BET Inhibitors Target the SCLC-N subtype of Small Cell Lung Cancer by Blocking NEUROD1 Transactivation

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Supplemental files

Contents:

1. Supplemental Methods
2. Supplemental Table S1. The list of the SCLC cell lines (N=52) used in this study.
3. Supplemental Table S2. The list of 384 genes that constitute the NEUROD1 gene signature.
4. Supplemental Table S3. The siRNA screen results in H446 and COR-L279 cells.
5. Supplemental Table S4. Sensitivity (IC50) of SCLC lines (N=52) to JQ1 and NHWD-870.
6. Supplemental Figure S1. Knockout of NEUROD1 by CRISPR in H446 cells.
7. Supplemental Figure S2. Validation of the H446 NEUROD1 ChIP-seq.
8. Supplemental Figure S3. BET bromodomain proteins interact with NEUROD1 in H524 cells.
9. Supplemental Figure S4. BRD4 interacts with ASCL1 in H1963 cells.
10. Supplemental Figure S5. NEUROD1 KO affects the BRD4 occupancy only in the genomic regions co-occupied by NEUROD1.
11. Supplemental Figure S6. Neuronal-related pathways are enriched among the NEUROD1-target genes in H446 cells.
12. Supplemental Figure S7. Validation of the NEUROD1 gene signature using the SCLC RNA-seq dataset in the CCLE.
13. Supplemental Figure S8. Effects of BETi on NEUROD1 gene expression in H446 cells.
14. Supplemental Figure S9. The NEUROD1- and BRD4-loaded SEs identified in H446 control and NEUROD1-KO cells and their correlation.
15. Supplemental Figure S10. The SCLC-N lines are more susceptible to BETi.
16. Supplemental Figure S11. NEUROD1 KO renders DMS-273 cells resistant to BETi.
17. Supplemental Figure S12. Effects of NEUROD1 KO on MYC expression and transactivation.
18. Supplemental Figure S13. LSAMP expression is regulated by both NEUROD1 and BET bromodomain proteins.
19. Supplemental Figure S14. Correlation between the IC50 values of NHWD-870 and JQ1 in SCLC cell lines (N=28).

Supplemental Methods

RNA Isolation and Quantitative real-time PCR Assay. RNA was extracted from cells using an RNeasy Mini Kit (Qiagen), and cDNA was synthesized using an iScript cDNA Synthesis Kit (Bio-Rad). Quantitative gene expression analysis was performed on an ABI PRISM7500 real-time PCR system (ThermoFisher), using either TaqMan Fast Advanced Master Mix or SsoAdvanced™ Universal SYBR® Green Supermix (Biorad). The predesigned specific primers were purchased from ThermoFisher Scientific, Biorad, and Integrated DNA Technologies.

ChIP-seq – Peak Calling. An initial quality check of fastq files was performed using FastQC, and adapter sequences and low-quality base pairs were trimmed using Cutadapt. Trimmed reads were mapped to the human hg19 reference genome using the Backtrack algorithm of Burrows-Wheeler Aligner (BWA-Backtrack, RRID:SCR_010910) (1). Duplicated reads were filtered, and the peaks were called using the Model-based Analysis of ChIP-Seq (MACS, RRID:SCR_013291) with the corresponding input as control (2). Peaks of BRD2, BRD3, and BRD4 were called using default parameters, and NEUROD1 peak calling was performed using the parameters optimized for transcription factors (2). Genomic annotations of the NEUROD1 peaks were performed using the 'annotatePeaks' function of the Homer v4.11, RRID:SCR_010881 (3).

ChIP-seq – Metagene Analysis. The metagene profile of the NEUROD1 peaks was performed using the 'makeMetaGeneProfile' function of the Homer, with the NEUROD1 peaks as the input.

ChIP-seq – Peak Colocalization. Tag directories were first generated from the bam files of ChIP-seq samples and the corresponding input, using the 'makeTagDirectory' function of the Homer. Histograms on BET proteins occupancy within 2.5 KB bilateral of the centers of NEUROD1 peaks were generated using Homer's 'annotatePeaks' function with the parameter '-size 5000 -hist 10'. The results were plotted using the GraphPad Prism (v8.4.3).

ChIP-seq – Heatmap. BRD4 peaks colocalizing with NEUROD1 peaks were identified using Homer's 'mergePeaks' function with the setting '-d given'. Peak annotation was performed using Homer's 'annotatePeaks' function. The BRD4 peaks in the H446 control cells were separated into two

groups: those co-occupied by NEUROD1 and BRD4 and the others singly occupied by BRD4. The NEUROD1 or BRD4 occupancy near the BRD4 peaks was assessed using Homer's 'annotatePeaks' function, and the results were visualized in heatmaps using EaSeq (4).

ChIP-seq – Superenhancer (SE) Analysis. The H3K27Ac peaks were used to mark constituent enhancer sites in the H446 control cells and the NEUROD1-KO cells, and BRD4-loaded or NEUROD1-loaded SEs were called by ROSE (RRID:SCR_017390) using the default setting (5, 6). The identified SEs were annotated for the nearest genes using Homer's 'annotatePeaks' function.

ChIP-seq – de novo Motif Discovery. Motif discovery was performed using the 'findMotifsGenome' function of the Homer, with the setting of '-size 200 -mask' and the NEUROD1 peaks as the input.

Identification of NEUROD1-Target Genes. The NEUROD1 target genes were identified using the Binding and Expression Target Analysis (BETA, RRID:SCR_005396) algorithm (7). Input data were the NEUROD1 peaks identified from MACS and differential gene expression output from Limma (H446 NEUROD1-KO cells vs. control cells). The analysis was performed with the 'Plus' option in the setting of '--df 0.05 --bl --da 0.5'.

Western Blot Analysis. Whole-cell lysates were extracted using RIPA lysis buffer (Millipore), and membrane proteins were isolated using a Mem-PER Plus Membrane Protein Kit (ThermoFisher). Western blots were performed using a standard immunoblotting protocol. The antibodies and their dilutions were: NEUROD1 (BD Biosciences, #563000; RRID:AB_2737942; 1:2,000), BRD2 (Santa Cruz Biotechnology, #sc-393720; RRID:AB_430777; 1:200), BRD3 (Santa Cruz Biotechnology, #sc-81202; RRID: AB_1119692; 1:200), BRD4 (Novus, NBP2-52959, 1:1,000), LSAMP (Abcam, #ab89719, RRID:AB_2138207; 1:1,000), NaK-ATPase (Santa Cruz Biotechnology, #sc-71638, RRID:AB_1125500; 1:1,000), cleaved caspase 3 (Cell Signaling Technology, #9661, RRID:AB_2341188; 1:1,000), caspase 3 (Cell Signaling Technology, #9662, RRID:AB_331439; 1:1,000), cleaved caspase 9 (Cell Signaling Technology, #7237, RRID:AB_10895832; 1:1,000), caspase 9 (Cell Signaling Technology, #9502, RRID:AB_2068621; 1:1,000), Phos-ERK1/2 (Cell Signaling Technology, #9101, RRID:AB_331646; 1:1,000), ERK1/2 (Cell Signaling Technology, #9102, RRID:AB_330744; 1:2,000), MYC (Cell Signaling Technology, #5605, RRID:AB_1903938; 1:1,000), γ-tubulin (Sigma, #T6557, RRID:AB_477584; 1:10,000).

Co-immunoprecipitation (Co-IP) Assays. Nuclear extracts were prepared from H446, H524, and H1963 cells using a Nuclear Complex Co-IP Kit (Active Motif, #54001). Each IP, in a final volume of 500 µl, contained 200-500 µg nuclear extract, an antibody against the protein of interest (αBRD4, Bethyl, A301-985A, RRID:AB_1576498, 5 µg; αNEUROD1, Cell Signaling Technology, #4373, RRID:AB_10549071, 10 µl; αBRD2, Cell Signaling Technology, #5848, RRID:AB_10835146, 10 µl; αASCL1, BD Biosciences, #556604, RRID:AB_396479, 5 µg) or control IgG (Cell Signaling Technology, #3900, RRID:AB_1550038, 5 µg), 20 µl slurry of Magna ChIP protein A magnetic beads or Magna ChIP protein G magnetic beads, Millipore), and 1x IP High buffer from the Nuclear Complex Co-IP Kit supplemented with a protease inhibitor cocktail (Sigma). After overnight mixing on an orbital shaker at 4°C, antibody/antigen/bead complexes were washed three times with 1x High Buffer supplemented with 1mg/ml bovine serum albumin (BSA), followed by three washes with 1x High Buffer without BSA. The immunoprecipitated protein complexes were eluted by mixing the washed beads with Laemmli buffer supplemented with 5% 2-mercaptoethanol (Sigma) after heating at 95 °C for 5 min. The presence of the protein of interest was detected by western blot.

