



Original Articles

A human stem cell model of neuroblastoma identifies four chromosome 17q genes that drive tumor initiation and progression

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ABSTRACT

Neuroblastoma is a pediatric solid malignancy that primarily arises from poorly differentiated sympathoadrenal cells in the developing autonomic nervous system. The neuroblastoma genetic landscape is characterized by a paucity of somatic mutations and a far more common occurrence of chromosome copy number variations (CNVs). Two of the most common genetic abnormalities in neuroblastoma are the amplification of *MYCN* and the unbalanced gain of the long arm of chromosome 17 (17q+), both of which correlate with poor prognosis. Although mouse models exist for *MYCN*-driven neuroblastoma, no mouse models exist for 17q+. Thus, the mechanism by which 17q+ contributes to poor prognosis remains largely unknown. Here, we used a human pluripotent stem cell (PSC) model of neuroblastoma to show that 17q+ cooperates with *MYCN* amplification to accelerate tumorigenesis compared to *MYCN* amplification alone. We identified six genes on 17q that are upregulated in both our 17q+ PSC-derived tumors and 17q+ neuroblastoma patient tumors compared to WT 17q, correlate with poor prognosis in neuroblastoma patients, and are overexpressed at the protein level in our *MYCN*/17q+ tumors compared to *MYCN* alone. Of these six genes, knockdown of *BIRC5*, *CDC6*, *IGF2BP1*, or *TK1* in *MYCN*/17q+ cells increases tumor latency *in vivo* and contributes to tumor progression through diverse mechanisms. Furthermore, knockdown of *CDC6*, *IGF2BP1*, and *TK1* in neuroblastoma patient-derived xenografts with 17q+ slowed growth *in vivo*. Our findings provide new insights into potential candidate 17q drivers of neuroblastoma tumorigenesis and possible therapeutic vulnerabilities.

1. Introduction

Neuroblastoma is an embryonal neuroendocrine tumor originating in the sympathetic nervous system [1]. While neuroblastoma tumors can arise anywhere within the sympathetic nervous system, they most commonly occur in the adrenal medulla [2]. Since adrenal medullary cells are derived from sympathoadrenal cells, which are differentiated

from trunk neural crest cells (tNCCs), the cells of origin of neuroblastoma are believed to be poorly differentiated sympathoadrenal cells or tNCCs [3]. Although the overall survival of neuroblastoma has improved over the years, the five-year event-free survival of high-risk neuroblastoma patients remains around 50%, demonstrating an incredible need for novel therapies to improve outcomes [4].

Genomic analyses of neuroblastoma tumors have shown that

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recurring single-nucleotide variations are rare, with the most common being an anaplastic lymphoma kinase (ALK) mutation in approximately 10% of cases [5–7]. In contrast, *MYCN* amplification is more frequent, occurring in about 25% of neuroblastoma patients, including 40% of those with high-risk disease [8,9]. Initial mouse models of neuroblastoma were developed by misexpression of *MYCN* driven by the tyrosine hydroxylase (TH) promoter or the dopamine beta-hydroxylase (DBH) promoter [10,11], and ALK^{F1174L} driven by the dopamine beta-hydroxylase (DBH) promoter [12]. However, chromosome copy number variants (CNVs) are far more frequent in neuroblastoma [13,14], with the most common being the gain of chromosome 17q (17q+), which is strongly associated with worsened patient outcomes [15–17]. Recently, 17q + has been implicated as a pathognomonic marker of high-risk disease [18]. However, no mouse models have demonstrated whether 17q + plays a definitive role in tumorigenesis, in part due to the lack of alignment of genes between mouse and human chromosomes. To remedy this, we recently developed and optimized a human pluripotent stem cell (PSC) model of high-risk neuroblastoma [19]. Using this model, we show that 17q + can cooperate with amplification of the *MYCN* oncogene to accelerate neuroblastoma tumorigenesis *in vivo*. Furthermore, we identified four candidate genes of interest located on 17q (*BIRC5*, *CDC6*, *IGF2BP1*, and *TK1*) that are involved in tumor initiation in neuroblastoma, three of which (*CDC6*, *IGF2BP1*, and *TK1*) are also involved in tumor maintenance.

2. Materials and methods

2.1. Animals

Immunocompromised (NOD-scid IL2rgamma^{null} or NSG) 6–8-week-old female mice used for transplantation were purchased from Jackson Laboratories. Mice were maintained in the Animal Care Facility at CHLA in individually ventilated cages and subjected to 12-h light/12-h dark cycles. All experiments were performed in accordance with national guidelines and regulations and with the approval of the IACUC at CHLA. Analgesia was provided preoperatively via a subcutaneous injection of buprenorphine (Ethiqs XR, Fidelity Animal Health) at 3.25 mg/kg, and 0.25% bupivacaine hydrochloride (Hospira) was given at 7.5 mg/kg via a subcutaneous injection intraoperatively before the first incision. Mice were initially anesthetized with 3% isoflurane and 1.5 L O₂/minute and maintained at 2% isoflurane for the duration of the procedure through a nose cone. Mice were placed in right lateral recumbency. The left flank between fore- and hindlimb was shaved and sterilized, and a lateral incision of 15 mm was made in the abdomen to exteriorize the kidney.

For tumorigenesis experiments, 1e6 sympathoadrenal cells were resuspended in 100 µL of DPBS (–/–) (Thermo Fisher) and injected orthotopically into the left renal capsules of mice using 28G needles (Becton Dickinson). The kidney was returned to the abdominal cavity, and the incision was closed using coated Vicryl 4-0 sutures (Ethicon Surgical Technologies) for the peritoneum, and 9 mm AutoClips (Braintree Scientific) for the outer skin. Mice were recovered on a heating pad post-implantation until fully alert and ambulatory. Mice were fed Dox Diet (Bio-Serv) immediately after surgical recovery. Mice were euthanized at endpoint using CO₂ inhalation and cervical dislocation, which was either 2 cm in length of tumor, or 150 days post-implantation.

For patient cell line xenograft experiments, 1e6 tumor cells were resuspended in 100 µL of RPMI-1640 media (Thermo Fisher). Mice were fed with standard LabDiet 5061 (PMI Nutrition International) after surgical recovery. Mice were euthanized at endpoint, which was either 2 cm in length of tumor, or 300 days post-implantation.

2.2. ESC culture

Female H7 and H9 ESCs were obtained from WiCell Research Institute. The cell lines were authenticated by University of Arizona Genetics

Core by Short Tandem Repeat analysis in August 2024. Female H7 and H9 ESCs were maintained on GelTrex (Thermo Fisher) coated 6-well plates in StemFlex media (Thermo Fisher) in a humidified 37°C incubator with 5% CO₂. Cells were passaged every 4 days or when fully confluent using Accutase (Innovative Cell Technologies) and plated at approximately 30,000 cells/cm² in StemFlex with 2µM of ROCK inhibitor Thiazovivin (Stemcell Technologies), and changed to StemFlex without Thiazovivin the next day. All experiments using ESC in the Miller Huang lab were approved by Stem Cell Research Oversight and Institutional Review Board Committees at CHLA.

2.3. Patient cell line culture

COG-N-471nb and COG-N-623 PDX cell lines were obtained from the Children's Oncology Group (COG) Cell Culture and Xenograft Repository at Texas Tech University Health Sciences Center. COG-N-471nb cells were maintained in TC-treated 6-well or 10 cm plates in Neurobasal-A media (Thermo Fisher) supplemented with 50 ng/ml rhEGF, 50 ng/ml rhFGF2 (Peprotech), 2 mM L-glutamine (Thermo Fisher), 1X B27 without vitamin A (Thermo Fisher), and 1X N2 (Thermo Fisher). COG-N-623 cells were maintained in Iscove's Modified Dulbecco's Medium (Thermo Fisher) supplemented with 20% Fetal Bovine Serum (Thermo Fisher), 1% Penicillin/Streptomycin (Thermo Fisher), 4 mM L-glutamine (Thermo Fisher), 1X ITS (Corning) (5 µg/mL insulin, 5 µg/mL transferrin, 5 ng/mL selenous acid). Cells were passaged every 4–5 days using 0.02% Versene (Thermo Fisher) and plated at approximately 30,000 cells/cm².

2.4. Sympathoadrenal differentiation

Sympathoadrenal differentiation of ESCs was performed as described previously, see protocol 4¹⁹. For *in vivo* implantation studies, cells were dissociated using Accutase (Innovative Cell Technologies) on day 17. Live cells were counted in Trypan Blue (Lonza) using the TC20 automated cell counter (Bio-Rad) prior to implantation.

2.5. ESC-derived tumor cell line generation

Tumor tissue was extracted from mice at endpoint and placed in 1X PBS on ice. Tumor tissue was physically cut into small pieces with scalpels and then dissociated into single cells using Accutase at 37°C for 10 min. Cells were then plated in 6-well plates in 50% DMEM/F12 (Thermo Fisher), 50% Neurobasal-A media (Thermo Fisher), supplemented with 20 ng/ml rhEGF, 20 ng/ml rhFGF2 (Peprotech), 0.4% Penicillin/Streptomycin (Thermo Fisher), 1X Glutamax (Thermo Fisher), 1X N2 (Thermo Fisher), 1X B27 without vitamin A supplement (Thermo Fisher). Doxycycline (Sigma Aldrich) was added at 1 µg/mL to maintain expression of inducible *MYCN*. Media is changed every 2–3 days, and tumor cells were passaged using Accutase and plated at approximately 30,000 cells/cm².

2.6. Bioluminescence imaging

D-Luciferin Sodium Salt (Gold Biotechnology) was brought to room temperature and dissolved in DPBS (–/–) (Thermo Fisher) to a final concentration of 5 mg/mL and sterile-filtered. Intraperitoneal injections of 300 µL per mouse were administered. Approximately 10 min after substrate administration, the mice were anesthetized with 3% isoflurane and 1.5 L O₂/minute, and imaged with the Lago X 2023 SII (Spectral Instruments Imaging). The following parameters were used: binning = 4, exposure time = 180 s, f/stop = 2, field of view = 25 x 25 cm. Raw images were processed using Aura (Spectral Instruments Imaging).

2.7. Plasmids

The following plasmids are lentiviral constructs (pCDH backbone). The doxycycline-inducible *MYCN* plasmid was cloned as previously described, consisting of the following: UCOE-TRE3G-3xFLAG-MYCN-P2A-mScarlet and CAG-rta3G-P2A-Luciferase-T2A-Blasticidin [20]. Constitutive CRISPRi-mediated KD was achieved using dCas9-BFP-KRAB as previously described [21]. Constitutive CRISPRa-mediated OE was achieved using Cas9m4-VP64-BFP, which we cloned from Addgene plasmid #47319 into the pCDH backbone downstream of a CAG promoter. sgRNA primers (100 μ M) were annealed with T4 PNK (NEB) in T4 ligation buffer (NEB) in 10 μ L total volume using the following protocol: 37°C (30 min), 95°C (5 min), decrease 5°C per minute until 25°C. Annealed primers were then diluted 1:250 in water. 1 μ L of diluted primer was added to 100 ng of the backbone with 2 μ L of fast digest buffer (Thermo Fisher), 1 μ L *Bbs*I (NEB), 0.5 μ L T7 ligase (NEB), 0.5 mM DTT (Thermo Fisher), 0.5 mM ATP (NEB), total volume raised to 20 μ L in water. The ligation and digestion mixture was run for 6 cycles of a two-step process: 37°C (5 min) and 23°C (5 min). 2 μ L of ligation and digestion product was transformed into 20 μ L of Stbl3 competent cells (Thermo Fisher). Plasmid DNA was extracted using the Zippy Plasmid Miniprep Kit (Zymo Research) and sequence verified (Azena Life Sciences).

