157-7> (2023).
- Hatch, E. M., Fischer, A. H., Deerinck, T. J. & Hetzer, M. W. Catastrophic nuclear envelope collapse in cancer cell micronuclei. *Cell* **154**, 47–60 <https://doi.org/10.1016/j.cell.2013.06.007> (2013).

24. Von Hoff, D. D. et al. Hydroxyurea accelerates loss of extra-chromosomally amplified genes from tumor cells. *Cancer Res.* **51**, 6273–6279 (1991).
25. Von Hoff, D. D. et al. Elimination of extrachromosomally amplified MYC genes from human tumor cells reduces their tumorigenicity. *Proc. Natl Acad. Sci. USA* **89**, 8165–8169 <https://doi.org/10.1073/pnas.89.17.8165> (1992).
26. Shimizu, N., Itoh, N., Utiyama, H. & Wahl, G. M. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. *J. Cell Biol.* **140**, 1307–1320 <https://doi.org/10.1083/jcb.140.6.1307> (1998).
27. Giurgiu, M. et al. Reconstructing extrachromosomal DNA structural heterogeneity from long-read sequencing data using Decoil. *Genome Res.* **34**, 1355–1364 <https://doi.org/10.1101/gr.279123.124> (2024).
28. Bei, Y. et al. Passenger gene coamplifications create collateral therapeutic vulnerabilities in cancer. *Cancer Discov.* **14**, 492–507 <https://doi.org/10.1158/2159-8290.Cd-23-1189> (2024).
29. Tang, J. et al. Enhancing transcription–replication conflict targets ecDNA-positive cancers. *Nature* **635**, 210–218 <https://doi.org/10.1038/s41586-024-07802-5> (2024).
30. Hung, K. L. et al. Coordinated inheritance of extrachromosomal DNAs in cancer cells. *Nature* **635**, 201–209 <https://doi.org/10.1038/s41586-024-07861-8> (2024).
31. Nichols, A. et al. Chromosomal tethering and mitotic transcription promote ecDNA nuclear inheritance. *Mol. Cell* **85**, 2839–2853.e8 <https://doi.org/10.1016/j.molcel.2025.06.013> (2025).
32. Jaworski, J. J. et al. ecDNA replication is disorganized and vulnerable to replication stress. *Nucleic Acids Res.* **53**, gkaf711 <https://doi.org/10.1093/nar/gkaf711> (2025).
33. Trivedi, P., Steele, C. D., Au, F. K. C., Alexandrov, L. B. & Cleveland, D. W. Mitotic tethering enables inheritance of shattered micronuclear chromosomes. *Nature* **618**, 1049–1056 <https://doi.org/10.1038/s41586-023-06216-z> (2023).
34. Lin, Y. F. et al. Mitotic clustering of pulverized chromosomes from micronuclei. *Nature* **618**, 1041–1048 <https://doi.org/10.1038/s41586-023-05974-0> (2023).
35. Tanaka, T. & Shimizu, N. Induced detachment of acentric chromatin from mitotic chromosomes leads to their cytoplasmic localization at G₁ and the micronucleation by lamin reorganization at S phase. *J. Cell Sci.* **113**, 697–707 <https://doi.org/10.1242/jcs.113.4.697> (2000).
36. Xie, Y. et al. Cis and trans regulatory mechanisms of extra-chromosomal DNA segregation. *Nat. Cell Biol.* **28**, 1453–1463 <https://doi.org/10.1038/s41556-026-01982-0> (2026).

