Analysis of super enhancers with subgroup-specific enhancer activity (SSEA). To identify subgroup-specific enhancer activity, we employed the R/Bioconductor package QSEA v.0.0.11[23]. Previously calculated enhancer regions (see above) were provided as regions of interest and tiled into 500-bp windows. For each sample, H3K27ac ChIP–seq enrichments were calculated at these tiled enhancers and were library size-normalized by TMM. In addition, matched blood and tumour WGS data were imported and copy number variations were calculated for all ependymoma samples using the findCNV() function of the QSEA package. CNV-aware subgroup-specific enhancer activity was then calculated by comparing H3K27ac ChIP–seq enrichments in one subgroup against the other subgroups by fitting general linear models with respect to the presence of CNVs (non-default parameters are norm_method = "nrpkm", minRowSum = 10, fdr_th = 10^{-5}, direction = "gain"). We excluded 500-bp windows that were significant in more than one subgroup. For each subgroup, we stitched all significant 500-bp windows within a distance of 12.5 kb together, summed their normalized H3K27ac ChIP–seq enrichment values (nRPKM), and ranked them accordingly. Analogous to the definition of super enhancers, we define the first occurrence of a slope >1 (from high to low enrichment) as a threshold for distinguishing between extended stretches of significant SE-SSEAs and TE-SSEAs.

Calculating core regulatory networks for super-enhancer-associated transcription factors. To quantify the interaction network of transcription factor regulation, we calculated the inward and outward binding degree of all super-enhancer-associated transcription factors[14]. For all promoters within 100 kb, the most acetylated promoter was assigned as the target of the super enhancer (excluding promoters that overlap super enhancers). If there were no active promoters within 100 kb, the super enhancer was assigned to the nearest active promoter. All super-enhancer-associated promoters annotated to regulate a transcription factor were considered as the node-list for network construction. For any given transcription factor (TFi), the IN degree was defined as the number of transcription factors with an enriched binding motif at the proximal super enhancer or promoter of TFi. The OUT degree was defined as the number of transcription factor-associated super enhancers containing an enriched binding site for TFi. Within any given super enhancer, enriched transcription factor binding sites were determined at putative nucleosome-free regions (valleys) flanked by high levels of H3K27ac. Valleys were calculated using an adapted algorithm[13]. In these regions, we searched for enriched transcription factor binding sites using the FIMO59 algorithm with transcription factor position weight matrices defined in the TRANSFAC database[24]. An FDR cut-off of 0.01 was used to identify enriched transcription factor-binding sites.

Identification of regulatory networks at enhancers with subgroup-specific enhancer activity. Subgroup-specific transcription factor-regulatory networks were constructed as previously described with only a few amendments[8,25]. H3K27ac data of the samples within the same subgroup were combined. For each subgroup, nucleosome-free regions (NFRs) were identified using the findPeaks function of HOMER[26] (http://homer.salk.edu/homer/ngs/index.html) with option -nfr. ENCODE transcription factor motifs and their mapped positions in the genome were downloaded from http://compbio.mit.edu/encode-motifs/. For each transcription factor, contingency tables containing the number of NFRs overlapping and non-overlapping with the respective transcription factor were constructed. The significance of enrichment of transcription factors in NFRs of enhancers with subgroup-specific activity was determined using the χ^2 test. The resulting *P* values were corrected for multiple testing (FDR <0.01). Transcription factor enrichments were calculated as the ratio between observed counts over expected counts. To identify enhancer target genes, we accessed publicly available topology-associated domains (TADs) previously obtained in IMR90 cells. Each SSEA was assigned to its enclosing TAD and protein-coding genes within the same TAD were identified. Correlation tests (Spearman's rank correlation coefficient) for SSEA H3K27ac enrichment and gene expression level within the same TAD were performed. After repeating this procedure for each enhancer, all *P* values obtained were combined and corrected for multiple testing using the Bioconductor package qvalue. Correlations with an FDR less than 1% were preserved. To derive subgroup-specific transcription factor regulatory networks, we selected the top 50% enriched transcription factors in each subgroup, which also have the highest expression in the respective subgroup compared to the other subgroups. The resulting networks highlight transcription factors (red or orange nodes) whose binding sites are significantly enriched at enhancers with SSEA. By gene-enhancer correlation analysis restricted by TAD domains (see above), these transcription factors were assigned to their likely target genes (blue nodes). Networks were visualized using by Gephi (http://gephi.github.io/).