The doxycycline-inducible sgRNA plasmid was modified from pRDA-355 (Addgene plasmid #187159) to add a UCOE element [22] upstream of the H1 promoter. 5 μ g of the backbone was digested with 2.5 μ L *Bsm*BI v2 (NEB) in r3.1 buffer (NEB), total volume raised to 50 μ L in water. The reaction was incubated at 55°C for 4 h, and the digested backbone was gel-extracted. sgRNA primers (100 μ M) are annealed with r3.1 buffer (NEB) in 50 μ L total volume using the following protocol: 95°C (4 min), 70°C (10 min), decrease 5°C per minute until 25°C. Annealed primers were then diluted 1:100 in water. 1 μ L of diluted primer was added to 20 ng of the digested backbone with 1 μ L T7 ligase (NEB) in StickTo-gether buffer (NEB), total volume raised to 20 μ L in water, and incubated at 16°C for 3 h. 2 μ L of ligation and digestion product was transformed into 20 μ L of Stbl3 competent cells (Thermo Fisher). Plasmid DNA was extracted using the Zippy Plasmid Miniprep Kit (Zymo Research) and sequence verified (Azena Life Sciences).

For constitutive expression of sgRNAs, we cloned a human U6-sgRNA (from the PX330 plasmid [23]) upstream of CAGGS-hygromycin-P2A-BFP. Both the U6 and CAG portions of the plasmid are flanked by PiggyBac 5' and 3' inverted terminal repeats.

All sgRNA primers used in this study are presented in [Supplementary Table S1](#).

2.8. Lentiviral particle production and transduction

Lentiviruses were produced using HEK293T cells transfected at 80% confluent with the lentiviral expression vector and VSVG and PSPAX plasmids in Opti-MEM (Thermo Fisher), supplemented with TransIT-Lenti Transfection Reagent (Mirus Bio) according to the manufacturer's instructions. Lentivirus-containing medium was collected from the HEK293T cells 48 and 72 h after transfection, filtered using a 0.45- μ m filter (Whatman), and precipitated using Lentivirus Precipitation Solution (ALSTEM). Recipient cells were transduced and subjected to antibiotic selection 48 h after transduction.

2.9. Electroporation

500 ng of PiggyBac sgRNA expression plasmids and 500 ng of the Super PiggyBac Transposase expression vector (System Biosciences) were delivered to approximately 1e5 recipient cells via electroporation using the Neon NxT Electroporation System (Thermo Fisher) according to the manufacturer's instructions.

2.10. RT-qPCR

Total RNA was extracted from cells using the Quick-RNA MiniPrep kit (Cat# R1055, Zymo Research) and reverse transcribed with 4x iScript Reverse Transcription Supermix (Cat# 1708841, Bio-Rad) at a standard concentration of 500 ng RNA per 20 μ L sample. All qPCR primers used are presented in [Supplementary Table S2](#). All results were performed in triplicate; expression levels were detected via SsoAdvanced Universal SYBR Green Supermix (Cat# 1725274, Bio-Rad). The results are presented relative to the expression levels of ACTB.

2.11. Antibodies

Antibodies against Survivin (Cat# 10508-1-AP, RRID:AB_2064048), BRIP1 (Cat# 24436-1-AP, RRID:AB_2879548), CBX2 (Cat# 15579-1-AP, RRID:AB_2737362), CDC6 (Cat# 11640-1-AP, RRID:AB_2078519), IGF2BP1 (Cat# 22803-1-AP, RRID:AB_2879173), TK1 (Cat# 15691-1-AP, RRID:AB_2203613), and CSH2 (Cat# 16326-1-AP, RRID:AB_2083883) were purchased from Proteintech.

The antibody against KIF18B (Cat# A303-982A-T, RRID:AB_2620331) was purchased from Bethyl Laboratories.

Antibodies against EME1 (Cat# sc-53275, RRID:AB_2278026), and N-Myc (Cat# sc-53993, RRID:AB_831602) were purchased from Santa Cruz Biotechnology.

The antibody against cleaved PARP (Cat# 5625, RRID:AB_10699459) was purchased from Cell Signaling Technology.

Rhodamine-conjugated antibodies against GAPDH (Cat# 12004168, RRID:AB_2941791) and α -tubulin (Cat# 12004165, RRID:AB_2884950) were purchased from Bio-Rad.

All antibodies and dilutions used are presented in [Supplementary Table S3](#).

2.12. Western blotting

Cells were harvested under nondenaturing conditions and lysed using ice-cold RIPA buffer (Thermo Fisher) supplemented with 1X HALT protease and phosphatase inhibitor (Thermo Fisher). Samples were sonicated three times using the Model 50 Sonic Dismembrator (Thermo Fisher) at an amplitude setting of 15% for 3 s each and centrifuged at 13,000 rcf at 4°C for 15 min. Lysate supernatant concentrations were measured using the Pierce BCA kit (Thermo Fisher), and 15 μ g of protein sample was loaded per well. Western blotting was performed according to the manufacturer's protocol (Bio-Rad). Primary and secondary antibody incubations are performed as recommended, diluted in TBST (Bio-Rad). Membranes were developed using ECL (Bio-Rad).

2.13. Quantification of western blots

Western blot quantification was performed by ImageJ with the gel analysis tool. Lanes of band intensities were plotted as bell curves, and the areas under the curve were quantified. Band densities of the target proteins were normalized to their respective loading controls, and relative densities were normalized to the density of the target protein of the control cell line. Quantifications shown are the average of band densities in triplicate and standard deviations.

2.14. Cell proliferation assays

Cells were plated in triplicate in 12- or 24-well plates coated with GelTrex (Thermo Fisher). Cell proliferation was monitored and analyzed using the IncuCyte S3 live-cell analysis system (Sartorius) according to the manufacturer's instructions.

2.15. EdU incorporation assays

EdU incorporation assays were performed with the Click-iT™ EdU

Alexa Fluor™ 647 Flow Cytometry Assay Kit (Thermo Fisher) according to the manufacturer's instructions, and co-stained with DAPI (Thermo Fisher) at 1:20000 in DPBS (–/–) (Thermo Fisher) with 1% BSA (Sigma Aldrich).

2.16. LIVE/DEAD assays

LIVE/DEAD assays were performed with the LIVE/DEAD Fixable Far Red stain (Thermo Fisher) according to the manufacturer's instructions.

2.17. Annexin V staining assays

Annexin V Alexa Fluor™ 647 Conjugate (Thermo Fisher) staining was performed according to the manufacturer's instructions. SYTOX Green (Thermo Fisher) was co-stained at 1:20000.

2.18. Flow cytometry

Flow cytometry was performed with the FACS Canto II cytometer (BD Biosciences), and flow cytometry analysis was performed with the BD FACSDiva™ Software and FlowJo™ Software (BD Biosciences).

2.19. RNA sequencing sample preparation

Total RNA was extracted from 10 µL of flash-frozen tissue using the TissueLyser II (Qiagen) and AllPrep DNA/RNA Mini Kit (Qiagen). The quality of the total RNA samples was checked using an Agilent Bioanalyzer 2100 RNA Nano chip (Agilent) and then sent to Novogene (<https://en.novogene.com/>) for library preparation (polyA enrichment) and RNA sequencing (150 base pair paired-end reads, totaling 50 million reads).

2.20. RNA sequencing data analysis

Raw RNA sequencing reads were mapped to the human hg38 primary assembly reference genome using STAR (v2.7.2a) [24]. Differential Gene expression analysis was performed using the DESeq2 R package [25]. Gene Ontology analysis was performed using the clusterProfiler R package [26]. Differentially expressed genes (DEGs) were defined by meeting the following criteria: adjusted p-value (Benjamini–Hochberg false discovery rate) < 0.05 and absolute log2 fold change ($|\log_2FC|$) > 0.58496250072 (or 1.5-fold). The matrix of DEGs is presented in [Supplementary Table S4](#).

2.21. Patient RNA sequencing analysis

RNA sequencing (RNA-seq) data were obtained from two independent pediatric neuroblastoma cohorts: the Gabriella Miller Kids First Pediatric Research Program (GMKF) [27] and the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative [28]. For GMKF, gene-level expression quantifications were downloaded directly as RSEM-generated count matrices from the Gabriella Miller Kids First Data Resource Center. Corresponding clinical annotations were obtained from the same portal. For TARGET, harmonized open-access neuroblastoma RNA-seq data were obtained from the NCI TARGET initiative. Raw FASTQ files were processed to generate gene-level counts as described below. Raw FASTQ files from TARGET were processed using the nf-core RNA-seq pipeline (v3.14.0) [29] implemented in Nextflow [30]. Reads were aligned to the human hg38 reference genome using STAR (v2.7.11b) [24], and transcript quantification was performed with RSEM (v1.3.3) [31] to generate gene-level expected count matrices. In total, 169 TARGET RNA-seq samples were processed, of which 151 had matched CNV data available for downstream integrative analyses.

All downstream analyses were performed in R (v4.3.2). For both GMKF and TARGET cohorts, gene-level raw counts were imported into

edgeR (v3.40) [32]. Lowly expressed genes were filtered according to standard edgeR practices. Library sizes were normalized using the Trimmed Mean of M-values (TMM) method implemented in edgeR to account for compositional differences between samples. Exploratory data analysis was conducted using multidimensional scaling (MDS) plots generated from normalized expression values to assess sample relationships and detect potential outliers.

Differential gene expression analysis between 17q wild-type (17q WT) and 17q gain (17q+) patient groups was performed using the limma-voom framework (v3.66) [33]. Briefly, normalized count data were transformed using the voom method to estimate mean-variance relationships and generate precision weights. Linear models were fitted for each gene, followed by empirical Bayes moderation of standard errors using the eBayes function. Differentially expressed genes (DEGs) were defined by meeting the following criteria: adjusted p-value (Benjamini–Hochberg false discovery rate) < 0.05 and absolute log2 fold change ($|\log_2FC|$) > 1.

The matrix of DEGs for the TARGET database is presented in [Supplementary Table S5](#). The matrix of DEGs for the GMKF database is presented in [Supplementary Table S6](#).

2.22. Whole genome sequencing sample preparation

10 µL of flash-frozen tumor tissue was homogenized using the TissueLyser Adapter Set (Qiagen) in 2 ml microcentrifuge tubes with 5 mm stainless steel beads (Qiagen). gDNA was isolated from the tumor samples using the AllPrep DNA/RNA Mini Kit (Qiagen). Sequencing libraries were prepared from 1 ng of gDNA using the Nextera XT DNA Library Prep Kit (Illumina), and adapters were ligated using the DNA Unique Dual Index Kit (Illumina) according to the manufacturer's protocols. Library qualities were assessed on High Sensitivity DNA Bioanalyzer chips (Agilent Technologies). Libraries were pooled and sequencing (150 base pair paired-end reads) was performed using Novaseq X Plus (Illumina).

2.23. Low-coverage whole-genome sequencing analysis

Trimmomatic [34] was used for the adaptor removal and quality trimming of the raw reads. The clean reads were then mapped to the human hg38 reference genome using BWA, and Picard was used to mark the duplicated reads. The R package ACE [35] was used for the copy number and karyotyping analysis.

2.24. Public ChIP sequencing data analysis

SHEP21 MYCN and H3K27Ac ChIP RX data (sample IDs: GSM2113505, GSM2113507, GSM2113509, GSM2113511) were directly downloaded as bedGraph files from Gene Expression Omnibus (accession: GSE80154) [36], original data deposited by Zeid et al. [37] bedGraph files were converted to bigWig files and tracks were visualized using IGV.

2.25. Statistical analysis

For RT-qPCR and EdU incorporation assays, data bars represent the average of datapoints from at least 3 independent experiments. p-values were generated by two-tailed t-tests using GraphPad Prism. For the Incucyte assays, datapoints represent the average of at least 3 independent experiments ± standard error of mean. p-values were generated by two-tailed t-tests of the confluence values measured at the final timepoint using GraphPad Prism. For Kaplan Meier survival curves, p-values were calculated by the Log-rank (Mantel-Cox) test using GraphPad Prism. For all statistical analyses, a p-value less than 0.05 was interpreted as statistically significant.