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Methods

Patient samples and clinical data access

This study comprised the analysis of tumor and blood samples from patients diagnosed with neuroblastoma between 1991 and 2022. Patients were registered and treated according to the trial protocols of the German Society of Pediatric Oncology and Hematology (GPOH). Patients were registered in GPOH trials NB90 (ref. 37), NB97 (ref. 38), NB2004 (ClinicalTrials.gov identifier: [NCT00410631](#)) and NB2016-registry³⁹ (German Clinical Trials Register: [DRKS00023442](#)). All intervention studies (NB90, NB97, NB2004) have completed recruitment and the planned follow-up period for their primary endpoints. The NB2016-registry is an ongoing prospective neuroblastoma registry rather than an interventional clinical trial. All protocols were approved by the ethics committees of the Universities of Cologne as leading institutions, and approval was confirmed by the local ethics committees (application numbers 9764, 04-049, 16-432). Informed consent was obtained from the participants at the local treatment site and was a prerequisite to register in the respective trials. Trial participation was a requirement for inclusion in this analysis. This study was conducted in accordance with the World Medical Association Declaration of Helsinki (2013) and good clinical practice; informed written consent was obtained from all patients or their guardians. The collection and use of patient specimens and clinical data were approved by the institutional review boards of Charité–Universitätsmedizin Berlin and the Medical Faculty of the University of Cologne. Patients were enrolled in the German neuroblastoma clinical trials NB90, NB97 (ethics approval 9764; ClinicalTrials.gov identifier: [NCT00017225](#)), NB2004/NB2004-HR (ethics approval 04-049 for NB2004-HR; ClinicalTrials.gov identifier: [NCT00410631](#)) or the NB2016-registry (ethics approval 16-432; German Clinical Trials Register: [DRKS00023442](#)). Specimens and clinical data were archived and made available by Charité–Universitätsmedizin Berlin or the National Neuroblastoma Biobank and Neuroblastoma Trial Registry (University Children's Hospital Cologne) of the GPOH. The *MYCN* copy number was determined as a routine diagnostic method using FISH. DNA and total RNA were isolated from tumor samples with at least 60% tumor cell content as evaluated by a pathologist.

Cell culture and cell lines

Details on cell lines used and culturing practice can be found in the Supplementary Note.

Genetically induced ecDNA cell lines

The aNS cells were isolated from *Myc*^{ec/+}; *Trp53*^{fl/fl} and *Trp53*^{fl/fl} mice, as previously described⁴⁰ and cultured following a previously published protocol⁴¹. All animal experiments were approved by the Memorial Sloan Kettering Cancer Center Institutional Animal Care and Use Committee and adhered to institutional guidelines. Details can be found in the Supplementary Note.

Immunoblotting

Details on immunoblotting can be found in the Supplementary Note. Details on antibodies used and dilutions can be found in Supplementary Table 4.

Inducible shRNA knockdown plasmid cloning

The shRNA constructs were designed using the genetic perturbation platform of the Broad Institute and ordered from Eurofins. The constructs were cloned into pRSITEP-U6Tet-sh-EF1-TetRep-2A-Puro (Cellecta) following the manufacturer's instructions. We generated three independent shRNAs targeting *CIP2A* (1, TGCG-GCACTTGAGGTAATTT; 2, ATTTGTGACTTCGTACAATA; 3, GCTAGTAGACAGAGAACATAA), two targeting *TOPBP1* (1, GATAGATTTGGG-TAGTAATTT; 2, GCTGCAAAGAAGTGAATTTA) and one targeting *MDC1* (CGGACCAAACCTTAACCAAGAA). Successful insertion was confirmed by full plasmid sequencing (Plasmidsaurus).

Lentivirus production and cell transduction

Lentivirus production was carried out as previously described⁴². In brief, HEK293T cells were transfected with PEI MAX (Polysciences) in a 2:1:1 ratio of the lentiviral vector and psPAX2 and pMD2.G packaging plasmids (Addgene), according to the manufacturer's instructions. Viral supernatant was collected 48 h and 72 h after transfection. The supernatant was pooled, filtered and stored at −80 °C. Cells were transduced with virus particles in the presence of 5 µg ml^{−1} polybrene (Merck). Cells were transduced for 1 day in antibiotic-free medium and then grown in full medium for 1 day. Neuroblastoma cells were then selected for 2 days with puromycin hydrochloride (10 µg ml^{−1}) or geneticin disulphate (Roth, G418) (2 mg ml^{−1}). Plasmid expression was induced using 5 µg ml^{−1} doxycycline (Sigma-Aldrich, D9891) for 2 days. Western blot validation was performed as described above.