Cell Proliferation Assay and Viability Assay. After trypsinization, cell suspensions were passed through 40 µm cell strainers. Five thousand cells in 100 µl growth media were seeded into each well of a 96-well white plate with a clear bottom (Corning, #3610). Alternatively, 750 cells in 15 µl were seeded into each well of a 384-well white plate with a clear bottom (Corning, #3765).

For cell proliferation assay, cell viability was checked the next day (D1) and then every two days using CellTiter-Glo® 2.0 Reagent (Promega). A reflective foil seal (Bio-Rad, #MSF1001) was applied to the bottom of each plate to maximize the signal. The results were normalized to the reading on day 1. Four replicates were used for each data point.

For cell viability assay, JQ1 (Sigma), OTX-015 (Selleckchem) or NHWD-870 (a kind gift from Nenghui Wang) were serially diluted 2-fold in DMSO to generate nine consecutive concentrations. The compounds were first diluted 100 times in culture media, and the diluted drug solution was then delivered to the cells at 10% of the final volume to achieve a total of 1,000-time dilutions. After brief mixing, cells were incubated for 72 hours before cell viability was measured. Four replicates were used for each dose of drug treatment.

MYC reporter assay. An MYC Firefly luciferase reporter (pBV-Luc wt MBS1-4, Addgene plasmid #16564, RRID:Addgene_16564; a gift from Bert Vogelstein (8)) and a Renilla reporter (pRL-CMV; Promega) were transfected into H446 NEUROD1-KO and control cells at 8:1 ratio by lipofection. One day after transfection, the cells were trypsinized and seeded into a 384-well plate at 750 cells per well, and JQ1 treatment was administered subsequently. The Firefly and Renilla luciferase activities were measured at 24- and 48-hour intervals using a Dual-Glo Luciferase assay system (Promega). Four replicates were used for each dose.

References:

1. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinform* 2009;25:1754-1760.
2. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, et al. Model-based Analysis of ChIP-Seq (MACS). *Genome Biol* 2008;9:R137.
3. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 2010;38:576-589.
4. Lerdru M, Johansen JJ, Agrawal-Singh S, Hansen K. An interactive environment for agile analysis and visualization of ChIP-sequencing data. *Nat Struct Mol Biol*. 2016, 23:349-357.
5. Lovén J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc C, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 2013;153:320-334.
6. Whyte Warren A, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 2013;153:307-319.
7. Wang S, Sun H, Ma J, Zang C, Wang C, Wang J, et al. Target analysis by integration of transcriptome and ChIP-seq data with BETA. *Nat Protoc* 2013;8:2502-2515.
8. Hermeking H, Rago C, Schuhmacher M, Li Q, Barrett JF, Obaya AJ, et al. Identification of CDK4 as a target of c-MYC. *Proc Natl Acad Sci USA* 2000;97:2229-2234.

Supplemental Table S1. The list of the SCLC cell lines (N=52) used in this study.

Cell Line	RRID	Subtype	Source*	Catalog #	Growth media
COLO-668	CVCL_1128	ASCL1	Sigma-Aldrich	87061209	RPMI + 10% FBS
COR-L47	CVCL_2415	ASCL1	Sigma-Aldrich	92031915	RPMI + 10% FBS
COR-L88	CVCL_1141	ASCL1	Sigma-Aldrich	92031917	RPMI + 10% FBS
DMS 454	CVCL_2438	ASCL1	Sigma-Aldrich	95062832	Waymouth + 1x glutamax + 10% FBS
DMS 53	CVCL_1177	ASCL1	ATCC	CRL-2062	RPMI + 10% FBS
DMS 79	CVCL_1178	ASCL1	Sigma-Aldrich	95062824	RPMI + 10% FBS
H1092	CVCL_1454	ASCL1	ATCC	CRL-5855	HITES† + 5% FBS
H1105	CVCL_1455	ASCL1	ATCC	CRL-5856	HITES† + 5% FBS
H1417	CVCL_1469	ASCL1	ATCC	CRL-5872	RPMI + 10% FBS
H1436	CVCL_1471	ASCL1	ATCC	CRL-5871	HITES† + 5% FBS
H146	CVCL_1473	ASCL1	ATCC	HTB-173	RPMI + 10% FBS
H1836	CVCL_1498	ASCL1	ATCC	CRL-5898	HITES† + 5% FBS
H187	CVCL_1501	ASCL1	ATCC	CRL-5804	RPMI + 10% FBS
H1876	CVCL_1503	ASCL1	ATCC	CRL-5902	HITES† + 5% FBS
H1882	CVCL_1504	ASCL1	ATCC	CRL-5903	HITES† + 5% FBS
H1930	CVCL_1507	ASCL1	ATCC	CRL-5906	RPMI + 10% FBS
H1963	CVCL_1510	ASCL1	ATCC	CRL-5982	RPMI + 10% FBS
H2029	CVCL_1516	ASCL1	ATCC	CRL-5913	HITES† + 5% FBS
H2081	CVCL_1522	ASCL1	ATCC	CRL-5920	HITES† + 5% FBS
H209	CVCL_1525	ASCL1	ATCC	HTB-172	RPMI + 10% FBS
H2107	CVCL_1527	ASCL1	ATCC	CRL-5983_FL	HITES† + 5% FBS
H2141	CVCL_2653	ASCL1	ATCC	CRL-5927	HITES† + 5% FBS
H2196	CVCL_1539	ASCL1	ATCC	CRL-5932	HITES† + 5% FBS
H2227	CVCL_1542	ASCL1	ATCC	CRL-5934	HITES† + 5% FBS
H345	CVCL_1558	ASCL1	ATCC	HTB-180	HITES† + 5% FBS
H378	CVCL_1560	ASCL1	ATCC	CRL-5808	RPMI + 10% FBS
H510A	CVCL_1565	ASCL1	ATCC	HTB-184	F12K + 10% FBS
H64	CVCL_1573	ASCL1	ATCC	CRL-5976	HITES† + 5% FBS
H69	CVCL_1579	ASCL1	ATCC	HTB-119	RPMI + 10% FBS

Cell Line		Subtype	Source*	Catalog #	Growth media‡
H889	CVCL_1598	ASCL1	ATCC	CRL-5817	RPMI + 10% FBS
HCC-33	CVCL_2058	ASCL1	ATCC	ACC 487	RPMI + 20% FBS
COR-L279	CVCL_1140	NEUROD1	Sigma-Aldrich	96020724	RPMI + 10% FBS
CPC-N	CVCL_1146	NEUROD1	DSMZ	ACC 306	McCoy's 5A + 10% FBS
DMS 273	CVCL_1176	NEUROD1	Sigma-Aldrich	95062830	Waymouth + 1x glutamax + 10% FBS
H1694	CVCL_1489	NEUROD1	ATCC	CRL-5888	HITES† + 5% FBS
H2066	CVCL_1520	NEUROD1	ATCC	CRL-5917	HITES† + 5% FBS
H2171	CVCL_1536	NEUROD1	ATCC	CRL-5929	HITES† + 5% FBS
H446	CVCL_1562	NEUROD1	ATCC	HTB-171	RPMI + 10% FBS
H524	CVCL_1568	NEUROD1	ATCC	CRL-5831	RPMI + 10% FBS
H82	CVCL_1591	NEUROD1	ATCC	HTB-175	RPMI + 10% FBS
SCLC-21H	CVCL_0024	NEUROD1	DSMZ	ACC 372	DMEM + 10% FBS
SCLC-22H	CVCL_2186	NEUROD1	DSMZ	ACC 373	DMEM + 10% FBS
COR-L311	CVCL_2412	POU2F3	Sigma-Aldrich	96020721	RPMI + 10% FBS
H1048	CVCL_1453	POU2F3	ATCC	CRL-5853	HITES† + 5% FBS
H211	CVCL_1529	POU2F3	ATCC	CRL-5824	RPMI + 10% FBS
H526	CVCL_1569	POU2F3	ATCC	CRL-5811	RPMI + 10% FBS
DMS 114	CVCL_1174	YAP1	ATCC	CRL-2066	Waymouth + 1x glutamax + 10% FBS
H1341	CVCL_1463	YAP1	ATCC	CRL-5864	ACL-4‡ + 5% FBS
H196	CVCL_1509	YAP1	ATCC	CRL-5823	RPMI + 10% FBS
H841	CVCL_1595	YAP1	ATCC	CRL-5845	HITES† + 5% FBS
SBC3	CVCL_1678	YAP1	JCRB	JCRB0818	EMEM + 10% FBS
SBC5	CVCL_1679	YAP1	JCRB	JCRB0819	EMEM + 10% FBS

*ATCC, American Type Culture Collection; DSMZ, German Collection of Microorganisms and Cell Cultures; JCRB, Japanese Collection of Research Bioresources Cell Bank.