3. Results

3.1. Chromosome 17q gain cooperates with MYCN to drive faster tumorigenesis than MYCN alone

To test whether 17q + promotes neuroblastoma tumorigenesis, we used a human pluripotent stem cell model that we previously optimized [19] (Fig. 1A). We obtained two human embryonic stem cell (ESC) lines (H7 & H9) that spontaneously acquired a gain of 17q and were clonally derived [38], with 17q breakpoints at q11 (H7) and q21.1 (H9), similar to breakpoints found in patients [39] (Fig. S1A). We first tested whether 17q + influences differentiation to candidate cells of origin for neuroblastoma, tNCC and sympathoadrenal cells. Both H7 and H9 ESC lines

with either the wild-type (WT) chromosome 17q or 17q+ were differentiated toward sympathoadrenal cells as previously described [19] and analyzed for expression of tNCC markers at day 10 and sympathoadrenal markers at day 17. While RT-qPCR showed significant differences in *TFAP2A* (higher in H7 and H9 17q + compared to 17q WT), *NGFR* (higher in H7 17q + compared to 17q WT), and *SOX10* (lower in H9 17q + compared to 17q WT), the majority of tNCC markers (*SOX9*, *FOXD3*, *SNAI2*, *HOXC8-9*) showed no significant differences between WT and 17q + cells at the tNCC stage (day 10) (Fig. 1B–S1B). Similarly, although RT-qPCR showed significant differences in *HAND2* and *TH* (lower in H9 17q + compared to 17q WT), the majority of sympathoadrenal markers (*PHOX2B*, *ASCL1*, *GATA3*, and *DBH*) showed no significant difference between WT and 17q + cells at the sympathoadrenal stage (day 17)

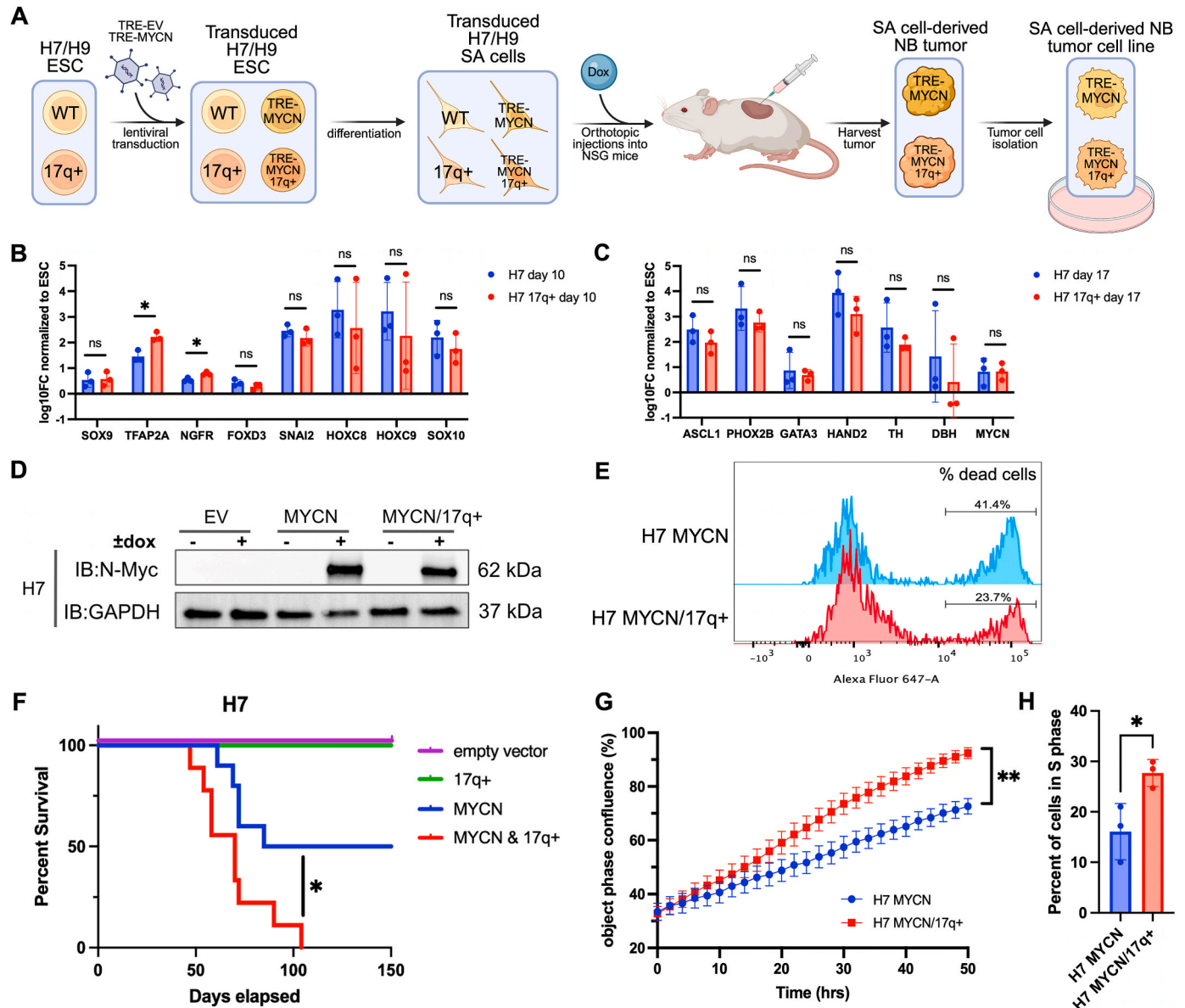


Fig. 1. Chromosome 17q gain cooperates with MYCN amplification to drive more aggressive tumorigenesis than MYCN alone. (A) Schematic of experimental design of neuroblastoma tumor generation. (B) Mean tNCC marker expression of H7 cells normalized to ESC at day 10 of differentiation (n = 3 for each cell line), *p < 0.05, unpaired t-test. (C) Mean sympathoadrenal marker and MYCN expression of H7 cells normalized to ESC at day 17 of differentiation (n = 3 for each cell line). (D) Western blot of N-Myc in representative empty vector (EV) ESCs ± doxycycline, and H7 and H7 17q + ESCs + doxycycline. (E) LIVE/DEAD assay flow cytometry histograms of H7 MYCN and MYCN/17q + cells at 24hr post doxycycline induction of MYCN expression at day 10 of differentiation. (F) Kaplan-Meier curves of tumor generation in H7. n = 10 per group. *p = 0.0112, Log-rank test. (G) Object phase confluence of H7 MYCN and H7 MYCN/17q + cells, imaged every 2 h for 48 h. Confluence calculated by IncuCyte, error bars = standard error of image for 16 images. **p = 0.0011, unpaired t-test. (H) EdU assay of H7 MYCN and H7 MYCN/17q + cells, n = 3. *p = 0.0314, unpaired t-test.

(Fig. 1C–S1C). *MYCN* levels also showed no significant difference between WT and 17q + cells (Fig. 1C–S1C). Thus, we concluded that 17q + did not have a significant impact on differentiation toward tNCCs or sympathoadrenal cells. Next, we transduced H7 and H9 ESC (both WT 17q and 17q+) with empty vector or doxycycline-inducible *MYCN* (Fig. 1D–S1D), and

differentiated them into sympathoadrenal cells. Interestingly, when *MYCN* expression was induced at day 10 of differentiation, *MYCN*/17q + cells tolerated the induction of *MYCN* overexpression with less cell death at 24 h post-induction compared to the *MYCN* cells (Fig. 1E–S1E), indicating that the presence of 17q + confers a survival advantage in the presence of increased replication stress and apoptotic signals upon

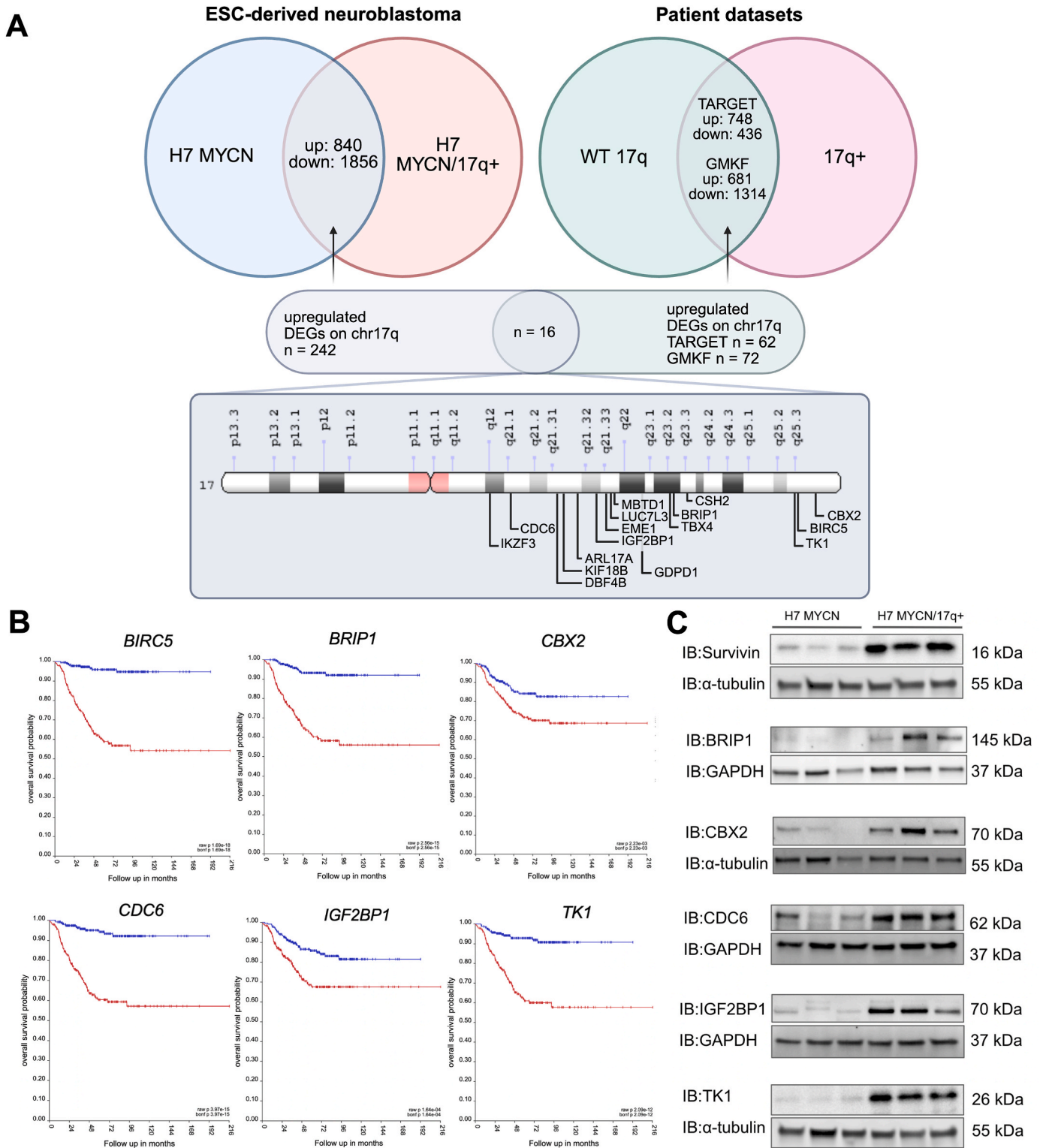


Fig. 2. 17q-encoded *BIRC5*, *BRIP1*, *CBX2*, *CDC6*, *IGF2BP1* and *TK1* are upregulated in *MYCN*/17q + tumors and in patients, and correlate with poor survival. (A) Schematic of DEG filtering for upregulated 17q candidate genes. (B) Patient Kaplan-Meier curves by expression of 17q candidate genes, Kocak Cohort (n = 476), R2 database. (C) Western blot of final candidate gene expression in H7 tumors.

MYCN induction [40].

Each sympathoadrenal cell line was orthotopically injected into the renal capsule of NSG mice at day 17 of differentiation. Although 17q + alone was insufficient to generate neuroblastoma tumors by day 150 post-implantation, *MYCN*/17q + tumors, compared to *MYCN* tumors, exhibited higher penetrance (H7: 100% vs 60%; H9: 100% vs 50% at day 104 post-implantation) and shorter latency (median survival H7: 70 vs 90 days, $p = 0.0186$; H9: 69 vs 114 days, $p = 0.0001$, $n = 10$ per arm), suggesting that *MYCN* and 17q + cooperate in tumorigenesis (Fig. 1F–S1F). In support of this, *MYCN*/17q + tumor cells displayed significantly faster cell proliferation than *MYCN* tumor cells *in vitro* by both IncuCyte analysis (Fig. 1G–S1G) and EdU incorporation assays (Fig. 1H–S1H).