ATAC-seq chromatin preparation and sequencing. Freshly cultured ependymoma cells were prepared for ATAC-seq as described[27]. In brief, nuclei were prepared from ~50,000 cells by spinning at 600*g* for 10 min at 4 °C, followed by a PBS wash and centrifugation at 600*g* for 5 min. Cells were lysed using ice-cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1%), and centrifuged for 10 min at 600*g* at 4 °C. The supernatant was removed and pellet re-suspended in 50 μl transposase mix (25 μl 2×TD buffer, 2.5 μl transposase, 22.5 μl water) (FC-121-1030 Illumina) for 30 min at 37 °C. Library amplification was performed using the NEBnext High Fidelity 2×PCR Master Mix (#M0541S New England Biolabs) according to previously published PCR conditions[27]. PCR reactions were purified using a QIAGEN miniElute kit, and a following size selection step using standard gel extraction protocol to isolate ~240–360 bp. ATAC-seq library preparations were sequenced using single-end 50-bp reads on the Illumina HiSeq 2000 platform. Raw reads were adaptor-trimmed using Trim Galore (v0.2.5) and aligned to the genome with Bowtie (v1.0.1) with the m1 option enabled to allow only uniquely aligned high-quality reads. Peaks were called using the MACS2 software (v2.1.0.20140616) with the options –q 0.05 to retain significant peaks, –shiftsize 50 to account for the transposase fingerprint, and otherwise default parameters were used. Tag count libraries and bedgraph files were constructed using HOMER software (v4.7).

Ependymoma culture experiments. Ependymoma cell cultures were isolated from patients and cultured on laminin (Sigma) and in neurobasal medium (Invitrogen) consisting of: sodium pyruvate (Invitrogen), B27 (Invitrogen), glutamine (Cleveland Clinic Media Core), human EGF (Invitrogen), human basic FGF (Invitrogen), and penicillin/streptomycin (Cleveland Clinic Media Core). Medium was replenished every other day while leaving ~50% conditioned medium to encourage continued cell proliferation. Cell viability assays were performed in 96 wells using an Alamar Blue stain (Invitrogen) according to the manufacturer's instructions. Drug-response assays were performed by seeding cells overnight, treating the following day with increasing drug concentrations, and reading by Alamar Blue Absorption following 72 h of treatment. AZD4547 and MK1775 were obtained from Selleck Chemicals. JQ1 was provided by the laboratory of J. E. Bradner (Harvard). All cell lines were STR profiled for authenticity and confirmed to be mycoplasma free using a PCR-based detection strategy with positive and negative controls.

RNA interference of enhancer-associated genes. Lentiviral shRNA clones (Sigma Mission RNAi) targeting super-enhancer-associated genes, and two non-targeting controls (SHC002, SHC007) were purchased from Sigma. (Supplementary Table 23). These vectors were co-transfected into HEK 293FT cells with the packaging vectors psPAX2 (Addgene) and pCI-VSVG (Addgene) using a calcium phosphate method to produce viable lentivirus. Knockdown efficiency of different lentiviral shRNA clones in cells was determined by quantitative reverse transcription PCR. Cells infected with lentivirus expressing the indicated shRNAs were plated in 96-well plates at 1,000 cells per well. Cell viability was determined after the indicated number of days after plating using Alamar Blue Assay (Life Technologies) or CellTitreGlo (Promega).

CRISPR–Cas9-mediated repression of enhancer regions. CRISPR–Cas9 sgRNAs were identified and designed using the MIT CRISPR design tool, and control (pLenti-Guide-Puro D103) non-targeting sgRNAs were selected from the GeCKOv2 library. All sgRNA sequences may be found in Supplementary Table 23. sgRNAs were cloned into plenti-Guide-Puro (Addgene, 52963). Lentivirus expressing dCAS9-KRAB (gift from M. Meyerson laboratory)[28] were used to infect EP1-NS, following which cells were selected for 48 h with 10 μg/ml blasticidin. These cells were then infected with selected lentiGuide-Puro sgRNA constructs and selected for 48 h with 1 μg/ml puromycin. These cells were plated for 48 h following selection in 96-well plates and cell viability was assessed using an Alamar Blue Stain (Life Technologies).

***In vivo* animal experiments.** We followed the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research from the National Research Council to estimate the minimal number of animals necessary to assess statistical significance. The number of animals per arm was based upon the following calculation: $N = 1 + 2C(s/d)^2$ where n is the number of animals per arm, $C = 7.85$ when $\alpha = 0.05$ and $1-\beta = 0.8$ (significance level of 5% with a power of 80%), s is standard deviation, and d is the difference to be detected. All animal experiments were performed in accordance with local IACUC regulations and protocols. Animal experiments were conducted in a single-blinded fashion, and endpoints were assessed by an independent animal technician in the laboratory. 250,000 H612 cells were xenografted intracranially into NOD/SCID/γ female mice. Tumours were allowed to develop for 14 days then independently randomized into a treatment or vehicle group. AZD4547 (25 mg/kg/d) or vehicle (Sigma: 1% Tween-80) were administered daily by oral gavage. Survival of mice was plotted using a Kaplan–Meier curve and quantified using a log-rank test. Our study did not measure tumour size or volume directly. We monitored neurological signs and behaviours associated with brain tumour development in accordance with our IACUC protocols and regulations.