Targeted DNA damage induction by CRISPR–Cas9

To induce targeted DNA damage on amplicons, which we refer to as a DNA segment that has undergone focal, high-level amplification, in COLO 320DM/HSR and DMS273 cells, we used CRISPR–Cas9. The CRISPR RNA (crRNA) used in COLO 320DM/HSR cells was designed to target an intergenic region amplified in both cell lines (sequence: chr8:+127,572,615: TACAACGAATTTAACCG, TGG(pam)). The crRNA used to target *MYC*-containing ecDNA in DMS273 cells was GGAGA-GCTTGTGGACCGAGC. A non-targeting control was also included (IDT, 1072544). Equal amounts of crRNA and tracer RNA were mixed and annealed before mixing with Alt-R.S.p. Cas9 Nuclease V3 (IDT, 1081059) to make the RNP complex. The RNP complex was stabilized by incubating at room temperature (20–25 °C) for 15 min, after which it was delivered into cells using electroporation with the Nucleofector system from Lonza, using program CM-138 and solution SF (Lonza, V4XC-2012). After electroporation, cells were immediately seeded onto coverslips previously coated with fibronectin (10 µg ml^{−1}). Cells were fixed at the indicated time points and subjected to multiplex immunofluorescence and DNA FISH staining.

FISH

Metaphase spreads, or cells seeded on coverslips for interphase FISH, were fixed in methanol/acetic acid for 20 min at −20 °C, followed by a PBS wash for 5 min at room temperature. Details on metaphase spread preparation can be found in the Supplementary Note. The wells were removed, and slides were digested in pepsin solution (0.001 N HCl) with the addition of 10 µl pepsin (1 g per 50 ml) at 37 °C for 10 min. After a wash in 0.5× SSC for 5 min, slides were dehydrated by washing in 70%, 90% and 100% cold ethanol stored at −20 °C (3 min in each solution). Dried slides were stained with either 5 µl of Vysis LSIN-MYC Spectrum-Green/CEP 2 SpectrumOrange Probes (Abbott), ZytoLight Spec CDK4/CEN 12 Dual Color Probe (ZytoVision) or ZytoLight SPEC MDM2/CEN 12 Dual Color Probe (ZytoVision), ZytoLight SPEC MYC/CEN 8 Dual Color Probe (ZytoVision), covered with a coverslip and sealed with rubber cement. Denaturing was performed in a Thermobrite (Abbott) for 5 min at 72 °C, followed by incubation at 37 °C overnight. The slides were washed for 5 min at room temperature in 2× SSC/0.1% IGEAL, followed by 3 min at 60 °C in 0.4× SSC/0.3% IGEAL (Sigma-Aldrich) and further 3 min in 2× SSC/0.1% IGEAL at room temperature. Dried slides were stained with 12 µl Hoechst 33342 (10 µM, Thermo Fisher) for 10 min and washed with PBS for 5 min. After drying, a coverslip was mounted on the slide and sealed with nail polish. Images were taken using a Leica Stellaris Confocal microscope. For ecDNA copy number estimation, we counted foci using the Find Maxima function in FIJI. Nuclear boundaries were marked as regions of interest. The threshold for signal detection within the regions of interest was determined manually and used for all images analyzed within one group. Please see Supplementary Note for details on preparation and analysis of triple FISH staining.

Indirect Immunofluorescence

Cells were fixed in 4% paraformaldehyde (Electron Microscopy Science) prepared in PHEM buffer (Sigma-Aldrich) for 10 min at room temperature, with fresh fixative applied after the first 2 min. Fixed cells were washed three times in 125 mM glycine/PHEM for 5 min each, followed by permeabilization with 0.5% Triton X-100 (Sigma-Aldrich) in PHEM for 10 min at room temperature. After a single 5-min wash in PHEM, cells were blocked in 5% fetal calf serum (FCS, Sigma-Aldrich) containing 0.1% Tween-20 (Bio-Rad) in PHEM for 30 min at room temperature. Primary antibodies were diluted in 5% FCS/0.1% Tween-20/PHEM and applied to cells overnight at 4 °C. The following day, cells were washed three times in 0.1% Tween-20/PHEM for 10 min each and incubated with secondary antibodies diluted in 5% FCS/0.1% Tween-20/PHEM for 2 h at room temperature. Details on antibodies used and dilutions can be found in Supplementary Table 4. After incubation, cells were washed three additional times in 0.1% Tween-20/PHEM for 10 min each. Nuclei were counterstained with 5 μ M Hoechst 33342 (Thermo Fisher Scientific) in PHEM for 10 min, followed by three washes in PHEM for 5 min each. Cells were air-dried for 5 min and mounted onto SuperFrost glass slides (Eppendorf) using 5 μ l of ProLong Glass Antifade Mountant (Thermo Fisher Scientific). For double immunofluorescence, both primary and secondary antibodies were applied simultaneously.

Combined FISH and immunofluorescence

Details on combined FISH and immunofluorescence can be found in the Supplementary Note.