Next, we examined whether *MYCN* or *MYCN*/17q + tumors acquired additional chromosome CNVs *in vivo*. Genomic DNA from both sets of tumors was analyzed by low-coverage whole-genome sequencing and revealed that no additional chromosome CNVs occurred during transformation, allowing us to investigate the effect of 17q+ in isolation (Fig. S1I). Additionally, to confirm whether these tumors resemble neuroblastoma, we performed bulk RNA sequencing on H7 *MYCN* and *MYCN*/17q + tumors. Compared to transcriptomes of various embryonal and neural crest-derived tumors, the tumors we generated most closely resembled neuroblastoma (Fig. S1J). Taken together, these data demonstrate that while 17q + does not have a significant impact on differentiation, 17q + accelerates tumorigenesis and increases tumor cell proliferation, confirming its significance in neuroblastoma.

3.2. Identification of genes on chromosome 17q that are upregulated in 17q + tumors and correlate with poor prognosis

We next sought to determine which genes on chromosome 17q are functionally relevant in tumorigenesis by first identifying genes that are upregulated in 17q + tumors. Using RNA sequencing data from our H7 *MYCN* and *MYCN*/17q + tumors, we observed striking transcriptome-level differences, with 840 upregulated and 1856 downregulated differentially expressed genes (DEGs) (Fig. 2A, S2A–B, Table S4). Notably, chromosome 17 was the only chromosome with more upregulated genes than downregulated genes in *MYCN*/17q + tumors compared to *MYCN* alone tumors, with a total of 242 DEGs upregulated on 17q (Fig. 2A, Fig. S2C). GO analysis showed an enrichment in DNA replication and ribosome biogenesis in *MYCN*/17q + tumors, indicating that 17q + results in a more metabolically active and replication-addicted phenotype to support the *in vivo* phenotype of faster growth of these tumors (Fig. S2D).

To establish patient relevance, we analyzed neuroblastoma patient tumor sequencing data from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) [28] and the Gabriella Miller Kids First (GMKF) [27] databases, and grouped the samples based on their 17q status (Fig. S2E). GO analysis of patient DEGs reveals similarities in biological processes to our stem cell model, such as an enrichment in regulation of G2/M phase transition and sister chromatid separation, which confirms the phenotype of replication addiction in 17q + tumors (Fig. S2F). By comparing patient tumors that are WT 17q against 17q + tumors, we found 62 upregulated DEGs located on 17q from the TARGET dataset, and 72 upregulated DEGs located on 17q from the GMKF dataset (Tables S5–6). After pooling all sets of DEGs and filtering for overlap, we found a final list of 16 unique protein-coding genes located on 17q that are upregulated in both our ESC-derived 17q + tumors and 17q + neuroblastoma patient tumors (Fig. 2A–S3A–C). Next, we determined which of these 16 genes, when upregulated, correlate with poor prognosis in neuroblastoma patients using the Kocak patient cohort ($n = 649$) in the R2 database (<https://r2.amc.nl/>) (Fig. 2B). We also checked which genes were overexpressed in *MYCN*/17q + tumors compared to *MYCN* tumors at the RNA and protein levels (Fig. 2C–S3D–G). Overall, six genes were confirmed to be overexpressed in 17q + tumors, both in our model and in neuroblastoma

patients, and correlate with worse prognosis: *BIRC5*, *BRIP1*, *CBX2*, *CDC6*, *IGF2BP1*, and *TK1*.

3.3. *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1* affect tumorigenesis and tumor latency *in vivo*

To determine whether the six candidate genes (*BIRC5*, *BRIP1*, *CBX2*, *CDC6*, *IGF2BP1*, and *TK1*) had functional roles in tumorigenesis *in vivo*, we transduced H7 *MYCN*/17q + ESCs with plasmids encoding dCas9-BFP-KRAB to enable CRISPR interference (CRISPRi) [41], and doxycycline-inducible sgRNAs (either control sgRNA or sgRNA that targeted each of the six candidate genes individually). The presence of the knockdowns (KD) was validated at the ESC level (Fig. S4A–B). These ESCs were then differentiated toward sympathoadrenal cells, during which we confirmed the presence of the knockdowns (KD) on the protein level at the differentiation endpoint (day 17) (Fig. S4C). We then orthotopically implanted these cells into NSG mice and fed the mice doxycycline chow to induce KD of the candidate gene (Fig. 3A, S4D). Bioluminescence imaging (BLI) at day 45 post-implantation revealed a significant reduction of signal in the mice carrying tumors derived from the *MYCN*/17q + sympathoadrenal cells with candidate sgRNA compared to the control sgRNA, indicating slower tumorigenesis and tumor growth (Fig. 3B–C). While all mice developed tumors, *MYCN*/17q + sympathoadrenal cells with KD of *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1* showed significantly longer latency of tumor formation (median survival *IGF2BP1*: 63 days; *CDC6*: 62 days; *TK1*: 59.5 days; *BIRC5*: 66 days) compared to control sgRNA (median survival: 45 days, $n = 10$ per arm), suggesting these genes individually contribute to tumorigenesis (Fig. 3D). KD of *IGF2BP1*, *CDC6*, *TK1*, and *BIRC5* revealed a significant effect in prolonging tumor latency *in vivo*, with Log-rank p -values of 0.013, 0.0132, 0.0142, and 0.0332, respectively. Therefore, our data suggest that *IGF2BP1*, *CDC6*, *TK1*, and *BIRC5* contribute to tumorigenesis in 17q + tumors.

3.4. *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1* contribute to neuroblastoma tumor cell growth by influencing cell cycle progression, survival, and *MYCN* expression

Previous studies have shown *BIRC5* (member of chromosome passenger complex), *CDC6* (member of pre-replication complex), and *TK1* (thymidine kinase) directly drive the cell cycle, and *IGF2BP1* indirectly influences the cell cycle by influencing *MYCN* expression [42–45], and we wanted to evaluate whether these 17q genes have similar functions in *MYCN*/17q + tumors. To do this, we selected CRISPRi sgRNA sequences [41] that reduced the protein level of each candidate gene in the H7 *MYCN*/17q + tumor cells to the levels found in H7 *MYCN* cells. Conversely, we chose CRISPR activation (CRISPRa) sgRNA sequences [41] that increased protein expression of the target gene in the *MYCN* cells at levels most similar to expression in *MYCN*/17q + cells (Fig. S5A). We first performed CRISPRi-mediated KD of these genes in H7 *MYCN*/17q + tumor cells and validated the KD by both RT-qPCR and Western blot (Figs. S5B and 4A). Decreased levels of *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1* in *MYCN*/17q + tumors each impaired *in vitro* tumor cell proliferation (Fig. 4B) and significantly decreased S-phase occupancy compared to control sgRNA (Fig. 4C). Conversely, we used CRISPRa to overexpress (OE) the same candidate genes in H7 *MYCN* tumor cells (WT 17q) (Figs. S5C and 4D). OE of *BIRC5*, *CDC6*, *IGF2BP1*, or *TK1* resulted in increased cell proliferation and S-phase occupancy in the H7 *MYCN* cells (Fig. 4E–F). These results confirm that these genes not only contribute to accelerating tumor transformation *in vivo*, but also directly contribute to maintaining the growth of the tumor cells after transformation.

Next, we investigated whether N-Myc levels would change due to modulation of *IGF2BP1* levels, as it was previously reported that *IGF2BP1* protein influences *MYCN* expression via stabilization of the *MYCN* mRNA [45]. Indeed, KD of *IGF2BP1* in H7 *MYCN*/17q + cells

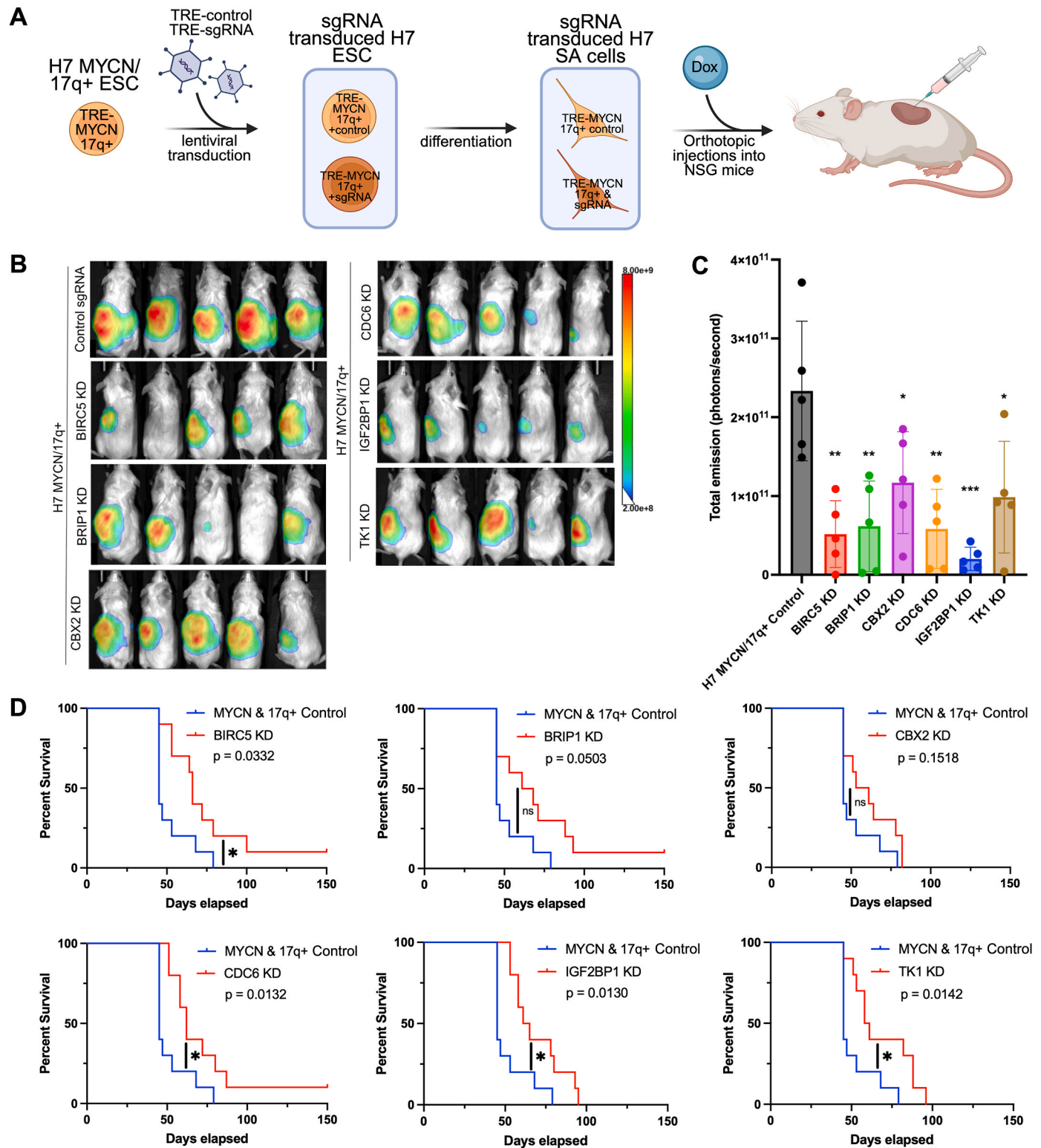
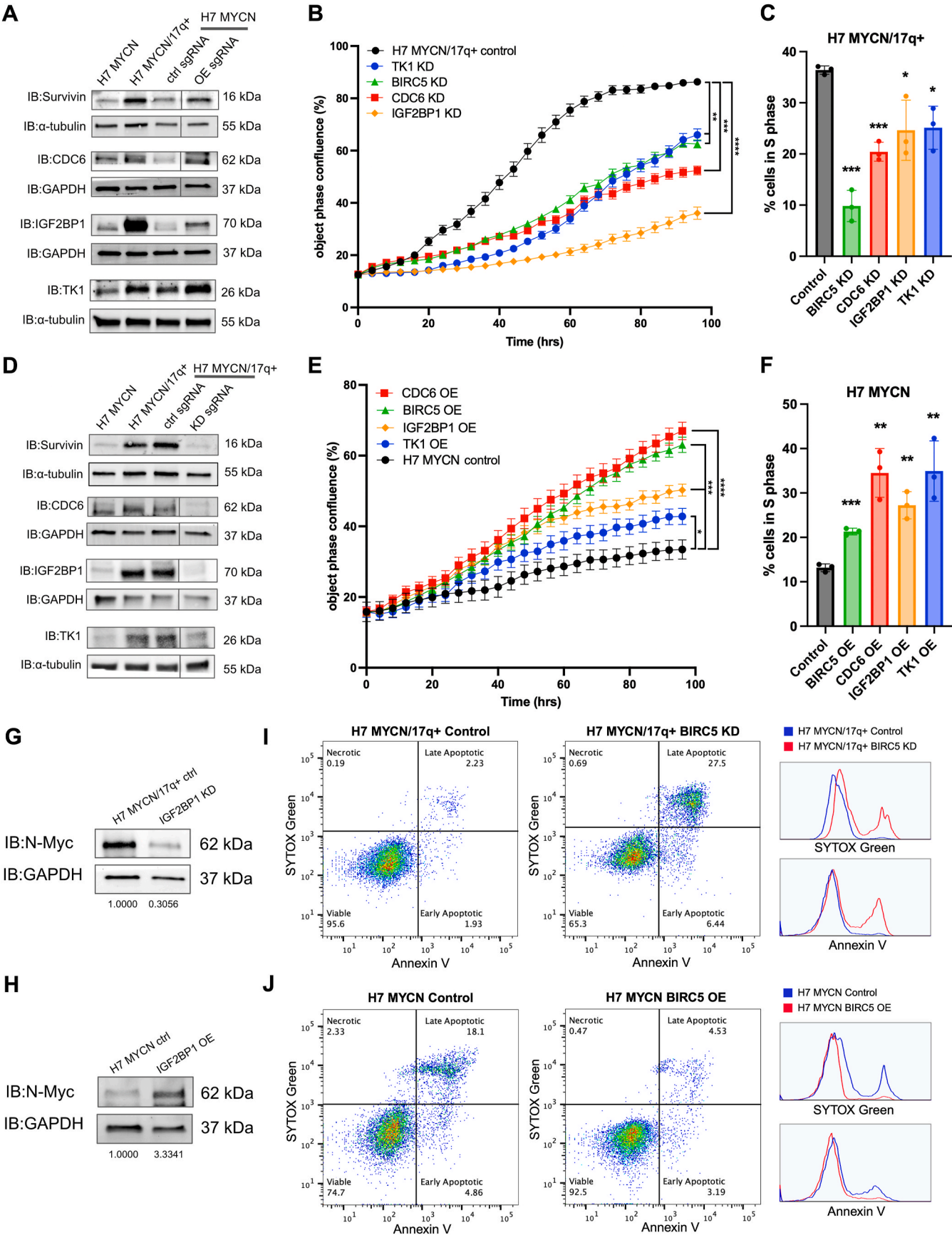