† HITES contains DMEM:F12 medium (ATCC, #30-2006; 500 ml), Insulin (Sigma, #I1882; 0.005mg/ml), apo-transferrin (Sigma, #T5391 or T1428; 0.01 mg/ml), sodium selenite (Sigma, #S9133; 30 nM), hydro-cortisone (Sigma, #H0135; 10nM), β-estradiol (Sigma, #E2257; 10nM), glutamax (Gibco, #35050-061; 1x).

‡ ACL4 contains DMEM:F12 medium (ATCC, #30-2006; 500 ml), insulin (Sigma, #I1882; 0.02mg/ml), apo-transferrin (Sigma, #T5391 or T1428; 0.01 mg/ml), sodium selenite (Sigma, #S9133; 25 nM), hydrocortisone (Sigma, #H0135; 50nM), EGF (Sigma, #SRP3027; 1ng/ml), glutamax (Gibco, #35050-061; 1x), ethanolamine (Sigma, #E0135; 0.01mM), phosphorylethanolamine (Sigma, #P0503; 0.01mM), triiodothyronine (Sigma, #T6397; 100pM), HEPES (Sigma, #H0887; 10mM), sodium pyruvate (Gibco, #11360-070; 0.5mM).

Supplemental Table S2. The list of 384 genes that constitute the NEUROD1 gene signature.

NELL1	STAB2	LBX1-AS1	COLCA2	KCNJ3	PROX1	FGF12	PPP1R16B	FGD4	SHF	KCNC4-AS1
GLI2	AMER3	TOX3	RASSF5	VWC2	C1orf194	CCSER1	NAALADL2	KIAA0408	NRSN1	NAALADL2-AS2
LRRTM2	C6orf99	STK32A	MEGF10	LRTM2	FRMD3	GPR19	STARD13-AS	TACR2	WSCD1	MAMDC2-AS1
LRRN1	LINC00907	ANKRD33B	OTOR	KCNH2	JAKMIP2	NEUROD2	LINC00940	TSPAN15	HES6	WWTR1-AS1
ALPL	CHODL	C1orf220	MLLT4-AS1	RHBDL3	PCSK1	KLHL32	LINC00989	CERKL	RPRML	
RHOJ	RXRG	DOK5	BMF	THRSP	NEUROD1	RIPPLY2	SEC16B	RAB39A	CAMK4	
ACE	IGDCC3	ITGA9	HEPACAM2	WNT16	GPR68	P4HA2	CXCL11	EDA	CPLX2	
SYK	AKNAD1	IGSF21	KCNH6	L1CAM	KCND3	LINC00052	ENC1	GNG4	GRIA2	
ROBO4	SLC18A1	KLF1	USH1G	ARHGEF28	ONECUT2	DUSP5	MAMDC2	CBFA2T3	LINC00948	
DACT1	TMEM179	C1QTNF7	TMEM178A	ZNF704	RFPL1S	KALRN	CPO	CORO2B	ARHGAP15	
TAGAP	PTPRT	CELF2	NES	SLC18A3	HNMT	NPTXR	ENHO	VWC2L	TMEM132A	
FNDC1	MGAT5B	KIAA1462	CXCL12	DCHS1	INA	ADRA2A	CXADR	PAK1	PHACTR3	
VWA5B2	CNBD2	HCN1	FHL5	FIGF	OAS3	SSTR2	RUNDC3A	AK5	ZDHHC22	
EXOC3L1	PWWP2B	FRMPD4	PHACTR2	CKMT1A	DRD2	SLCO4C1	OAS1	PCSK2	SNCAIP	
RASGRP1	IL12RB1	PRELP	S100A5	SNTG1	PGBD5	CLSTN2	STMN3	LRP8	PRKG2	
CDH20	PHACTR1	LINC00087	CACNA2D4	PCDH8	KCNH8	ATOH8	SH3GL2	HS3ST5	CBFA2T2	
WSCD2	OTOGL	TUBA3FP	PTCHD2	NTF3	NYAP2	ADAMTS14	HRH3	KCTD16	MARCH4	
VEPH1	SLC6A17	ISLR2	SLC6A3	MC5R	KIAA1324	NAA11	GRIK2	VLDLR	UNC13C	
ALOX5	RAB37	RTBDN	SEMA5B	STARD13	GRIK3	DCX	FLRT3	LRRN2	OPRD1	
IQSEC3	ZC2HC1B	COL16A1	SYNGR4	VPS37D	ADAM11	TLE6	C8orf46	COL19A1	CBLN1	
KCNF1	FCN2	ADCYAP1R1	ADAMTS5	KCNAB1	LINC00642	MAP6	BAI2	KIAA1456	KCNB2	
SPOCK3	GDNF	TMEM151B	KCTD12	POU2AF1	MAPT	RASL11B	LINC00348	GABRP	STRADB	
HK3	DUOX1	SLC6A11	MYO7A	NXPH2	GOLGA7B	RNF112	ANKRD30B	FOXO6	CHGB	
FAM181B	LAMC2	CDH15	KCNC2	RALYL	CNR1	BCAN	LINC00951	SRRM4	LPPR1	
S100A3	FAM78B	NEUROD4	PTX3	GALNT5	CABP7	HLF	XKR7	GLCE	NAPB	
C15orf59	LRRC10B	LBX1	PPP1R17	KCNS2	TMEM63C	SYTL3	SYNPR	CAMKK1	STX1A	
LRMP	OLFML3	OLFM1	PRIMA1	PDZD4	NYNRIN	C11orf53	RTN1	PRDM8	OLFM3	
HIF3A	PPP1R14A	HTR1B	CCM2L	SYNGR1	GADD45G	TESC	KIF1A	GOLGA7B	FAM19A5	
BAI1	SYT14	ELMO1	CRYM	MFAP4	ACSL6	ASXL3	GPR12	TMOD2	DCC	
FOXN4	SPHKAP	SLC4A10	OLFM4	GPR112	SNCB	C1orf95	TDRG1	DISC1	STXBP5L	
EGFEM1P	FAM196A	NTRK1	CNTN2	MRAP2	SYN2	KCNMB4	RELL1	CHD5	ST8SIA3	
DYSF	ACE2	APCDD1	SYNDIG1	MPPED1	IGSF11	PPM1L	EIF4E1B	PREX1	NPTX1	
SYT4	FRMPD3	WDR72	TEX35	FAT3	ARPP21	TPH2	COLCA1	UNC5A	CRMP1	
WNT5A	CDCP1	SAG	ASPDH	TCF15	FUT9	SOX5	FBLL1	SYT2	CHGA	
GAP43	PAPPA2	MYOZ3	RAP1GAP	SLC15A3	SNAP91	XKR4	BAI3	CSRNP3	NXPH1	
TSHZ2	VSTM4	KIAA1549L	SPTBN4	GPR111	KCNH7	CDH7	ARHGEF3	STMN2	GNAO1	
CRHR1	SYT6	CXCR4	LINC01088	S100A4	CKMT1B	GPR85	PLD5	C1orf87	SCG3	
MYBPHL	SPON2	RLN1	ANGPTL1	LURAP1L	GNAZ	GK2	PDE9A	RPH3A	KIAA1614	