Live-cell imaging

Live-cell imaging presented in Supplementary Fig. 10a was performed as previously described³⁰. The stably engineered COLO 320DM cell line³⁰ was used to conduct live-cell imaging. Before cell seeding, 96-well glass-bottom plates were coated using poly-D-lysine. The medium was replenished with FluoroBrite DMEM (Gibco, A1896701), with 10% FBS, 1 $\times$ GlutaMAX and ProLong Live Antifade reagent, diluted at a 1:200 ratio (Invitrogen, P36975), 30 min before imaging. The 96-well plate was fitted onto a top-stage incubator (Okolab) and imaged on a Leica DMi8 widefield microscope using a $\times 63$ oil objective. Temperature (37 °C), humidity and CO₂ (5%) were stably maintained throughout the experiment.

MN-harboring cell fitness analysis by live-cell imaging

COLO 320DM-Tg19 cells were seeded in a coated well of a 96-well glass-bottom plate and treated with 50 μ M HU to enrich MN formation. After 24 h, HU was removed and replaced with FluoroBrite DMEM (Gibco, A1896701), with 10% FBS, 1 $\times$ GlutaMAX and ProLong Live Antifade reagent diluted at a 1:200 ratio (Invitrogen, P36975). Z-stack images were acquired every 3 h for a total of 60–72 h, and images were processed using Small Volume Computational Clearing. Maximum intensity projections were made on cropped frames of daughter cells immediately after initial division and for which at least one daughter cell contained an MN. The MN amplicon status (presence or absence of ecDNA⁺ or ecDNA⁻ MNs) and MN size (denoted as large if diameter is greater than 2 μ m) of each daughter cell were recorded. The fate of daughter cells was manually tracked and assigned to the following three categories: continued division, cell death or remaining undivided for the 72 h imaging period. If the daughter cell divided, the timepoint at which the next division occurred was recorded to quantify time to next division. Cropped maximum intensity projections were imported into Aivia (v.12.0.0). Outlines were manually drawn around primary nuclei and their corresponding MN. A machine learning-based pixel classifier and recipe were used to identify TetR–mNeonGreen foci, by which ecDNA copy number in the primary nuclei and MNs was quantified. The ecDNA status of MNs was classified as high or low relative to the median ecDNA content within an MN among all MNs.

Microscopy

Details on image acquisition and analysis can be found in the Supplementary Note.

Nanopore-based replication fork dynamics

Details on sample preparation and the analysis of nanopore-sequencing data can be found in the Supplementary Note.

Laser microdissection and whole-genome amplification of single (micro-)nuclei

TR-14 cells were directly grown on poly-D-lysine-coated (Gibco, A3890401) PEN-membrane metal frame slides (Leica, 11600289). Immunofluorescence was performed as described above. Slides were stored in 1 $\times$ PHEM in the dark at 4 °C until dissection. Single MNs, pooled MNs (5–10 MNs) and single primary nuclei were manually visualized and identified on a Leica microdissection microscope (LMD7 or LMD7000, Leica Microsystems). We collected primary nuclei and MNs from independent cells and primary nuclei and MNs from the same cell in a subset of samples (Supplementary Fig. 9d). Dissected membrane shapes were collected into adhesive PCR caps (MicroDissect, MDCA8W1K20). Whole-genome amplification was performed as in the ImmunoGAM method, with minor modifications^{43,44}. Sequencing libraries were prepared using the NEBNext Ultra II FS kit (New England Biolabs, E7805) according to the manufacturer's protocol with reduced reaction volumes (1/4). Samples were barcoded for Illumina sequencing using the NEBNext Multiplex Oligos for Illumina kit (New England Biolabs, E6444) and sequenced on a NextSeq2000 sequencer (Illumina) or NovaSeq X (Illumina) in 2 $\times$ 150 nt or 2 $\times$ 100 nt paired-end mode. Details on the analysis of single-MN sequencing data can be found in the Supplementary Note.

Chromatin immunoprecipitation with sequencing

Details on the preparation of chromatin immunoprecipitation-sequencing samples can be found in the Supplementary Note.

Reads were trimmed to remove low-quality bases and adaptor sequences using Trim Galore⁴⁵ (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^{46,47} (v.1.2.2).

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.

RNA sequencing

Details on the preparation of RNA-sequencing samples can be found in the Supplementary Note.