Fig. 3. Knockdown of *BIRC5*, *CDC6*, *IGF2BP1* and *TK1* slows *MYCN*/17q + tumor growth in vivo. (A) Schematic of experimental design of generation of neuroblastoma tumors with candidate gene KD. (B) Representative images of pseudocolored total bioluminescence emission of mice at day 45 post-implantation. n = 5 per condition, exposure time = 1.5 s. (C) Quantification of bioluminescence intensity from Fig. 3C. (D) Kaplan-Meier curves of tumor generation in H7 *MYCN*/17q+ with control sgRNA compared to candidate gene KD. n = 10 per group, p-value calculated by Log-rank test.

showed a decrease in N-Myc protein abundance compared to the control sgRNA (Fig. 4G). Conversely, H7 *MYCN* cells with OE of *IGF2BP1* showed increased N-Myc protein levels compared to the control sgRNA (Fig. 4H). These findings corroborate previous reports that *IGF2BP1* influences *MYCN* expression, which results in faster tumor progression

[45].

In addition, given that *BIRC5* is an essential anti-apoptotic factor [46], we performed an Annexin V/SYTOX staining and Western blot of cleaved PARP to look at apoptosis in relation to *BIRC5* expression. KD of *BIRC5* in H7 *MYCN*/17q + cells resulted in a significant increase in



(caption on next page)

Fig. 4. *BIRC5*, *CDC6*, *IGF2BP1* and *TK1* contribute to neuroblastoma tumor growth by influencing cell cycle progression, cell survival, and *MYCN* expression. (A) Western Blot confirmation of candidate gene KD in H7 *MYCN*/17q + cells. (B) Object phase confluence of H7 *MYCN*/17q + cells with control sgRNA or CRISPRi KD of candidate genes, imaged every 4 h for 96 h. Confluence calculated by IncuCyte, error bars = standard error of image for 16 images. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, unpaired t -test. (C) EdU assay of H7 *MYCN*/17q + control sgRNA and KD cells, $n = 3$ per condition. * $p < 0.05$, ** $p < 0.01$, unpaired t -test. (D) Western Blot confirmation of candidate gene OE in H7 *MYCN* cells. (E) Object phase confluence of H7 *MYCN* tumor cells with control sgRNA or CRISPRa OE of candidate genes, imaged every 4 h for 96 h. Confluence calculated by IncuCyte, error bars = standard error of image for 16 images. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, unpaired t -test. (F) EdU assay of H7 *MYCN* control sgRNA and OE cells, $n = 3$ per condition. * $p < 0.05$, ** $p < 0.01$, unpaired t -test. (G) Western Blot of N-Myc in H7 *MYCN*/17q + tumor cells with control sgRNA or *IGF2BP1* KD. (H) Western Blot of N-Myc in H7 *MYCN* tumor cells with control sgRNA or *IGF2BP1* OE. (I) Annexin V/Sytox Green staining of H7 17q+/MYCN control sgRNA and *BIRC5* KD. (J) Annexin V/Sytox Green staining of H7 *MYCN* control sgRNA and *BIRC5* OE. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Annexin V+ cells and cleaved PARP compared to control sgRNA. Conversely, OE of *BIRC5* in H7 *MYCN* cells significantly decreased Annexin V+ cells and cleaved PARP compared to control sgRNA (Fig. 4I-J, S5D-E). Therefore, 17q + -mediated upregulation of *BIRC5* promotes cell survival in *MYCN*/17q + tumors.

Finally, to confirm whether *MYCN* influences the transcription of *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1*, we analyzed public ChIP-RX data of *MYCN* and H3K27Ac in the Tet-off *MYCN* neuroblastoma cell line SHEP21 [37]. Indeed, we found that *MYCN* ChIP signal is higher at 0 h (*MYCN* on) compared to 24 h post doxycycline treatment (*MYCN* off) at the promoter regions of *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1*, and that *MYCN* binding to the promoter corresponds to higher signal of the activating mark H3K27Ac (Fig. S5F).

Overall, these data suggest that 17q genes *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1* influence tumor growth through three main mechanisms: by increasing cell cycle progression, inhibiting apoptosis, and stabilizing

MYCN, which in turn increases the transcription of the chromosome 17q candidate genes.

3.5. *CDC6*, *IGF2BP1*, and *TK1* affect the growth of neuroblastoma patient tumors

We next sought to determine whether *BIRC5*, *CDC6*, *IGF2BP1*, and *TK1* can influence the growth of neuroblastoma patient tumors *in vitro* and *in vivo*. We generated CRISPRi-mediated KD of these candidate genes in two neuroblastoma patient-derived xenograft cell lines, COG-N-471nb (female), and COG-N-623 (male) (Fig. 5A, S6A). These cell lines were specifically selected due to the presence of *MYCN* amplification and chromosome 17q gain, with the absence of other common cancer-related somatic mutations such as *ALK*, *TP53*, *BRAF*, and *RAS* family genes; thus, these cell lines maintain a similar genotype to the ESC-derived tumors in our model (Table 1). We found that KD of *CDC6*,

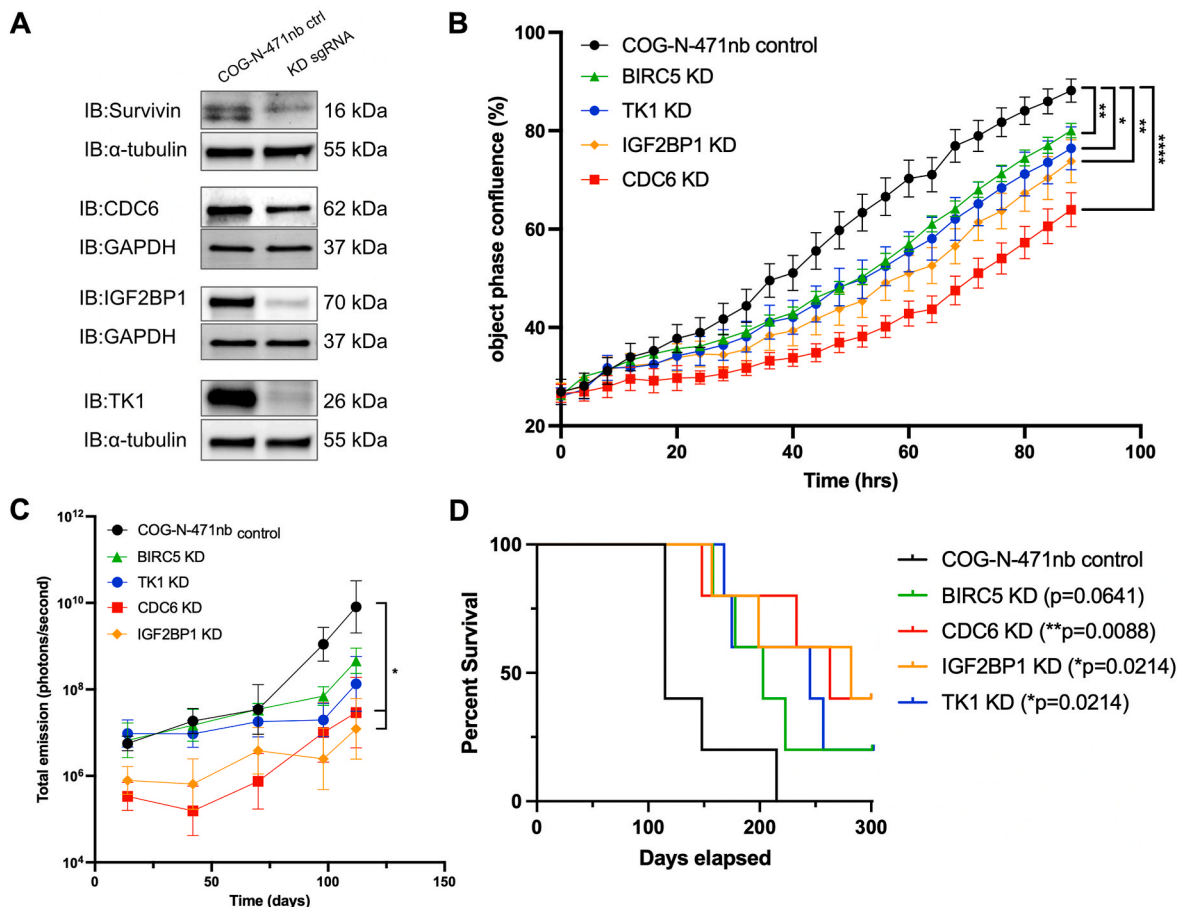


Fig. 5. Knockdown of *BIRC5*, *CDC6*, *IGF2BP1* and *TK1* in COG-N-471nb PDX slows tumor growth *in vitro* and *in vivo*. (A) Western Blot confirmation of candidate gene KD in COG-N-471nb cells. (B) Object phase confluence of COG-N-471nb cells with control sgRNA or CRISPRi KD of candidate genes, imaged every 4 h for 88 h. Confluence calculated by IncuCyte, error bars = standard error of image for 16 images. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, unpaired t -test. (C) Quantification of bioluminescence intensity, $n = 5$ per condition, error bars = standard error of mean. * $p < 0.05$, unpaired t -test. (D) Kaplan-Meier curves of tumor generation in COG-N-471nb control sgRNA compared to candidate gene KD. $n = 5$ per group, p -values calculated by Log-rank test.

Table 1
Children's oncology group patient-derived xenograft sample attributes and Molecular profile.