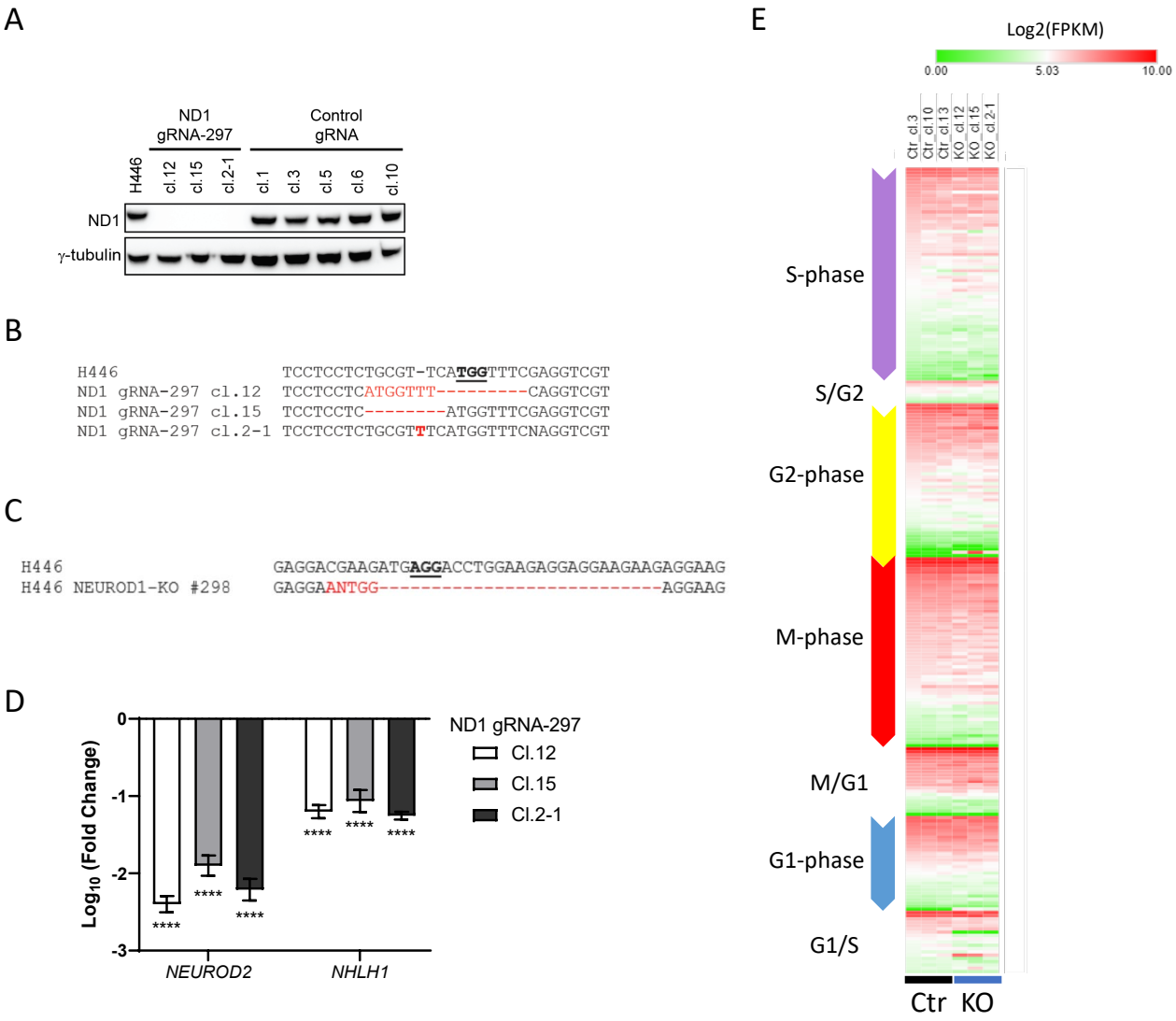
Supplemental Table S3. The siRNA screen results in H446 and COR-L279 cells. The averages show the cell numbers of the siRNA-treated samples relative to non-targeting siRNA control. Stdev shows the standard deviation of three replicates.

	H446				COR-L279			
	siRNA 1		siRNA 2		siRNA 1		siRNA 2	
Target	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
BLNK	0.667	0.037	0.682	0.019	0.269	0.024	0.881	0.024
TSPAN18	0.562	0.028	0.974	0.034	0.408	0.017	0.952	0.021
SHISA9	1.354	0.081	1.021	0.074	1.004	0.047	0.809	0.014
MC5R	0.388	0.041	0.938	0.023	0.222	0.007	0.780	0.010
PHEX	1.306	0.104	0.875	0.108	1.107	0.035	0.543	0.017
ANK2	0.761	0.036	0.440	0.021	0.801	0.040	0.335	0.027
FRMD4A	1.163	0.033	0.761	0.030	1.079	0.045	0.689	0.010
ARHGEF28	0.912	0.057	1.319	0.026	1.185	0.060	1.157	0.062
GPR68	0.989	0.073	0.436	0.026	1.505	0.027	0.273	0.003
SEMA5B	0.799	0.036	1.172	0.155	0.830	0.009	1.101	0.021
SLC9A9	0.989	0.041	0.573	0.021	0.319	0.007	0.590	0.027
GAS2	0.889	0.026	0.973	0.026	0.883	0.029	0.876	0.029
PTPRZ1	0.585	0.026	0.741	0.070	0.153	0.011	0.788	0.029
HNMT	0.711	0.069	1.404	0.080	0.678	0.048	1.276	0.063
COL16A1	0.714	0.068	1.094	0.076	0.570	0.033	0.872	0.053
NDP	0.903	0.108	0.892	0.065	0.797	0.028	0.939	0.028
CNR1	0.992	0.055	1.134	0.120	1.135	0.022	0.956	0.032
TTC30B	0.536	0.051	1.059	0.048	0.367	0.017	1.157	0.096
LSAMP	0.794	0.064	0.701	0.019	0.717	0.065	0.403	0.014
BRINP1	1.165	0.033	0.560	0.038	0.856	0.024	0.579	0.010
CORO2B	1.126	0.078	1.097	0.028	1.011	0.030	0.487	0.028
COL8A2	1.075	0.019	0.417	0.035	1.185	0.135	0.420	0.012
CDH5	1.098	0.020	1.212	0.051	0.785	0.032	1.304	0.039
COL19A1	0.672	0.011	0.757	0.025	0.470	0.026	0.358	0.014
ROBO2	1.014	0.031	0.591	0.013	1.053	0.048	0.221	0.004
VLDLR	0.738	0.096	0.579	0.092	0.299	0.030	0.181	0.028
CERKL	1.125	0.080	1.305	0.048	1.044	0.020	1.274	0.045
SLC15A3	0.633	0.027	0.368	0.015	0.182	0.009	0.475	0.036
TACR2	0.360	0.004	0.717	0.059	0.269	0.006	0.718	0.069
LRRN2	0.370	0.033	0.580	0.026	0.189	0.008	0.521	0.016
GABRA1	1.049	0.079	0.913	0.043	1.274	0.028	0.704	0.025
ARHGAP15	1.135	0.058	0.986	0.055	1.288	0.079	0.985	0.020
MYT1L	0.974	0.041	0.140	0.008	0.951	0.022	0.189	0.022
PHACTR3	1.246	0.041	1.023	0.057	0.854	0.034	0.958	0.033
FREM1	1.161	0.060	0.809	0.071	1.117	0.020	0.387	0.023
FAM19A5	0.986	0.079	1.094	0.050	0.816	0.024	1.191	0.028
CHRNA3	0.621	0.031	0.634	0.016	0.562	0.031	1.107	0.040
FAM181B	0.446	0.008	0.735	0.014	0.464	0.011	0.794	0.008
ST18	1.068	0.075	0.558	0.024	0.987	0.052	0.293	0.018
GRIK3	0.544	0.048	0.730	0.018	0.608	0.016	0.645	0.010
CA10	0.998	0.059	1.004	0.060	0.772	0.050	1.284	0.054
OAS1	0.522	0.060	0.643	0.020	0.334	0.012	0.478	0.039
RPH3A	0.743	0.063	1.194	0.066	0.719	0.030	0.964	0.009
SEZ6L	1.146	0.043	1.025	0.044	1.337	0.034	0.753	0.021
SYT4	0.927	0.034	0.100	0.018	0.255	0.014	0.259	0.017

Supplemental Table S4. Sensitivity (IC_{50}) of SCLC lines (N=52) to JQ1 and NHWD-870.

Cell Line	JQ1 IC_{50} (μM)	NHWD-870 IC_{50} (μM)	Cell Line	JQ1 IC_{50} (μM)	NHWD-870 IC_{50} (μM)
H446	0.5	0.011	COR-L47	1.7	nd
SCLC-21H	0.19	0.014	COR-L88	> 20	nd
H2066	0.23	0.015	CPC-N	2.97	nd
H1876	0.44	0.027	DMS-273	0.15	nd
COR-L311	0.495	0.037	DMS454	8	nd
H187	0.74	0.039	DMS53	1	nd
H2227	1	0.05	DMS79	> 20	nd
H211	1.1	0.055	H1092	> 20	nd
H2171	0.82	0.057	H1341	1	nd
COR-L279	0.54	0.064	H146	0.67	nd
H1694	0.74	0.104	H1882	> 20	nd
H64	3.8	0.18	H196	> 20	nd
H1417	2	0.203	H1963	0.83	nd
H524	3.5	0.318	H2081	17	nd
H209	1.2	0.5	H2107	5.1	nd
DMS114	1.5	0.74	H345	> 20	nd
H2029	4.7	0.74	H526	0.75	nd
H2141	7.84	0.805	H69	1.8	nd
H1436	20	3	H82	0.74	nd
H1930	14.5	3.83	H889	> 20	nd
H2196	16	4.55	HCC-33	1.6	nd
H510A	10	4.55	SBC3	0.6	nd
H378	6.6	4.94	SBC5	>20	nd
COLO-668	> 20	5.58	SCLC-22H	0.44	nd
H1105	> 10	10.7			
H1048	> 20	15.5			
H1836	> 20	16.9			
H841	>20	>20			

