Supplementary Figure Legends

Figure S1. (A) Western blot showing MYCN expression in the collection of neuroblastoma cell lines from the primary screen. (B) Western blot showing MYCN and c-MYC expression in a panel neuroblastoma cell lines.

Figure S2. *MYCN*-wildtype neuroblastoma is less sensitive to the effects of BET bromodomain inhibition. (A) Dose response of neuroblastoma cell line viability with BET bromodomain inhibitor treatment measured by a luminescent ATP detection assay. Data represent mean ± SEM for four biological replicates. (B) Two *MYCN*-wildtype neuroblastoma cell lines were used to determine the effects of JQ1 on growth. Values over time are shown relative to the day zero values, with errors bars representing the mean +/- SD of eight replicates per condition.

Figure S3. JQ1 treatment induces a G0/G1 arrest in neuroblastoma cell lines. Indicated neuroblastoma cell lines were treated with 1 μM JQ1 for 24, 48 and 72 hours before cell cycle analysis. Time 0 for each phase was compared to either 24, 48, or 72 hours based on paired T-test. *$P < 0.05$. Error bars represent the mean +/- SD of three replicates.

Figure S4. Effects of JQ1 treatment on cell cycle and apoptosis in *MYCN*-wildtype neuroblastoma cell lines. Indicated neuroblastoma cell lines were treated with 1 μM JQ1 for (A) 24, 48 and 72 hours before cell cycle analysis or

(B) 72 hours before measuring apoptosis by annexin V staining detected by flow cytometry.

Figure S5. Western blot showing c-MYC expression in a panel neuroblastoma cell lines treated with 1 μM JQ1 for 24h.

Figure S6. Consensus signatures for JQ1 treatment of cancer cell lines. **(A)** GSEA demonstrating enrichment in multiple myeloma and AML cells treated with JQ1 of gene sets derived from neuroblastoma cells treated with JQ1. **(B)** GSEA showing consistent regulation in JQ1-treated neuroblastoma cells of consensus gene sets derived from hematopoietic malignancies treated with JQ1. AML=acute myeloid leukemia, MM=multiple myeloma and NBL=neuroblastoma.

Figure S7. Structurally distinct BET bromodomain inhibitors share a transcriptional signature with JQ1 treatment of multiple cancer cell lines. Gene expression signatures for JQ1 treatment of neuroblastoma, multiple myeloma, and AML are all enriched in the treatment of AML cell lines with I-BET151. AML=acute myeloid leukemia, MM=multiple myeloma and NBL=neuroblastoma.

Figure S8. Heatmap of the top 50 genes exclusively downregulated in neuroblastoma following 24 hours of 1 μM JQ1 treatment meeting an absolute FC ≥ 2, and p-value and FDR ≤ 0.05. Data are presented row normalized.

Figure S9. Effects of *MYCN* downregulation on neuroblastoma cell lines. (A-C) Three shRNAs targeting *MYCN* or a control shRNA were transduced into three neuroblastoma cell lines. (A) Western blots showing MYCN knock-down four days post-transduction. (B) Viability relative to the time of seeding (day three post-transduction for BE(2)-C and Kelly cell lines or day five for LAN-1). (C) Apoptosis on day five (BE(2)-C), day six (Kelly), or day seven (LAN-1) post-transduction.

Figure S10. Effects of *BRD4* downregulation on *MYCN* non-amplified neuroblastoma cell lines. (A) Western blots showing BRD4 knock-down four days post-transduction. (B) Viability relative to the time of seeding (day four post-transduction for SK-N-AS cell line or day five for SH-SY5Y).

Figure S11. Testing of JQ1 in xenograft models neuroblastoma. (A) Body weight of mice with BE(2)-C subcutaneous xenografted tumors treated with vehicle versus JQ1 as indicated. Error bars indicate mean +/- SD of five mice across different time points. P = 0.69 calculated using non parametric Mann-Whitney test. ns = not significant (B) Effects of JQ1 treatment on survival in xenograft model. Day 0 indicates the first day of treatment and mice were treated until time of sacrifice. Statistical significance was determined by log-rank (Mantel-Cox) test for the survival curves as shown. Day 0 indicates the day of treatment initiation. ns = not significant (C) Mice were injected with the *MYCN*-wildtype SH-SY5Y cell line subcutaneously and treated with JQ1 or vehicle once tumors reached 100

mm^3. After 15 days, tumor volume was measured. Error bars indicate mean +/- SD of five mice per group. *P* = 0.34 calculated using non parametric Mann-Whitney test. ns = not significant.

Supplementary Table Legends

Supplementary Table S1. Results from the JQ1 cell line sensitivity screen reporting the inhibitory concentration 50 (IC_{50}) in Ln form and the Emax (the maximum effect corresponding to the minimum measured viability). "0<cn<8" represents *MYCN* non-amplified, "cn>=8", *MYCN* amplified, "-" represents no data available.

Supplementary Table S2. GI_{50} and E_{max} for multiple neuroblastoma cell lines treated with BET inhibitors and one inactive enantiomer (JQ1R). Data were generated for four biological replicates and a luminescent ATP detection assay was used to determine viability. ND indicates non Determined.

Supplementary Table S3. List of genes differentially expressed in JQ1- vs. vehicle-treated neuroblastoma cell lines.

Supplementary Table S4. *MYCN* Amplification Upregulation Signature in primary neuroblastoma tumors (Janoueix-Lerousey *et al*., GEO#:GSE12460). The signature was created by applying the Comparative Marker Selection

algorithm of GenePattern with the criteria of absolute fold change ratio of 2 or greater and p-value and FDR less than 0.05. We focused on genes whose expression is positively correlated with *MYCN* amplification.

Supplementary Table S5. Differential expression of the *MYCN* Affymetrix Probes in JQ1 versus DMSO (vehicle)-treated neuroblastoma cell lines. Data are presented in log2 and all vehicle-treated (BE(2)-C and Kelly) versus all JQ1-treated (BE(2)-C and Kelly) were considered in the 2-class analysis. The differential scores were computed by applying the Comparative Marker Selection algorithm from the GenePattern 3.5.0 software. A 2-sided SNR-test followed by 1000 permutations of phenotype labels was performed for this analysis.

Supplementary Table S6. GSEA results for the consensus JQ1 downregulation signature. Included are the significantly enriched gene sets in the annotated functional clusters from Figure 4C.

Supplementary Table S7. Extended list of gene set significantly enriched for the neuroblastoma specific JQ1 downregulation signature.

Supplementary Table S8. Statistical analysis of genotypes conferring sensitivity (enriched < 25% viability) and resistance (enriched > 75% viability), reveals *MYCN* as a strong determinant of response. NS indicates not significant.

Supplementary Table S9. shRNA Clone Information and Target Sequences. Shown are the sequences of the hairpins targeting *BRD4* and *MYCN*.

References

Janoueix-Lerosey, I., Lequin, D., Brugieres, L., Ribeiro, A., de Pontual, L., Combaret, V., Raynal, V., Puisieux, A., Schleiermacher, G., Pierron, G., et al. (2008). Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 455, 967-970.