Reads were trimmed to remove low-quality bases and adaptor sequences using Trim Galore (v.0.6.10) and aligned to the human reference assembly GRCh38 with an added EBV contig (NC_007605.1) using STAR⁴⁸ (v.2.7.11b) with parameters `--twopassMode Basic --outSAMtype BAM SortedByCoordinate`. Reads were counted per gene against GENCODE gene annotation (Release 47) using htseq⁴⁹ (v.2.0.5) htseq-count, with parameters `-r pos -s no`. Counts were normalized for library size and composition using SizeFactors from DESeq2 (v.1.38.3)⁵⁰. Counts were z-score normalized for visualization using the scale function in R-base (v.4.2.2). 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 performed using the MSigDB

hallmark gene sets with the GSEA function in ClusterProfiler⁵¹ (v.4.6.2) and visualized with DOSE⁵² (v.3.24.2).

Modeling of ecDNA segregation patterns

Details on modeling can be found in the Supplementary Note.

Clinical data analysis

For the analysis, follow-up information was evaluated to match tumor samples with the respective patients. The endpoints were overall survival and event-free survival. Overall survival was defined as death from any cause, and event-free survival was defined as disease progression or relapse, or death from any cause. Patients without these events were censored at last follow-up. Overall survival and event-free survival were calculated using the Kaplan–Meier estimator⁵³. The log-rank test was used for comparison. A Cox proportional hazards regression was conducted for ecDNA⁺ MN percentage. Statistical analysis was conducted using SPSS 29. Despite availability, only clinical data from samples collected at first diagnosis before the start of therapy were included to eliminate a potential bias owing to therapy-related changes in tumor genetics.

Statistical analysis

All experiments were performed a minimum of two times with a minimum of three independent measurements, unless otherwise stated. All statistical analyses were performed with R (v.3.6 or v.4.0) or Python (v.3.7). All data are represented as mean $\pm$ s.e.m. and were tested for normal distribution. Statistical significance was assessed at $\alpha = 0.05$ and indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, unless otherwise stated. Normality was assessed using the Shapiro–Wilk test.

AI-assisted copy editing

All text was written by the authors. Parts of the text were copy edited using ChatGPT to improve readability and clarity.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Sequencing data generated for this study have been deposited in ArrayExpress under accessions E-MTAB-16933 (RNA sequencing), E-MTAB-16944 (chromatin immunoprecipitation sequencing) and the European Nucleotide Archive under accessions PRJEB112294 (single-MN sequencing) and PRJEB111610 (long-read sequencing). Previously published peak calls for CHP-212 H3K27ac ChIP-seq data from ref. 54 were downloaded from the Gene Expression Omnibus (GSE90683). Source data are provided with this paper.

Code availability

Scripts for data analysis within the scope of this publication are available at GitHub (https://github.com/henssen-lab/ecDNA_microncous) and Zenodo (<https://doi.org/10.5281/zenodo.21207200>)⁵⁵.