nd, not determined

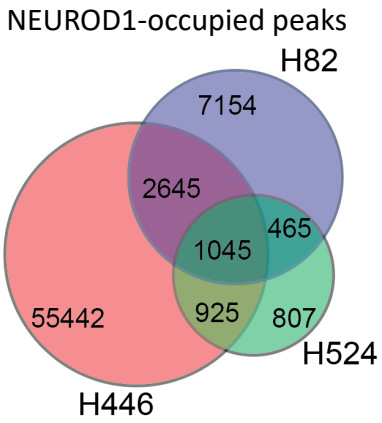


Supplemental Figure S1. Knockout of NEUROD1 by CRISPR in H446 cells. A) Western blots show a complete loss of NEUROD1 protein expression in three NEUROD1-KO clones (cl. 12, cl. 15, and cl. 2-1) receiving the gRNA #297. **B-C)** Sanger sequencing identified indels (highlighted in red) near PAM sequences (underlined) in the NEUROD1's coding sequence in the KO clones receiving the gRNA #297 (B) or the gRNA #298 (C). **D)** Expression of two NEUROD1-target genes (*NEUROD2* and *NHLH1*) in three H446 NEUROD1-KO clones (gRNA-297: cl. 12, cl. 15, and cl. 2-1) as measured by quantitative real-time PCR. **E)** A heatmap comparing the expression of cell-cycle signature genes (27) among H446 NEUROD1-KO and control cell clones (three clones for each group). Ctr, control; gRNA, guide RNA; KO, knockout; ND1, NEUROD1.

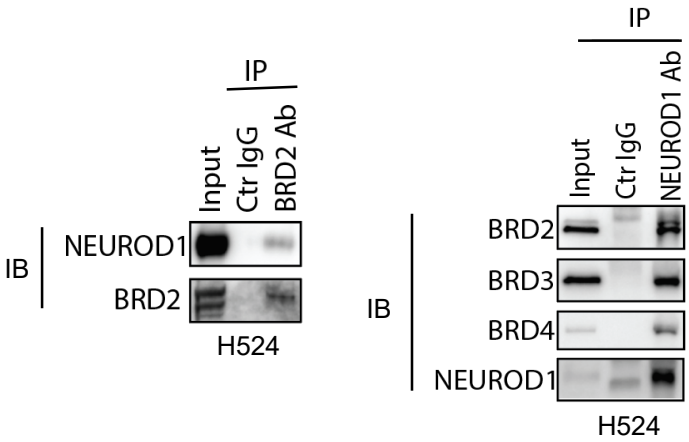
A

Rank	Motif	P-value	log P-pvalue	% of Targets	Best Match
1		1e-18233	-4.199e+04	73.08%	NEUROG2/NEUROD1
2		1e-3487	-8.031e+03	46.82%	NFIX
3		1e-664	-1.530e+03	7.58%	JunB
4		1e-556	-1.282e+03	8.39%	ASCL1
5		1e-521	-1.201e+03	21.82%	FOXA1
6		1e-205	-4.742e+02	13.85%	EMX2

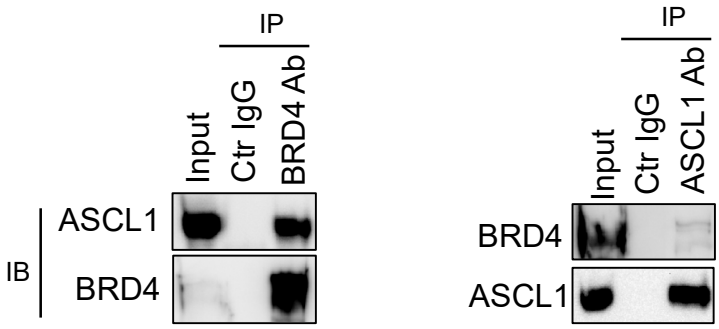
B



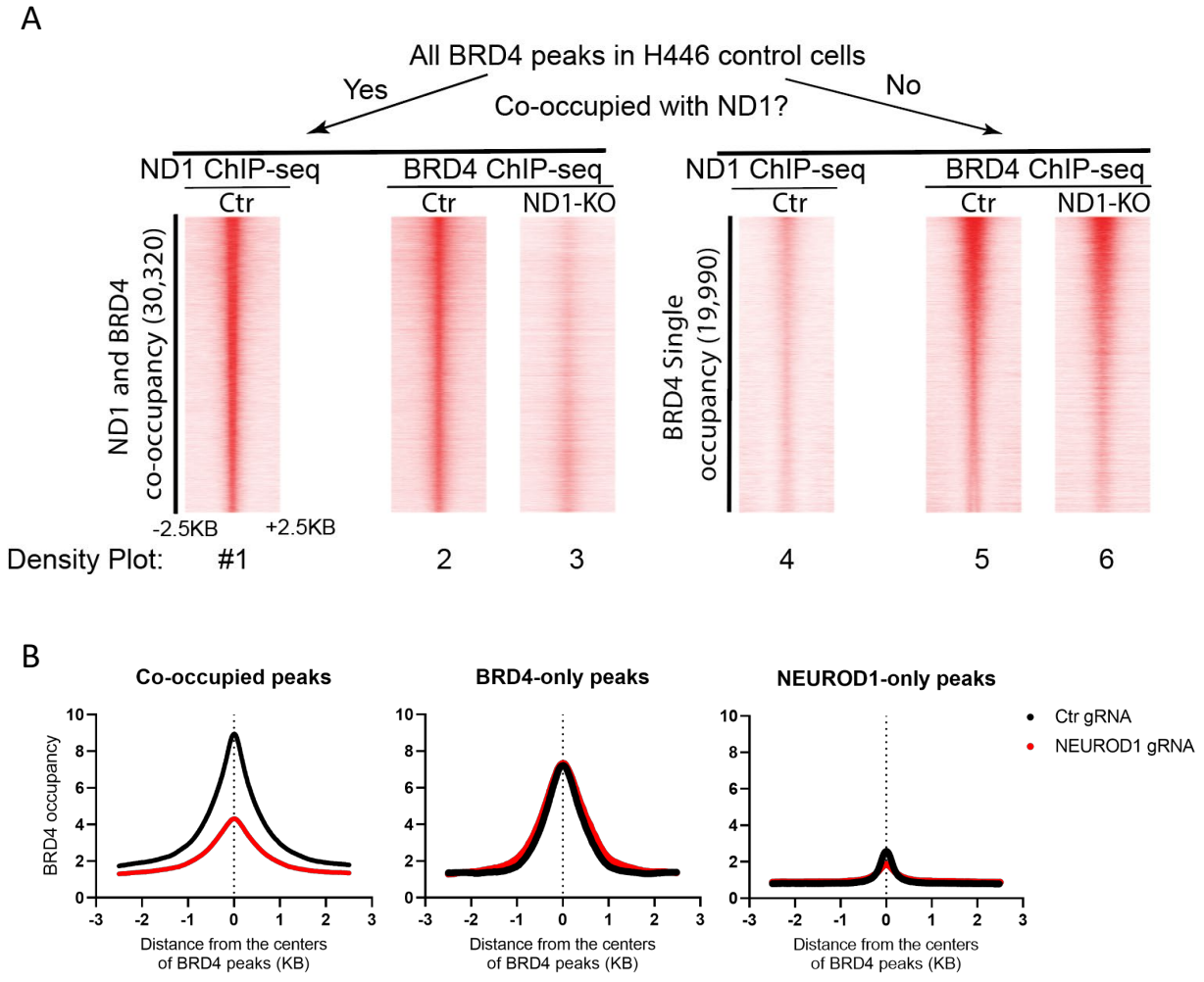
Supplemental Figure S2. **Validation of the H446 NEUROD1 ChIP-seq. A)** The top six motifs identified in the genomic sequences corresponding to the NEUROD1 peaks in H446 control cells. De novo motif discovery analysis was performed using the Homer. **B)** Comparison of the NEUROD1-occupied peaks among H446, H82, and H524 cells. The raw data files of NEUROD1 ChIP-seq in H82 (GSM1700641) and H524 (GSM1700642) were downloaded from NCBI GEO archive, and the NEUROD1-occupied peaks were identified using MACS. The overlaps of the NEUROD1 peaks among these SCLC-N lines were analyzed using the Homer.