MODEL	GENDER	PHASE	MYCN Status	ALK	HRAS	KRAS	NRAS	PTPN11	TP53	BRAF	chr17q_status_depthRatio	depthRatio_conf	chr17q_status_SNParray	SNParray_conf	chr17q_status_CNVkit	cnvkit_CN_conf
COG-N-415x	Female	Progressing Disease	Amplified	F1174L	WT	WT	WT	WT	WT	WT	Gain	*	Gain	**	Gain	*
COG-N-421x	Male	Relapse	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	*	Gain	**	Loss	no gain
COG-N-424x	Male	Diagnosis	Amplified	WT	WT	WT	WT	WT	WT	V600E	Gain	nc	Gain	*	Gain	***
COG-N-426x-Felix-PDX	Male	Relapse	Non-Amplified	F1245C	WT	WT	WT	WT	WT	WT	Gain	*	Gain	nc	Gain	**
COG-N-440x	Female	Relapse	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	nc	Gain	*	Gain	nc
COG-N-452x	Male	Relapse	Amplified	F1174L	WT	WT	WT	WT	WT	WT	Gain	**	Gain	**	Gain	****
COG-N-453x	Male	Relapse	Amplified	F1174L	WT	WT	WT	WT	WT	WT	Gain	**	Gain	*	Gain	nc
COG-N-471x	Female	Relapse	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	****	Gain	*	Gain	**
COG-N-480x	Male	Diagnosis	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	**	Gain	***	Gain	**
COG-N-496x	Female	Diagnosis	Amplified	WT	WT	WT	WT	WT	R342*	WT	Gain	***	Gain	nc	Gain	nc
COG-N-519x	Male	Relapse	Amplified	WT	WT	WT	WT	WT	G245S	WT	Gain	***	Gain	***	Gain	nc
COG-N-549x	Male	Progressing Disease	Non-Amplified	F1174L	WT	WT	WT	WT	WT	WT	Gain	nc	Gain	nc	Gain	*
COG-N-557x	Male	Diagnosis	Amplified	F1245L	WT	WT	WT	WT	WT	WT	Gain	nc	Gain	*	Gain	***
COG-N-560x	Female	Diagnosis	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	*	Gain	*	Gain	**
COG-N-561x	Female	Relapse	Amplified	F1245I	WT	WT	WT	WT	WT	WT	Gain	****	Gain	**	Loss	no gain
COG-N-564x	Female	Relapse	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	nc	Gain	*	Gain	*
COG-N-589x	Female	Diagnosis	Non-Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	***	Gain	***	Gain	*
COG-N-590x	Male	Diagnosis	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	*	Loss	no gain	Gain	**
COG-N-603x	Male	Diagnosis	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	*	Gain	***	Gain	*
COG-N-618x	Male	Progressing Disease	Non-Amplified	WT	WT	WT	WT	G503V	WT	WT	NA	NA	Gain	nc	NA	NA
COG-N-619x	Male	Progressing Disease	Non-Amplified	WT	WT	WT	WT	G503V	WT	WT	Gain	nc	Gain	nc	Gain	***
COG-N-620x	Male	Progressing Disease	Non-Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	***	Gain	****	Gain	*
COG-N-623x	Male	Progressing Disease	Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	**	Gain	***	Gain	****
COG-N-624x	Female	Progressing Disease	Non-Amplified	R1275Q	WT	WT	WT	WT	WT	WT	Gain	nc	Gain	nc	Loss	no gain
COG-N-625x	Male	Progressing Disease	Non-Amplified	WT	WT	WT	WT	WT	WT	WT	Gain	**	Gain	**	Gain	***
COG-N-628x	Female	Diagnosis	Amplified	WT	WT	WT	WT	D61Y	WT	WT	NA	NA	Gain	**	NA	NA
Fly-623m	NA	NA	Non-Amplified	R1275Q	WT	WT	WT	WT	WT	WT	NA	NA	Gain	**	NA	NA
NCH-NB-1	Female	Diagnosis	Non-Amplified	F1174L	WT	WT	WT	WT	WT	WT	Gain	**	Gain	****	Gain	**

IGF2BP1, and *TK1* all significantly impaired *in vitro* proliferation in both cell lines compared to control sgRNA, whereas KD of *BIRC5* only significantly affected proliferation in COG-N-471nb cells (Fig. 5B–S6B).

Lastly, we tested whether KD of these genes influences tumor growth *in vivo*. We orthotopically implanted COG-N-471nb cells carrying either control sgRNA or sgRNA for each respective 17q candidate gene in NSG mice. By day 112 post-implantation, BLI showed that tumor radiance was overall lower in the mice with candidate gene KD, and radiance was significantly decreased in tumor cells with *IGF2BP1* KD ($p = 0.0146$) and *CDC6* KD ($p = 0.0183$) compared to control sgRNA (Fig. 5C). KD of *CDC6*, *IGF2BP1*, and *TK1* significantly increased overall survival compared to control, with p -values of 0.0088, 0.0214 and 0.0214, respectively (Fig. 5D). Thus, this indicates that *CDC6*, *IGF2BP1*, and *TK1* play functional roles in promoting tumor growth and aggressiveness *in vivo*, and that their depletion impairs tumor progression and prolongs survival. Collectively, these data validate *CDC6*, *IGF2BP1*, and *TK1* as key drivers of neuroblastoma tumor maintenance and highlight them as potential therapeutic vulnerabilities worthy of further study.

4. Discussion

The association of chromosome 17q gain with poor prognosis in neuroblastoma has long been known [15]. Many studies have implicated genes located on 17q as candidate tumorigenesis drivers, such as *NME1*, *TBX2*, *BIRC5*, *ERBB2*, *TRIM37*, *PPM1D*, and *IGF2BP1*, by using genetically engineered mouse models, zebrafish models, or neuroblastoma patient cell lines [47]. However, a limitation of the animal models (particularly mouse) is that the same cell types from different species can respond differently to the same oncogenes. For instance, while mouse fibroblasts can be transformed by *HRAS* and *MYC*, human fibroblasts cannot [48]. Mouse cells also have longer telomeres than human cells, which can influence its susceptibility to transformation [49]. Furthermore, it has been difficult to investigate the effects of CNVs such as 17q+ in animal models, as genes on mouse and human chromosomes do not fully align. Although regions syntenic to human chromosome 17q on mouse chromosome 11 have been reported to show spontaneous gains in 30% of *MYCN*-driven mouse neuroblastoma tumors in a study by Łastowska et al. [50], it was not clear whether these chromosome CNVs contributed definitively to tumorigenesis. While some studies have shown several 17q genes regulate proliferation of human neuroblastoma tumors *in vitro* [47], this is distinct from a role in tumorigenesis *in vivo*.

Here, we circumvent these limitations by utilizing a human pluripotent stem cell model of neuroblastoma with 17q+. While the spontaneous acquisition of chromosome CNVs in human PSCs is typically an undesired outcome of long-term *in vitro* culture [51], this was useful for our purposes due to the high frequency of chromosome abnormalities in neuroblastoma. Indeed, 17q+ was among the first recurrent CNVs identified in human PSC culture [52], and 17q + confers a growth advantage for ESCs *in vitro* [53].

A recent study by Saldana-Guerro et al. examined the impact of chromosome CNVs (17q+, 1q+) with or without mis-expression of *MYCN* during sympathoadrenal development [62]. The combination of 1q+/17q+/MYCN had the strongest impact on disrupting the differentiation process, whereas 17q + alone had a statistically insignificant effect compared to WT cells. Indeed, we also found in our model that 17q + provides a mild differentiation block compared to 17q WT, though these differences are also not statistically significant (Fig. 1B–C, S1B–C). Saldana-Guerro et al. also examined the impact of chromosome CNV in tumorigenesis and found that while 1q+/17q + did not generate tumors, the combination of 1q+/17q+/MYCN did. However, it was not clear from this study whether *MYCN* cooperated with the chromosome CNVs. In contrast, our study demonstrates that *MYCN* and 17q + cooperate to accelerate tumorigenesis compared to *MYCN* alone, and we identify specific genes on 17q that contribute to tumor initiation and progression.

Our model also accurately recapitulates the order in which 17q+ and *MYCN* amplification are acquired in neuroblastoma, as it is postulated that 17q+ is a truncal aberration that occurs early in the first trimester of fetal development before *MYCN* amplification occurs [54]. Similarly, a study in medulloblastoma, another embryonal tumor, also found that chromosome CNVs are more likely the initiating event in tumorigenesis than amplification of oncogenes [55]. While 17q + has been observed in non-*MYCN* amplified neuroblastoma, it is notable that 17q + alone is insufficient to generate tumors using our model. One possible explanation is that the 17q + ESC used in our study has a gain of only 1 copy of 17q for a total of 3 copies, whereas neuroblastoma patients often present with a range of 3 to 9 copies of 17q gained at various loci [18]. Another possibility is that neuroblastoma patients tend to have, aside from 17q+, many additional genetic abnormalities, including chromosome CNVs commonly concurrent with 17q+, such as the deletion of 1p or 11q [14]. Finally, Körber et al. recently reported that chromosome CNV accumulation occurs early in fetal development and is likely a priming event that predisposes a premalignant state, driving early genetic diversification and providing a genetic reservoir in premalignant cells, which can later cooperate with other oncogenic drivers to progress aggressive disease, but is insufficient to drive tumorigenesis on its own [54].

It is important to note that the candidate 17q genes identified in this study should not be examined in isolation, as our model uses *MYCN* OE to drive tumorigenesis, thus pointing to the inherent cooperation between *MYCN* and 17q+ in neuroblastoma. It is well-known that *MYCN* drives the transcriptional regulation of many pathways involved in cell proliferation and survival [56]. Thus, in addition to the acquisition of a third copy of 17q, which can independently influence dosage-dependent expression, the OE of the 17q targets identified by our model is further upregulated by their interaction with *MYCN*. How *MYCN* and 17q + cooperate could be related to how *MYCN* influences the expression of genes on 17q, and conversely, how genes on 17q influence the expression of *MYCN*. Indeed, our 17q targets fall into these two categories: upstream regulators of *MYCN* expression and downstream *MYCN*-regulated target genes. *IGF2BP1* is unique in that it is in both categories: it is an mRNA-binding protein that functions upstream by stabilizing the 3' untranslated region of the *MYCN* transcript, thus increasing *MYCN* expression; it is also directly regulated by *MYCN* via *MYCN* binding to its promoter, forming a feed-forward loop [45]. In contrast, *BIRC5*, *CDC6*, and *TK1* are downstream effectors of *MYCN*, whose transcription may be directly induced by *MYCN* binding of their promoters, or indirectly regulated through *MYCN*-driven upregulation of E2F transcription factors that control G1/S cell-cycle progression [56,57]. Our study strongly corroborates previous studies arguing the importance of *IGF2BP1* in neuroblastoma [45,58], and also shows for the first time the importance of *BIRC5*, *CDC6*, and *TK1* in driving neuroblastoma tumorigenesis *in vivo* [59,60].

Given that our results show that all four candidate genes are upregulated in tumors with 17q+ and that KD of each can significantly extend tumor latency, these four genes are likely cooperating to produce the phenotypes we have observed with 17q+. In addition, *IGF2BP1* has been previously reported to bind and regulate the mRNA of *BIRC5* and *TK1* (in addition to *MYCN*) in other tumor models [61,62]. Thus, the combined upregulation of the four genes from increased gene dosage, along with the role of *IGF2BP1* in upregulating expression of other genes on 17q, suggest there is likely a cooperative or additive effect between the genes on 17q.

Aside from 17q+, there are several other chromosome alterations that frequently occur in neuroblastoma, including gain of 1q, 1p loss of heterozygosity (LOH), and 11q LOH, each of which frequently co-occurs with 17q+¹⁴. Although these other chromosome CNVs are less frequent than 17q+, they are still far more common than single-nucleotide variations. Additionally, chromosomal CNVs frequently occur not just in neuroblastoma, but also in other developmental tumor types such as medulloblastoma, pediatric gliomas, and sarcomas [63], as well as adult cancers like triple-negative breast cancer, high-grade ovarian

carcinoma, and colorectal cancer [64–66]. The presence of CNVs generally shows strong pan-cancer correlation with poor prognosis [67]. Thus, our human PSC model of neuroblastoma can be leveraged to further elucidate the impact of other common chromosome CNVs and uncover more insights into how neuroblastoma and other pediatric and adult cancers develop.