References

- 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 <https://doi.org/10.1186/1471-2407-11-21> (2011).
- 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 [https://doi.org/10.1016/S1470-2045\(05\)70291-6](https://doi.org/10.1016/S1470-2045(05)70291-6) (2005).
- Berthold, F., Spix, C., Kaatsch, P. & Lampert, F. Incidence, survival, and treatment of localized and metastatic neuroblastoma in Germany 1979–2015. *Paediatr. Drugs* **19**, 577–593 <https://doi.org/10.1007/s40272-017-0251-3> (2017).
- Pradella, D. et al. Engineered extrachromosomal oncogene amplifications promote tumorigenesis. *Nature* **637**, 955–964 <https://doi.org/10.1038/s41586-024-08318-8> (2025).
- Ahmed, A., Isaksen, T. J. & Yamashita, T. Protocol for mouse adult neural stem cell isolation and culture. *STAR Protoc.* **2**, 100522 <https://doi.org/10.1016/j.xpro.2021.100522> (2021).
- MacArthur, I. C. et al. Prohibitin promotes dedifferentiation and is a potential therapeutic target in neuroblastoma. *JCI Insight* **4**, e127130 <https://doi.org/10.1172/jci.insight.127130> (2019).
- Winick-Ng, W. et al. Cell-type specialization is encoded by specific chromatin topologies. *Nature* **599**, 684–691 <https://doi.org/10.1038/s41586-021-04081-2> (2021).
- Irastorza-Azcarate, I. et al. Extensive folding variability between homologous chromosomes in mammalian cells. *Mol. Syst. Biol.* **21**, 1 <https://doi.org/10.1038/s44320-025-00107-3> (2025).
- Krueger, F., James, F., Ewels, P., Afyounian, E. & Schuster-Boeckler, B. TrimGalore: v.0.6.10. Zenodo <https://zenodo.org/records/7598955> (2021).
- Kharchenko, P. V., Tolstorukov, M. Y. & Park, P. J. Design and analysis of ChIP-seq experiments for DNA-binding proteins. *Nat. Biotechnol.* **26**, 1351–1359 <https://doi.org/10.1038/nbt.1508> (2008).
- Landt, S. G. et al. ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. *Genome Res.* **22**, 1813–1831 <https://doi.org/10.1101/gr.136184.111> (2012).
- Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 <https://doi.org/10.1093/bioinformatics/bts635> (2013).
- Anders, S., Pyl, P. T. & Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**, 166–169 <https://doi.org/10.1093/bioinformatics/btu638> (2015).
- Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 <https://doi.org/10.1186/s13059-014-0550-8> (2014).
- Yu, G., Wang, L. G., Han, Y. & He, Q. Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* **16**, 284–287 <https://doi.org/10.1089/omi.2011.0118> (2012).
- Yu, G., Wang, L. G., Yan, G. R. & He, Q. Y. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics* **31**, 608–609 <https://doi.org/10.1093/bioinformatics/btu684> (2015).
- Kaplan, E. L. & Meier, P. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457–481 <https://doi.org/10.2307/2281868> (1958).
- Boeva, V. et al. Heterogeneity of neuroblastoma cell identity defined by transcriptional circuitries. *Nat. Genet.* **49**, 1408–1413 <https://doi.org/10.1038/ng.3921> (2017).
- Xu, R. henssen-lab/ecDNA_microncous: v.1.0. Zenodo <https://doi.org/10.5281/zenodo.21207200> (2026).

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Author contributions

L.B., R.X., J.T., A.G., A. Herrmann, I.T.W. and S.Z. contributed to study design, data collection and interpretation and paper writing. F.T., M.P., K.P., O.S., J.A., M.N.C., Q.Y., D.P., M.I., A.K., S.H., M.G.K., A.S.H., M. Fernandez, F.B., V.R.L., C.D., X.Y., D. Gamlin, S.K., D.G., M.R., N.W., G.M., N.W., R.S. and K.S. performed the experiments, analyzed data and reviewed the paper. L.B., R.X., J.T., A.G., A. Hermann, I.T.W. and S.Z. performed the experiments, analyzed and interpreted the data and edited the paper. D.T., A.L., F.D. and M. Fischer provided data. B.R.P., F.S. and A. Hamilton established essential methods. M.S. analyzed the clinical data. J.R.D., R.V., B.H., R.K.L., G.B., Y.L., V.B., G.W., R.M., S.P., B.S., A.V., A.P., R.P.K., W.H. and B.W. contributed to study design. A.G.H., P.S.M. and H.Y.C. led the study design, to which all authors contributed.

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Competing interests

A.G.H. and R.P.K. are founders and shareholders of Econic Biosciences. F.S., B.P., A. Hamilton and R.L. are employees of Econic Biosciences. P.S.M. is a co-founder of Boundless Bio. He has equity in the company and chairs the scientific advisory board, for which he is compensated. H.Y.C. is a co-founder of Accent Therapeutics, Boundless Bio, Cartography Biosciences and Orbital Therapeutics and was an advisor to 10x Genomics, Arsenal Biosciences, Chroma Medicine and Exai Bio until 15 December 2024. H.Y.C. is an employee and stockholder of Amgen as of 16 December 2024. G.B. is the founder and chief executive officer (part-time) of Function RX. A.P. holds a patent on 'Genome Architecture Mapping': Pombo, A., Edwards, P. A.W., Nicodemi, M., Scialdone, A. and Beagrie, R. A. Patent PCT/EP2015/079413 (2015). The other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Paul S. Mischel or Anton G. Henssen.

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Software and code

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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 Decoiler 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

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=AfmBOooiRXNwidcd4pnvAD9rLZEBQl0zjf4cNvdJS6Q9CS6VHjesdbH>
 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