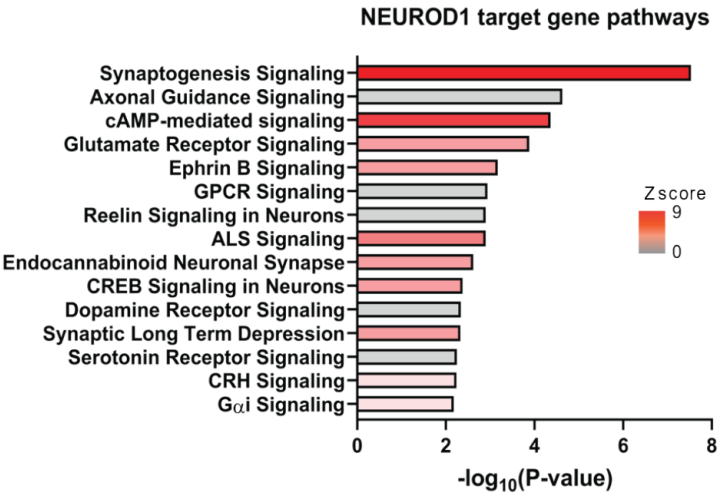
Supplemental Figure S3. **BET bromodomain proteins interact with NEUROD1 in H524 cells.** Nuclear extracts from H524 cells were subject to BRD2 (left) or NEUROD1 (right) immunoprecipitation (IP), and the presence of NEUROD1 (left) and BET bromodomain proteins (right) in the elute was detected by immunoblotting (IB). Ab, antibody.



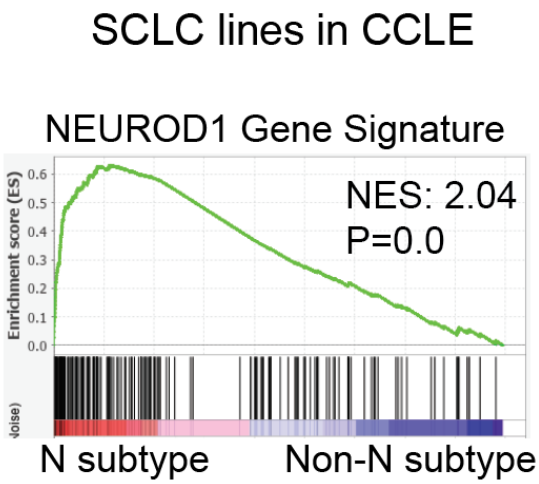
Supplemental Figure S4. **BRD4 interacts with ASCL1 in H1963 cells.** Nuclear extracts from H1963 cells were subject to BRD4 (left) or ASCL1 (right) immunoprecipitation (IP), and the presence of ASCL1 (left) and BRD4 (right) in the elute was detected by immunoblotting (IB). Ab, antibody.



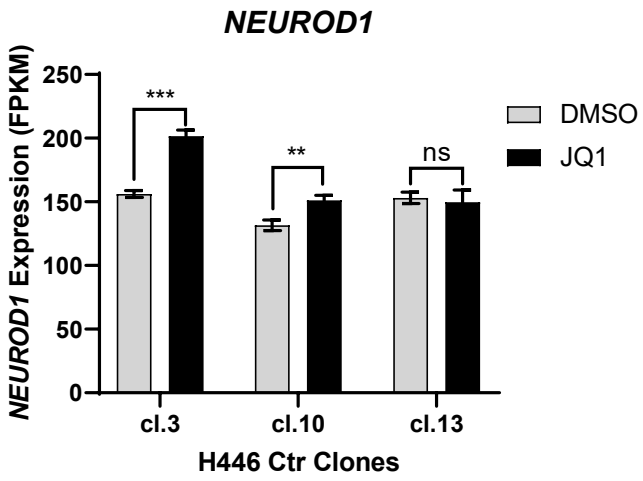
Supplemental Figure S5. **NEUROD1 KO affects the BRD4 occupancy only in the genomic regions co-occupied by NEUROD1. A)** BRD4 peaks in H446 control cells were separated into two groups on the basis of NEUROD1 (ND1) co-occupancy (density plot #1 vs. #4). NEUROD1 KO affected BRD4 occupancy in the NEUROD1/BRD4-cooccupied genomic regions (#2 vs. #3) but not in the BRD4 singly-occupied areas (#5 vs. #6). **B)** Aggregate signal plots showing the effects of NEUROD1 KO on BRD4 occupancy in the genomic regions co-occupied by NEUROD1 and BRD4 (left), singly occupied by BRD4 (middle), and singly occupied by NEUROD1 (right).



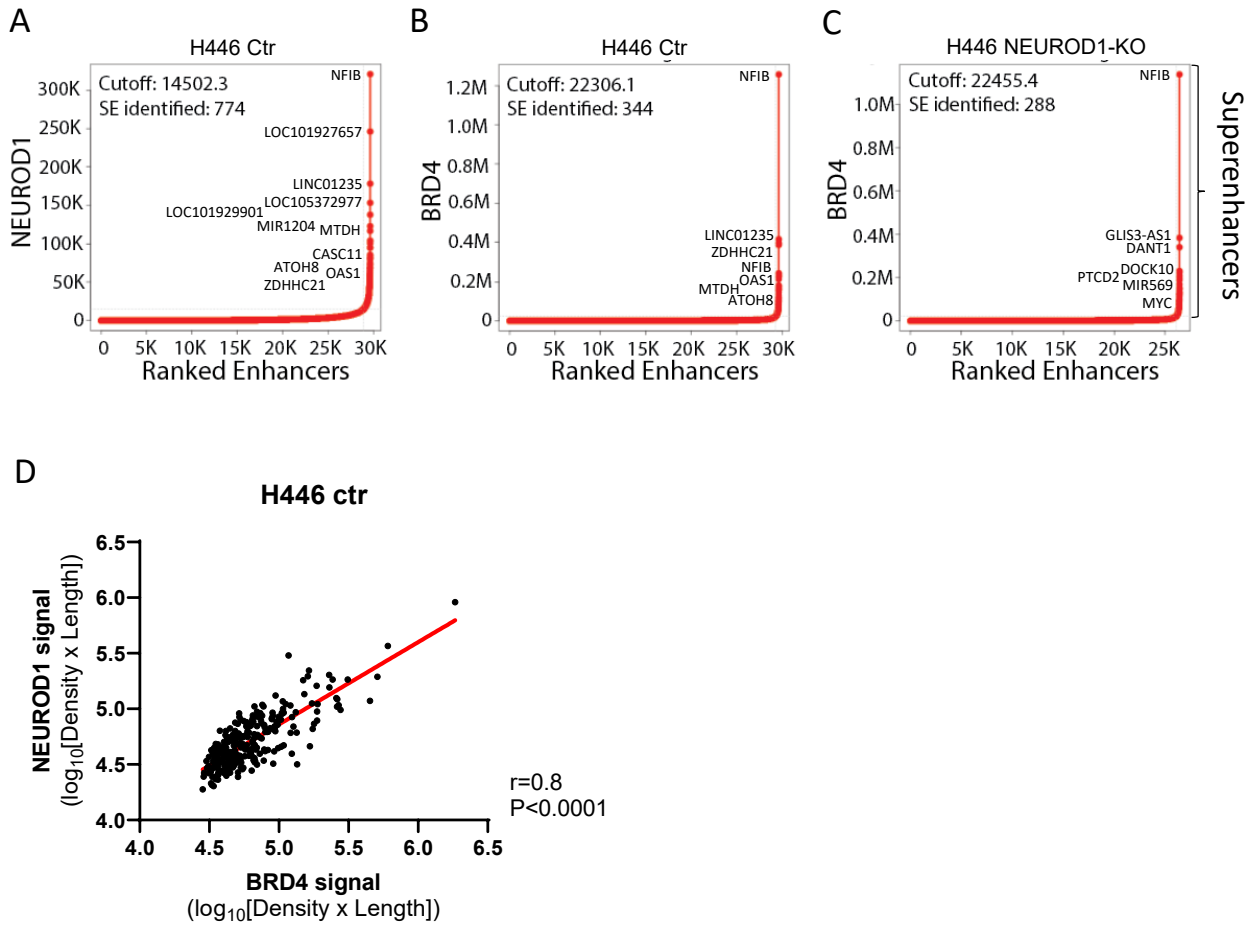
Supplemental Figure S6. Neuronal-related pathways are enriched among the NEUROD1-target genes in H446 cells. The top 15 pathways enriched among the NEUROD1-target genes as identified by the Ingenuity Pathway Analysis (RRID:SCR_008653). ALS, amyotrophic lateral sclerosis; CREB, cAMP response element-binding protein; CRH, corticotrophin-releasing hormone; GPCR, G protein coupled receptor.