In conclusion, our study demonstrates a unique application of a human PSC model of cancer, namely, investigating the impact of human patient-relevant chromosome copy number changes in promoting malignant phenotypes. Our model identifies candidate oncogenes in neuroblastoma with functional roles in tumor initiation and progression, and that represent promising therapeutic targets for future study.

CRediT authorship contribution statement

Wanqi Fang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bo Cheng:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Steven Pastor:** Investigation, Formal analysis, Data curation. **Michael J. Zobel:** Writing – review & editing, Investigation. **Tania Porras:** Investigation. **Hong-Wei Wu:** Investigation. **Taneesh Kondapally:** Validation, Investigation. **Stephen S. Roberts:** Conceptualization. **Chintan Parikh:** Supervision, Funding acquisition. **Jessica W. Tsai:** Writing – review & editing, Resources. **Anestis Tsakiridis:** Resources, Funding acquisition. **Ivana Barbaric:** Resources, Funding acquisition. **Shahab Asgharzadeh:** Supervision, Resources, Funding acquisition. **John M. Maris:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Miller Huang:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Ethics declaration

This study was performed in compliance with relevant laws, regulatory frameworks and guidelines where the research took place. This study was approved by the Institutional Review Board. (Approval No. CHLA-24-0025)

This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This study was approved by the Institutional Animal Care and Use Committee. (Approval No. 414-25)

Data availability

RNA sequencing analysis of *MYCN* and *MYCN*/17q + tumors is available in the dbGAP database (study accession: phs004436.v1.p1). This paper does not report original code. Further information and requests for resources, materials, and reagents used in this study should be directed to and will be fulfilled by the corresponding author, Miller Huang (mhuang@chla.usc.edu).

Generative AI

Generative AI technologies were NOT used in the preparation of this study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.canlet.2026.218781>.

Abbreviations

CNV	copy number variation
17q+	gain of chromosome 17q
tNCC	trunk neural crest cell
ALK	anaplastic lymphoma kinase
PSC	pluripotent stem cell
ESC	embryonic stem cell
TH	tyrosine hydroxylase
DBH	dopamine beta hydroxylase
WT	wild-type
NSG	NOD-scid IL2rgamma ^{null}
DEG	differentially expressed gene
CRISPRi	CRISPR interference
KD	knockdown
CRISPRa	CRISPR activation
OE	overexpress/overexpression
LOH	loss of heterozygosity

References

- [1] K.K. Matthey, J.M. Maris, G. Schleiermacher, A. Nakagawara, C.L. Mackall, L. Diller, et al., Neuroblastoma, *Nat. Rev. Dis. Primers* 2 (2016 Nov 10) 16078, <https://doi.org/10.1038/nrdp.2016.78>. PubMed PMID: 27830764.
- [2] A. Nakagawara, M. Ohira, Comprehensive genomics linking between neural development and cancer: neuroblastoma as a model, *Cancer Lett.* 204 (2) (2004 Feb 20) 213–224, [https://doi.org/10.1016/S0304-3835\(03\)00457-9](https://doi.org/10.1016/S0304-3835(03)00457-9). PubMed PMID: 15013220.
- [3] M. Ponzoni, T. Bachetti, M.V. Corrias, C. Brignole, F. Pastorino, E. Calarco, et al., Recent advances in the developmental origin of neuroblastoma: an overview, *J. Exp. Clin. Cancer Res.* 41 (1) (2022 Mar 11) 92, <https://doi.org/10.1186/s13046-022-02281-w>.
- [4] M.S. Irwin, A. Naranjo, F.F. Zhang, S.L. Cohn, W.B. London, J.M. Gastier-Foster, et al., Revised neuroblastoma risk classification system: a report from the children's oncology group, *J. Clin. Oncol.* 39 (29) (2021 Oct 10) 3229–3241, <https://doi.org/10.1200/JCO.21.00278>. PubMed PMID: 34319759; PubMed Central PMCID: PMC8500606.

- [5] Y.P. Mossé, M. Laudenslager, L. Longo, K.A. Cole, A. Wood, E.F. Attiyeh, et al., Identification of ALK as a major familial neuroblastoma predisposition gene, *Nature* 455 (7215) (2008 Oct) 930–935, <https://doi.org/10.1038/nature07261>.
- [6] R.E. George, T. Sanda, M. Hanna, S. Fröhling, W.L. II, J. Zhang, et al., Activating mutations in ALK provide a therapeutic target in neuroblastoma, *Nature* 455 (7215) (2008 Oct) 975–978, <https://doi.org/10.1038/nature07397>.
- [7] T.J. Pugh, O. Morozova, E.F. Attiyeh, S. Asgharzadeh, J.S. Wei, D. Auclair, et al., The genetic landscape of high-risk neuroblastoma, *Nat. Genet.* 45 (3) (2013 Mar) 279–284, <https://doi.org/10.1038/ng.2529>. PubMed PMID: 23334666; PubMed Central PMCID: PMC3682833.
- [8] G.M. Brodeur, R.C. Seeger, M. Schwab, H.E. Varmus, J.M. Bishop, Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage, *Science* 224 (4653) (1984 Jun 8) 1121–1124, <https://doi.org/10.1126/science.6719137>.
- [9] R.C. Seeger, G.M. Brodeur, H. Sather, A. Dalton, S.E. Siegel, K.Y. Wong, et al., Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas, *N. Engl. J. Med.* 313 (18) (1985 Oct 31) 1111–1116, <https://doi.org/10.1056/NEJM198510313131802>.
- [10] W.A. Weiss, K. Aldape, G. Mohapatra, B.G. Feuerstein, J.M. Bishop, Targeted expression of MYCN causes neuroblastoma in transgenic mice, *EMBO J.* 16 (11) (1997 Jun 2) 2985–2995, <https://doi.org/10.1093/emboj/16.11.2985>. PubMed PMID: 9214616; PubMed Central PMCID: PMC1169917.
- [11] K. Althoff, A. Beckers, E. Bell, M. Nortmeyer, T. Thor, A. Sprüßel, et al., A Cre-conditional MYCN-driven neuroblastoma mouse model as an improved tool for preclinical studies, *Oncogene* 34 (26) (2015 Jun) 26, <https://doi.org/10.1038/onc.2014.269>.
- [12] L.C. Heukamp, T. Thor, A. Schramm, K. De Preter, C. Kumps, B. De Wilde, et al., Targeted expression of mutated ALK induces neuroblastoma in transgenic mice, *Sci. Transl. Med.* 4 (141) (2012 Jul 4), <https://doi.org/10.1126/scitranslmed.3003967>, 141ra91–141ra91.
- [13] K. Uryu, R. Nishimura, K. Kataoka, Y. Sato, A. Nakazawa, H. Suzuki, et al., Identification of the genetic and clinical characteristics of neuroblastomas using genome-wide analysis, *Oncotarget* 8 (64) (2017 Nov 18) 107513–107529, <https://doi.org/10.18632/oncotarget.22495>. PubMed PMID: 29296183; PubMed Central PMCID: PMC5746085.
- [14] S.W. Brady, Y. Liu, X. Ma, A.M. Gout, K. Hagiwara, X. Zhou, et al., Pan-neuroblastoma analysis reveals age- and signature-associated driver alterations, *Nat. Commun.* 11 (1) (2020 Oct 14) 1, <https://doi.org/10.1038/s41467-020-18987-4>.
- [15] N. Bown, S. Cotterill, M. Lastowska, S. O'Neill, A.D. Pearson, D. Plantaz, et al., Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma, *N. Engl. J. Med.* 340 (25) (1999 Jun 24) 1954–1961, <https://doi.org/10.1056/NEJM199906243402504>. PubMed PMID: 10379019.
- [16] I. Janoueix-Lerosey, G. Schleiermacher, E. Michels, V. Mosseri, A. Ribeiro, D. Lequin, et al., Overall genomic pattern is a predictor of outcome in neuroblastoma, *J. Clin. Oncol.* 27 (7) (2009 Mar 1) 1026–1033, <https://doi.org/10.1200/JCO.2008.16.0630>. PubMed PMID: 19171713.
- [17] J. Theissen, A. Oberthuer, A. Hombach, R. Volland, F. Hertwig, M. Fischer, et al., Chromosome 17/17q gain and unaltered profiles in high resolution array-CGH are prognostically informative in neuroblastoma, *Gene Chromosome Cancer* 53 (8) (2014) 639–649, <https://doi.org/10.1002/gcc.22174>.
- [18] I. Soria-Bretones, M. Casas-Selves, M. Samanta, D. Groff, J. Murray, J.I. Fletcher, et al., The PLK4 inhibitor RP-1664 demonstrates potent efficacy in neuroblastoma preclinical models through a dual mechanism of sensitivity, *Nat. Commun.* (2026 Jun 13), <https://doi.org/10.1038/s41467-026-74061-5>.
- [19] B. Cheng, W. Fang, S. Pastor, A.R. March, T. Porras, H.W. Wu, et al., Comparison of human pluripotent stem cell differentiation protocols to generate neuroblastoma tumors, *Sci. Rep.* 14 (1) (2024 Oct 4) 23050, <https://doi.org/10.1038/s41598-024-73947-y>.
- [20] M. Huang, W. Fang, A. Farrel, L. Li, A. Chronopoulos, N. Nasholm, et al., ALK upregulates POSTN and WNT signaling to drive neuroblastoma, *Cell Rep.* 43 (3) (2024 Mar 26) 113927, <https://doi.org/10.1016/j.celrep.2024.113927>. PubMed PMID: 38451815; PubMed Central PMCID: PMC1101011.
- [21] L.A. Gilbert, M.H. Larson, L. Morsut, Z. Liu, G.A. Brar, S.E. Torres, et al., CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes, *Cell* 154 (2) (2013 Jul 18) 442–451, <https://doi.org/10.1016/j.cell.2013.06.044>.
- [22] U. Müller-Kuller, M. Ackermann, S. Kolodziej, C. Brendel, J. Fritsch, N. Lachmann, et al., A minimal ubiquitous chromatin opening element (UCOE) effectively prevents silencing of juxtaposed heterologous promoters by epigenetic remodeling in multipotent and pluripotent stem cells, *Nucleic Acids Res.* 43 (3) (2015 Feb 18) 1577–1592, <https://doi.org/10.1093/nar/gkv019>. PubMed PMID: 25605798; PubMed Central PMCID: PMC4330381.
- [23] L. Cong, F.A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, et al., Multiplex genome engineering using CRISPR/cas systems, *Science* 339 (6121) (2013 Feb 15) 819–823, <https://doi.org/10.1126/science.1231143>. PubMed PMID: 23287718; PubMed Central PMCID: PMC3795411.
- [24] A. Dobin, C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, et al., STAR: ultrafast universal RNA-seq aligner, *Bioinformatics* 29 (1) (2013 Jan 1) 15–21, <https://doi.org/10.1093/bioinformatics/bts635>.
- [25] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, *Genome Biol.* 15 (12) (2014) 550, <https://doi.org/10.1186/s13059-014-0550-8>. PubMed PMID: 25516281; PubMed Central PMCID: PMC4302049.
- [26] G. Yu, L.G. Wang, Y. Han, Q.Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, *OMICS* 16 (5) (2012 May) 284–287, <https://doi.org/10.1089/omi.2011.0118>. PubMed PMID: 22455463; PubMed Central PMCID: PMC3339379.
- [27] Gabriella Kids First Pediatric Research Program Data Resource Center [Internet]. [cited 2024 February 22]. Available from: <https://kidsfirstdrc.org/>.
- [28] National Cancer Institute (NCI), TARGET: therapeutically applicable research to generate effective treatments dbGaP study [Internet]. [cited 2024 Feb 29]. Available from: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000218.v26.p8.
- [29] P.A. Ewels, A. Peltzer, S. Fillinger, H. Patel, J. Alneberg, A. Wilm, et al., The nf-core framework for community-curated bioinformatics pipelines, *Nat. Biotechnol.* 38 (3) (2020 Mar) 276–278, <https://doi.org/10.1038/s41587-020-0439-x>. PubMed PMID: 32055031.
- [30] P. Di Tommaso, M. Chatzou, E.W. Floden, P.P. Barja, E. Palumbo, C. Notredame, Nextflow enables reproducible computational workflows, *Nat. Biotechnol.* 35 (4) (2017 Apr) 316–319, <https://doi.org/10.1038/nbt.3820>.
- [31] B. Li, C.N. Dewey, RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, *BMC Bioinf.* 12 (1) (2011 Aug 4) 323, <https://doi.org/10.1186/1471-2105-12-323>.
- [32] Chen Y, Chen L, Lun ATL, Baldoni PL, Smyth GK. edgeR v4: powerful differential analysis of sequencing data with expanded functionality and improved support for small counts and larger datasets [Internet]. [cited 2026 February 17]. Available from: <https://dx.doi.org/10.1093/nar/gkaf018>.
- [33] M.E. Ritchie, B. Phipson, D. Wu, Y. Hu, C.W. Law, W. Shi, et al., Limma powers differential expression analyses for RNA-sequencing and microarray studies, *Nucleic Acids Res.* 43 (7) (2015 Apr 20) e47, <https://doi.org/10.1093/nar/gkv007>.
- [34] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, *Bioinformatics* 30 (15) (2014 Aug 1) 2114–2120, <https://doi.org/10.1093/bioinformatics/btu170>. PubMed PMID: 24695404; PubMed Central PMCID: PMC4103590.
- [35] J.B. Poell, M. Mendelev, D. Sie, A. Brink, R.H. Brakenhoff, B. Ylstra, ACE: absolute copy number estimation from low-coverage whole-genome sequencing data, *Bioinformatics* 35 (16) (2019 Aug 15) 2847–2849, <https://doi.org/10.1093/bioinformatics/bty1055>.
- [36] GEO Accession Viewer [Internet]. [cited 2026 April 22]. Available from: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE80154>.
- [37] R. Zeid, M.A. Lawlor, E. Poon, J.M. Reyes, M. Pulcinetti, M.A. Lopez, et al., Enhancer invasion shapes MYCN-dependent transcriptional amplification in neuroblastoma, *Nat. Genet.* 50 (4) (2018 Apr) 515–523, <https://doi.org/10.1038/s41588-018-0044-9>.
- [38] C.J. Price, D. Stavish, P.J. Gokhale, B.A. Stevenson, S. Sargeant, J. Lacey, et al., Genetically variant human pluripotent stem cells selectively eliminate wild-type counterparts through YAP-mediated cell competition, *Dev. Cell* 56 (17) (2021 Sep 13) 2455–2470.e10, <https://doi.org/10.1016/j.devcel.2021.07.019>. PubMed PMID: 34407428; PubMed Central PMCID: PMC8443275.
- [39] N. Ho, H. Peng, C. Mayoh, P.Y. Liu, B. Atmadibrata, G.M. Marshall, et al., Delineation of the frequency and boundary of chromosomal copy number variations in paediatric neuroblastoma, *Cell Cycle* 17 (6) (2018 Mar 19) 749–758, <https://doi.org/10.1080/15384101.2017.1421875>. PubMed PMID: 29353549.
- [40] S.B. McMahon, MYC and the control of apoptosis, *Cold Spring Harb. Perspect. Med.* 4 (7) (2014 Jul) a014407, <https://doi.org/10.1101/cshperspect.a014407>. PubMed PMID: 24985130; PubMed Central PMCID: PMC4066641.
- [41] L.A. Gilbert, M.A. Horlbeck, B. Adamson, J.E. Villalta, Y. Chen, E.H. Whitehead, et al., Genome-Scale CRISPR-mediated control of gene repression and activation, *Cell* 159 (3) (2014 Oct 23) 647–661, <https://doi.org/10.1016/j.cell.2014.09.029>. PubMed PMID: 25307932.
- [42] G. Siragusa, L. Tomasello, C. Giordano, G. Pizzolanti, Survivin (BIRC5): implications in cancer therapy, *Life Sci.* 350 (2024 Aug 1) 122788, <https://doi.org/10.1016/j.lfs.2024.122788>.
- [43] L.R. Borlado, J. Méndez, CDC6: from DNA replication to cell cycle checkpoints and oncogenesis, *Carcinogenesis* 29 (2) (2008 Feb 1) 237–243, <https://doi.org/10.1093/carcin/bgm268>.
- [44] E.E. Bitter, M.H. Townsend, R. Erickson, C. Allen, K.L. O'Neill, Thymidine kinase 1 through the ages: a comprehensive review, *Cell Biosci.* 10 (2020 Nov 27) 138, <https://doi.org/10.1186/s13578-020-00493-1>. PubMed PMID: 33292474; PubMed Central PMCID: PMC7694900.
- [45] S. Hagemann, D. Misiak, J.L. Bell, T. Fuchs, M.I. Lederer, N. Bley, et al., IGF2BP1 induces neuroblastoma via a druggable feedforward loop with MYCN promoting 17q oncogene expression, *Mol. Cancer* 22 (1) (2023 May 29) 88, <https://doi.org/10.1186/s12943-023-01792-0>.
- [46] G. Ambrosini, C. Adida, D.C. Altieri, A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma, *Nat Med* 3 (8) (1997 Aug) 917–921, <https://doi.org/10.1038/nm0897-917>. PubMed PMID: 9256286.
- [47] V. Mlakar, I. Dupanloup, F. Gonzales, D. Papanangelopoulou, M. Ansari, F. Gumy-Pause, 17q gain in neuroblastoma: a review of clinical and biological implications, *Cancers* 16 (2) (2024 Jan 11), <https://doi.org/10.3390/cancers16020338>.
- [48] W.C. Hahn, C.M. Counter, A.S. Lundberg, R.L. Beijersbergen, M.W. Brooks, R. A. Weinberg, Creation of human tumour cells with defined genetic elements, *Nature* 400 (6743) (1999 Jul) 464–468, <https://doi.org/10.1038/22780>.
- [49] C. Jones-Weinert, L. Mainz, J. Karlseder, Telomere function and regulation from mouse models to human aging and disease, *Nat. Rev. Mol. Cell Biol.* 26 (4) (2025 Apr) 297–313, <https://doi.org/10.1038/s41580-024-00800-5>. PubMed PMID: 39614014; PubMed Central PMCID: PMC12096892.
- [50] M. Lastowska, Y.J. Chung, N. Cheng Ching, M. Haber, M.D. Norris, U.R. Kees, et al., Regions syntenic to human 17q are gained in mouse and rat neuroblastoma,

