**Supplementary materials and methods**

Cell culture

Four alveolar rhabdomyosarcoma (ARMS) cell lines, all with the *PAX3-FOXO1* fusion gene and with varying MYCN expression, were selected for *in vitro* studies. Cell lines were cultured in Dulbecco’s modified Eagle’s medium (Thermo Fisher Scientific, Waltham, MA, USA) (RH30, RMS-01, RH4) or Roswell Park Memorial Institute 1640 (RPMI) medium (Thermo Fisher Scientific, Waltham, MA, USA)(RH41) supplemented with 10% fetal calf serum (Gibco, Life Technologies Ltd., Thermo Fisher Scientific, Waltham, MA, USA), 2 mM L-glutamine (Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). Mycoplasma tests were performed every two months using the MycoAlert® mycoplasma detection kit (Lonza, Cat. LT07-318) and cell line identities were regularly confirmed using short-tandem repeat (STR) profiling.

Flow Cytometry

Cells were stained with 5µgml-1 Hoechst 33342 at 37°C for the time pre-determined by a time course series (the time that gave the lowest CV [co-efficient of variation] of the G0/G1 peak was selected). Cells were sorted according to phase as determined on a cell cycle histogram after gating out doublets on a peak vs area or width plot for Hoechst staining using either a BD FACSAria (BD Biosciences) or a Beckman Coulter MoFlo Astrios (Beckman Coulter) using a 100µm nozzle with a sheath pressure of 25psi in both cases. Purity of sorted fractions was determined by re-running a small aliquot of the sorted samples.

Western blot

Cells were harvested by detaching using trypsin (Sigma Aldrich) before the addition of cell lysis buffer (Cell Signalling Technology) for protein extraction. Protein was quantified using the BCA assay (Bio-Rad) and equal amounts of protein (20μg) were resolved in a 3-8% Tris-Acetate acrylamide gel and transferred onto PVDF membranes using the X-Cell II and iBlot systems according to manufacturer’s instructions (Invitrogen). Membranes were blocked for 1 hr at room temperature using 5% BSA in TBS-T before the addition of primary antibodies for overnight incubation. Primary antibodies used: anti-MYCN (Abcam, 1:1000), anti-GAPDH (Millipore, 1:1000), anti-HA (Roche Diagnostics, 1:1000), anti-FOXO1 (Cell Signaling Technology, 1:1000), anti-CDK4 (Santa Cruz Biotechnology, 1:1000), anti-KDM4B (Cell Signaling Technology, 1:1000). After washing in TBS-T, membranes were incubated in secondary antibody for 1 hr. Immunostained bands were detected via chemiluminescence.

Chromatin Immunoprecipitation and ChIP-sequencing

The *MYCN* transcript was HA tagged and cloned from RMS cells into the pCI-Neo vector (Promega) as per our previous publication. RH30 cells were stably transfected with a HA-*MYCN* construct [15] and ChIP performed using the MAGnify ChIP kit (Invitrogen) according to manufacturer’s instructions. ChIP was carried out using anti-HA antibody (Roche Diagnostics), or mouse IgG control antibody (Roche Diagnostics). DNA was quantified by PicoGreen (ThermoFisher Scientific). ChIP DNA was measured for enrichment by either qPCR or Sybr green PCR using primers spanning the *CDK4* and *KDM4B* promoter regions. ChIP DNA was sent to Source Bioscience for library preparation and sequencing on an Illumina GAII sequencer. A 'signal over background' normalization method was used to calculate fold change over IgG control. ChIP signals are divided by the no-antibody signals, representing the ChIP signal as the fold increase in signal relative to the background signal. Three replicates were performed, and fold change, percentage of input and standard deviation calculated relative to input and IgG control.

Bioinformatic Analysis

ChIP

FastQ files for each samples were aligned to GRCh37 with BWA v0.5.8a (http://bio-bwa.sourceforge.net/), non-uniquely aligning reads were removed using samtools v0.1.8 and duplicates were marked using PICARD v1.5.2 [18]. MACS v1.4.2 was used to identify enrichment peaks between the ChIP and control datasets using default parameters [19]. Enrichment peaks unique to the HA antibody were defined using an FDR threshold of 5%. Nearest genes were identified using PeakAnnotator (Salmon-Divon et al 2010 BMC bioinformatics) and classified by proximity to the transcription start site (TSS): proximal promoter (within 5kb of TSS), distal (5 to +/-50kb of TSS), or intergenic (>50kb from any TSS).

Data from Gryder *et* al. [20] were retrieved from the NCBI Sequence Read Archive (accessions SRR3720780 and SRR3720781 for control and SRR3720783 for MYCN enrichment). FastQ files were aligned to GRCh38 using Bowtie v2.2.9. Reads with a mapping quality of <30 and duplicates were removed using samtools v1.3.2 and Picard Tools v2.8.3 respectively. MACS v2.2.3 was used to identify enrichment peaks between the ChIP and control datasets using default parameters.

Gene Expression Analysis

Gene ontology analysis of ChIP-sequencing (ChIP-seq) targets and MYCN-bound DNA motifs was conducted using MetaCore (GeneGo, Thompson Reuters). Statistical-significance threshold levels for all enrichment analyses was set at p < 0.05. KEGG pathway analysis was carried out using DAVID [21, 22].

For analysis of genes correlating with MYCN expression in patient samples two datasets were analyzed within R2 (<http://hgserver1.amc.nl/cgi-bin/r2/main.cgi>), Intergroup Rhabdomyosarcoma Study Group/Pediatric Cooperative Human Tissue Network (IRSG/PCHTN) and InnovativeTherapies for Children with Cancer/Carte d’Identité des Tumeurs (ITCC/CIT) datasets, for correlations with *MYCN* where R> +/-0.339 and p<0.01.

Luciferase Reporter Assays

pGL3 Promoter, pGL3 Control and pRL-CMV (Promega) were used for luciferase assays. DNA containing the putative MYCN binding site within the CDK4 promoter was amplified from RH30 genomic DNA and cloned into pGL3 Promoter. Cells were transfected using Lipofectamine 2000 according to manufacturer’s protocol. Dual luciferase assays were performed at 72h using Dual-Glo luciferase reporter assay system (Promega). For each assay, 3 separate experiments were performed. T-tests were performed for statistical signficance.