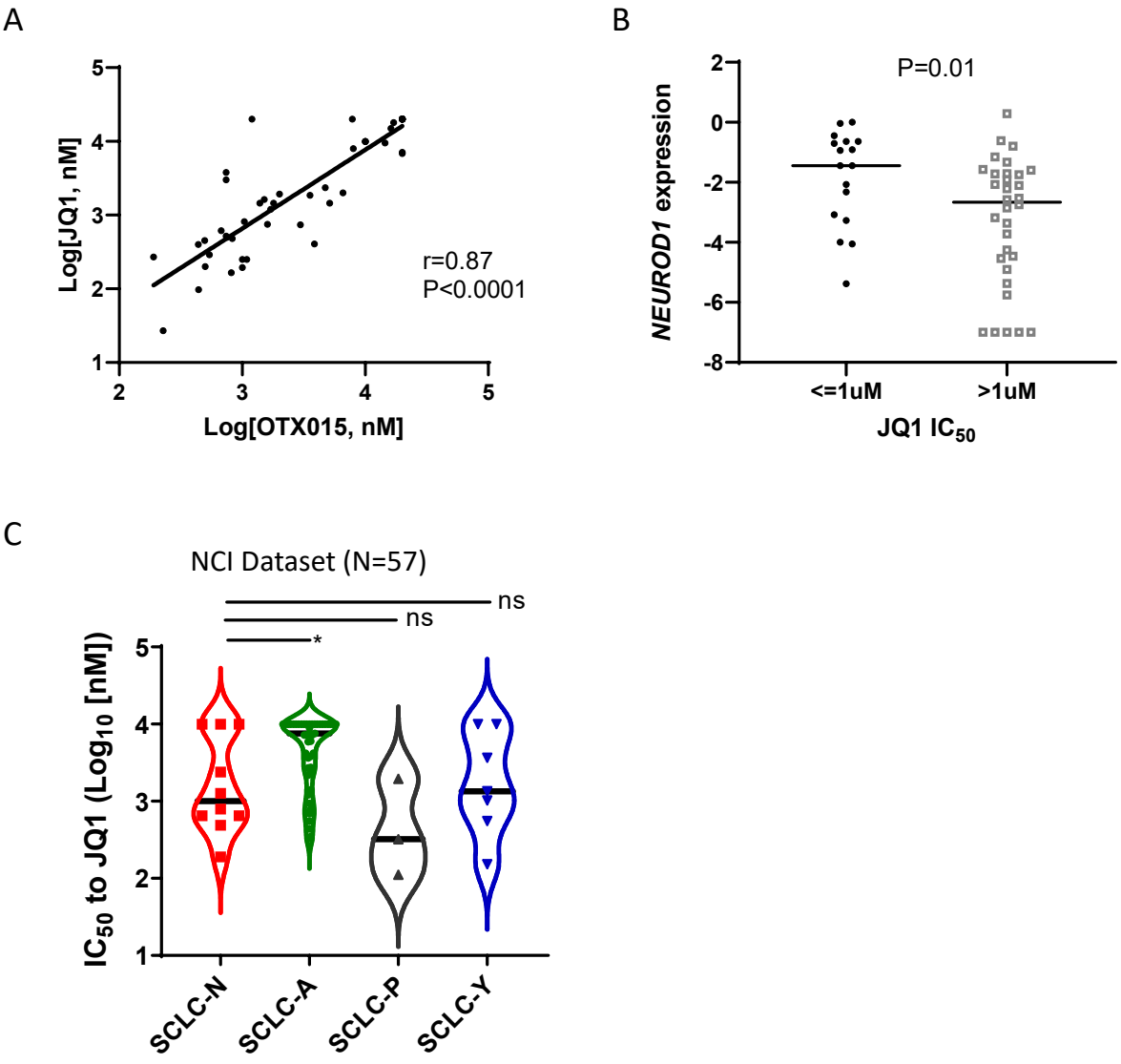
Supplemental Figure S7. Validation of the NEUROD1 gene signature using the SCLC RNA-seq dataset in the CCLE. A GSEA plot showing enrichment of the NEUROD1 gene signature in the SCLC-N cell lines (N subtype) compared to all other SCLC lines (non-N subtype) in the Cancer Cell Line Encyclopedia (CCLE, RRID:SCR_013836). NES, normalized enrichment score.



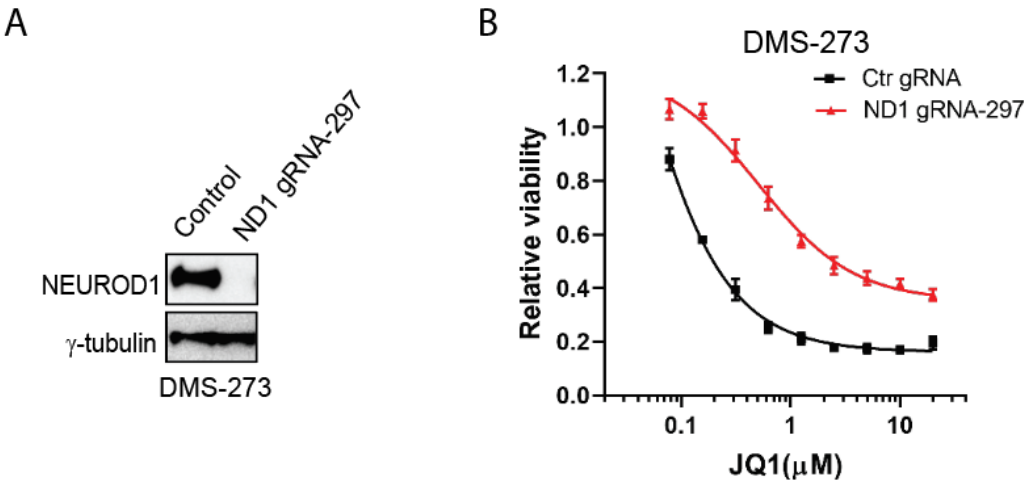
Supplemental Figure S8. Effects of BETi on NEUROD1 gene expression in H446 cells. The bar graph shows the transcript levels of *NEUROD1* in three H446 control clones following JQ1 treatment (1 μM, 24 hrs). The data were extracted from the RNA-seq dataset shown in Figure 3C and Supplemental Figure S1E. The significance of the two-group comparison was determined using the Student's t-test with multiple test correction through a false discovery rate approach. **, P<0.01; ***, P<0.001; ns, not significant. Ctr, control; FPKM, Fragments Per Kilobase of transcript per Million mapped reads.