- Gene Chromosome Cancer 40 (2) (2004) 158–163, <https://doi.org/10.1002/gcc.20031>.
- [51] R.J. Lund, E. Närvä, R. Laheesmaa, Genetic and epigenetic stability of human pluripotent stem cells, *Nat. Rev. Genet.* 13 (10) (2012 Oct) 732–744, <https://doi.org/10.1038/nrg3271>.
- [52] J.S. Draper, K. Smith, P. Gokhale, H.D. Moore, E. Maltby, J. Johnson, et al., Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells, *Nat. Biotechnol.* 22 (1) (2004 Jan) 53–54, <https://doi.org/10.1038/nbt922>.
- [53] L.M. Mus, S. Van Haver, M. Popovic, W. Trypsteen, S. Lefever, N. Zeltner, et al., Recurrent chromosomal imbalances provide selective advantage to human embryonic stem cells under enhanced replicative stress conditions, *Genes Chromosomes Cancer* 60 (4) (2021 Apr) 272–281, <https://doi.org/10.1002/gcc.22931>. PubMed PMID: 33336840; PubMed Central PMCID: PMC11646134.
- [54] V. Körber, S.A. Stainczyk, R. Kurilov, K.O. Henrich, B. Hero, B. Brors, et al., Neuroblastoma arises in early fetal development and its evolutionary duration predicts outcome, *Nat. Genet.* 55 (4) (2023 Apr) 619–630, <https://doi.org/10.1038/s41588-023-01332-y>.
- [55] K. Okonechnikov, P. Joshi, V. Körber, A. Rademacher, M. Bortolomeazzi, J. P. Mallin, et al., Oncogene aberrations drive medulloblastoma progression, not initiation, *Nature* 642 (8069) (2025 Jun) 1062–1072, <https://doi.org/10.1038/s41586-025-08973-5>.
- [56] M. Huang, W.A. Weiss, Neuroblastoma and MYCN, *Cold Spring Harb. Perspect. Med.* 3 (10) (2013 Oct) a014415, <https://doi.org/10.1101/cshperspect.a014415>. PubMed PMID: 24086065; PubMed Central PMCID: PMC3784814.
- [57] Y. Liu, D. Liu, W. Wan, MYCN-induced E2F5 promotes neuroblastoma cell proliferation through regulating cell cycle progression, *Biochem. Biophys. Res. Commun.* 511 (1) (2019 Mar 26) 35–40, <https://doi.org/10.1016/j.bbrc.2019.01.087>. PubMed PMID: 30765227.
- [58] J.M. Biegel, M. Dhamdhare, S. Gao, C.P. Gowda, Y.I. Kawasaki, V.S. Spiegelman, Inhibition of the mRNA-Binding protein IGF2BP1 suppresses proliferation and sensitizes neuroblastoma cells to chemotherapeutic agents, *Front. Oncol.* 11 (2021), <https://doi.org/10.3389/fonc.2021.608816>. PubMed PMID: 33796454.
- [59] F. Lamers, I. van der Ploeg, L. Schild, M.E. Ebus, J. Koster, B.R. Hansen, et al., Knockdown of survivin (BIRC5) causes apoptosis in neuroblastoma via mitotic catastrophe, *Endocr. Relat. Cancer* 18 (6) (2011 Oct) 657–668, <https://doi.org/10.1530/ERC-11-0207>. PubMed PMID: 21859926.
- [60] L. Feng, J.R. Barnhart, R.C. Seeger, L. Wu, N. Keshelava, S.H. Huang, et al., Cdc6 knockdown inhibits human neuroblastoma cell proliferation, *Mol. Cell. Biochem.* 311 (1–2) (2008 Apr) 189–197, <https://doi.org/10.1007/s11010-008-9709-5>. PubMed PMID: 18259842.
- [61] Y. Fan, Y. Pan, L. Jia, S. Gu, B. Liu, Z. Mei, et al., BIRC5 facilitates cisplatin-chemoresistance in a m6A-dependent manner in ovarian cancer, *Cancer Med.* 13 (1) (2023 Dec 19) e6811, <https://doi.org/10.1002/cam4.6811>. PubMed PMID: 38112021; PubMed Central PMCID: PMC10807614.
- [62] Q. Shen, Z. Xu, G. Sun, H. Wang, L. Zhang, TFAP4 activates IGF2BP1 and promotes progression of non-small cell lung cancer by stabilizing TK1 expression through m6A modification, *Mol. Cancer Res.* 20 (12) (2022 Dec 2) 1763–1775, <https://doi.org/10.1158/1541-7786.MCR-22-0231>.
- [63] S.N. Gröbner, B.C. Worst, J. Weischenfeldt, I. Buchhalter, K. Kleinheinz, V. A. Rudneva, et al., The landscape of genomic alterations across childhood cancers, *Nature* 555 (7696) (2018 Mar) 321–327, <https://doi.org/10.1038/nature25480>.
- [64] R. Gao, A. Davis, T.O. McDonald, E. Sei, X. Shi, Y. Wang, et al., Punctuated copy number evolution and clonal stasis in triple-negative breast cancer, *Nat. Genet.* 48 (10) (2016 Oct) 1119–1130, <https://doi.org/10.1038/ng.3641>. PubMed PMID: 27526321; PubMed Central PMCID: PMC5042845.
- [65] J. Debatista, L. Grech, C. Scerri, G. Grech, Copy number variations as determinants of colorectal tumor progression in liquid biopsies, *Int. J. Mol. Sci.* 24 (2) (2023 Jan 16) 1738, <https://doi.org/10.3390/ijms24021738>. PubMed PMID: 36675253; PubMed Central PMCID: PMC9866722.
- [66] D. Bell, A. Berchuck, M. Birrer, J. Chien, D.W. Cramer, F. Dao, et al., Integrated genomic analyses of ovarian carcinoma, *Nature* 474 (7353) (2011 Jun) 609–615, <https://doi.org/10.1038/nature10166>.
- [67] E. van Dijk, T. van den Bosch, K.J. Lenos, K. El Makrini, L.E. Nijman, H.F.B. van Essen, et al., Chromosomal copy number heterogeneity predicts survival rates across cancers, *Nat. Commun.* 12 (1) (2021 May 27) 3188, <https://doi.org/10.1038/s41467-021-23384-6>.