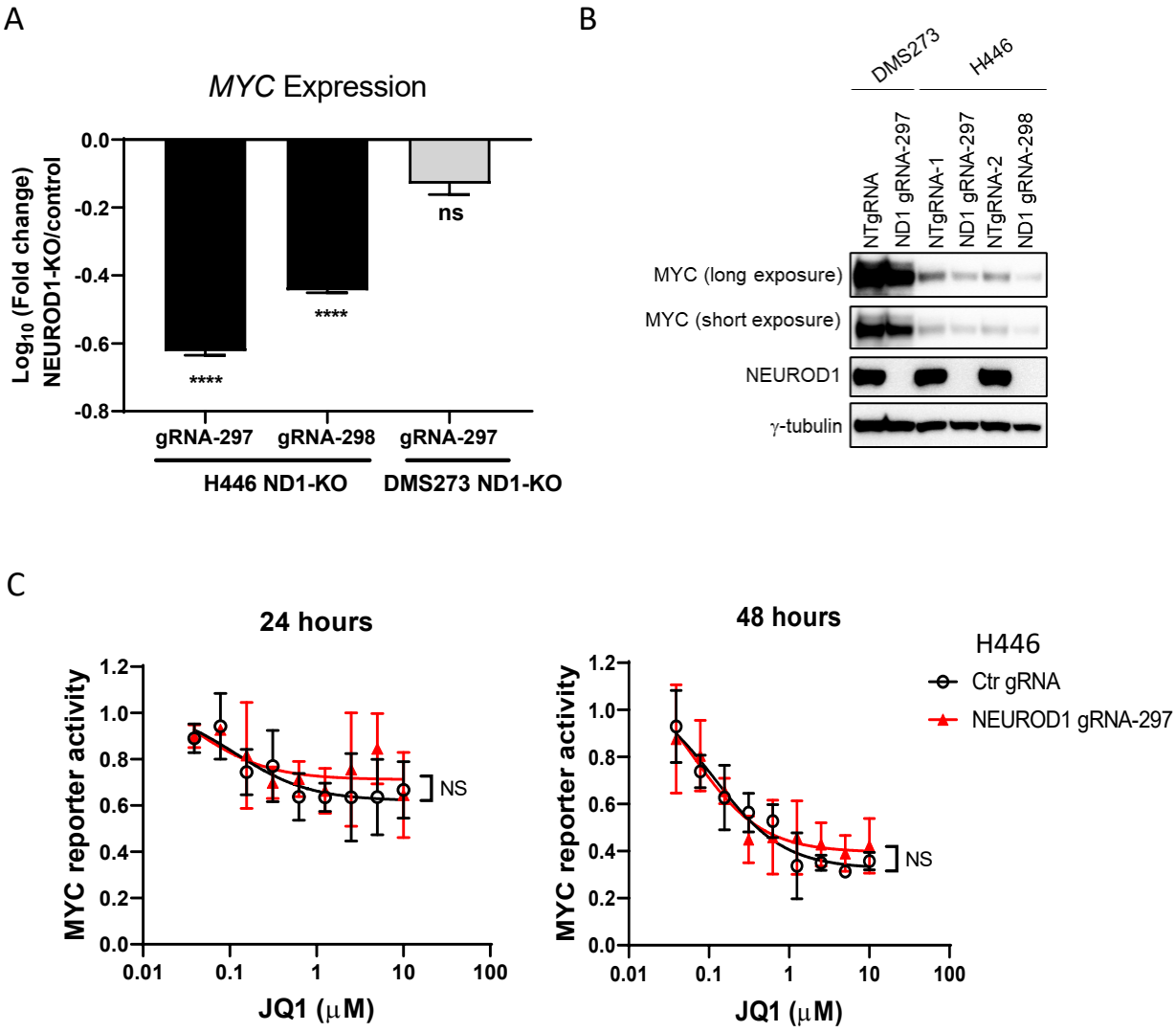
Supplemental Figure S9. The NEUROD1- and BRD4-loaded SEs identified in H446 control and NEUROD1-KO cells and their correlation. **A-B)** Stitched NEUROD1 (A) and BRD4 (B) enhancers in an increasing rank order on the basis of ChIP-seq signal minus the input signal in H446 control cells. **C)** Stitched BRD4 enhancers in an increasing rank order in the H446 NEUROD1-KO cells. **D)** Correlation between NEUROD1 and BRD4 signals (Density x Length) at the SEs co-occupied by these two genes in the H446 control cells. Pearson correlation coefficient (r) and P value were shown.



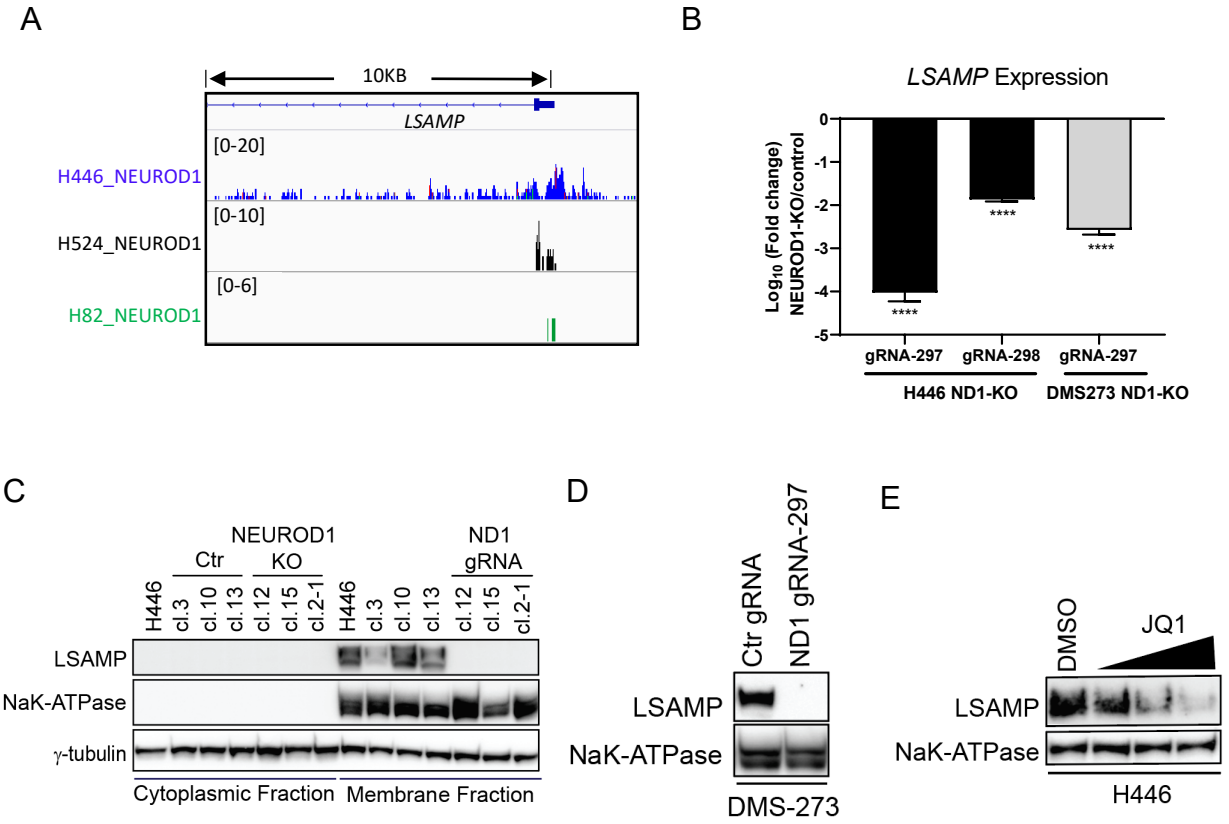
Supplemental Figure S10. The SCLC-N lines are more susceptible to BETi. A) Correlation between the IC_{50} values of OTX-015 and JQ1 in SCLC cell lines (N=50). Pearson correlation coefficient (r) and P value were shown. **B)** A comparison of *NEUROD1* expression between the JQ1-sensitive and -resistant SCLC lines (N=52) with the cutoff of IC_{50} = 1 μM JQ1. **C)** Comparison of JQ1 sensitivity (IC_{50}) among the four molecular subtypes of SCLC cell lines (N=57) in a public high-throughput drug screen dataset (30). The significance of the two-group comparisons was determined using the Student's t-test (B) or the ANOVA test with Dunnett's multiple test correction (C). *, P<0.05; ns: not significant.



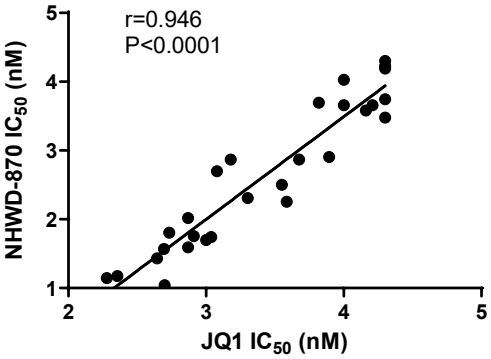
Supplemental Figure S11. NEUROD1 KO renders DMS-273 cells resistant to BETi. A) Immunoblots showing loss of NEUROD1 in DMS-273 cells after CRISPR gene editing. **B)** Viability of the DMS-273 NEUROD1-KO cells versus the control cells after JQ1 treatment (72 hrs). Error bars represent the SD of four replicates. Ctr, control; gRNA, guide RNA.



Supplemental Figure S12. Effects of NEUROD1 KO on MYC expression and transactivation. A-B) NEUROD1 KO modestly decreased *MYC* gene (A) and protein expression (B) in the H446 but not in DMS 273 cells. **C)** JQ1 suppressed MYC transactivation to a similar degree in H446 NEUROD1-KO and control cells (left, 24-hr time interval; right, 48-hr time interval).



Supplemental Figure S13. LSAMP expression is regulated by both NEUROD1 and BET bromodomain proteins. A) Integrated Genomics Viewer (IGV) snapshots showing NEUROD1 occupancy at the TSS site of LSAMP gene in H446, H82 (GSM1700641) and H524 (GSM1700642). **B)** A drastic decrease of *LSAMP* gene expression in the H446 and DMS-273 cells upon KO of NEUROD1. The gene expression was measured by qRT-PCR and plotted relative to control cells. **C)** LSAMP protein is present only in the membrane-bound protein fractions of H446 control cells but not in the NEUROD1-KO cells. **D)** Loss of LSAMP protein expression in DMS-273 cells upon KO of NEUROD1. **E)** A dose-dependent decrease of LSAMP protein expression in H446 cells 24 hours after JQ1 treatment (0.25, 0.5, and 1 μM).



Supplemental Figure S14. Correlation between the IC_{50} values of NHWD-870 and JQ1 in SCLC cell lines (N=28). Pearson correlation coefficient (r) and P value were shown.