Supporting Information

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SI Materials and Methods

Human Samples Collection. Control postmortem brain samples were purchased from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland. Control brain specimens from epileptic patients and glioblastoma samples were obtained from the Florida Center for Brain Tumor Research (IRB 134–2006). Additional glioblastoma samples were obtained through the University of Miami Miller School Of Medicine (IRB 20120067).

Helicos SMS. Total, DNase-treated, and rRNA-depleted RNA from glioblastoma and control samples was sequenced using the Helicos single-molecule system essentially as described previously (1, 2).

RNA Extraction and RT-qPCR. The RNA was extracted from tissues using the TRIzol-Chloroform protocol followed by a purification step with columns (QIAGEN RNAeasy Kit) and DNase treatment. cDNA was synthesized according to the manufacturer's protocol (Applied Biosystem cat. no. 4368814) and amplified using TaqMan Gene Expression Assays (Life Technologies). qPCR data were analyzed by Comparative Cq Method (ΔΔCq).

siRNA Transfections. Gene silencing was achieved by reverse transfection of siRNA using lipofectamine RNAiMax (Invitrogen cat. no. 13778–100) according to the manufacturer's protocol. siRNAs were purchased from Ambion [siHOTAIR1 (3), siHOTAIR2 (3), siBRD4-s23901, siBRD3-s15545, and siBRD2-s12070] and the control siRNA from Qiagen (SI03650318).

Proliferation and Apoptosis Assays. Proliferation was tested by a clonegenic assay plating of transfected cells at low confluency. The colonies were stained with crystal violet after 2 wk. The number of cells in S-phase was determined by incorporation of EdU in transfected cells and imaged using the Click-iT EdU Imaging Kit Alexa594 (Molecular Probes cat. no. C10339). The measurement of late and early apoptotic cells was done by staining the cells with APC Annexin V (BD Pharmigen cat. no. 550474) and 7-AAD (BD Pharmigen cat. no. 559925) and then analyzing them by flow cytometry.

Lentiviral Vector Cloning and Lentivirus Production. shControl and shHOTAIR (sequences below) were cloned in the lentiviral vector pLentiLox3.7. Lentiviral particles were produced cotransfecting LentiX293 cells with pLL3.7, paPAX2, and pVSVG.

The following shControl sequences were used: sense strand, TGCGGTAACTCCACTCAATATTCAAGAGATATTGAGTGGAGTTACCGCTTTTTTC; antisense strand, TCGAGAAAAAAGCGGTAACTCCACTCAATATCTCTTGAATATTGAGTGGAGTTACCG. The following shHOTAIR sequences were used: sense strand, TGCAGATGGAGATTACCATTATCAAGAGTAATGGTAATCTCCATCTGCTTTTTTC; antisense strand, TCGAGAAAAAAGCAGATGGAGATTACCATTACTCTTGATAATGGTAATCTCCATCTGCA.

The inducible lentiviral vector to overexpress HOTAIR (pLVX-mCMV-rtTA-TightPromoter-HOTAIR-IRES-ZsGreen) was purchased from Creative Biogene. The expression of HOTAIR was induced by DOX (5 μg/mL).

Luciferase-Expressing Cells and in Vivo Experiments. U87MG cells were transduced with Firefly Luciferase Lentivirus (Capital Biosciences). U87MGLuc cells were transduced with lentivirus expressing shControl or shHOTAIR, and the efficiency of transduction was confirmed by the expression of GFP (reporter of the pLL3.7 vector) visible at the fluorescence microscope. A previously published protocol was used to assess tumor growth (4). NU/NU-Nude mice (Crl:NU-*Foxn1*^{nu}; Charles River) were anesthetized, and a sagittal incision over the parieto-occipital bone was performed. A 25-gauge needle was used to puncture the skull at 2 mm to the right of the bregma and 1 mm anterior to the coronal suture. Using a 26-gauge Hamilton syringe, 10^5 cells were injected at 3 mm depth. At 7 and 14 d after the transplant, mice were injected with D-luciferin at 150 mg/kg intraperitoneally (Caliper Life Sciences), anesthetized with isofluorane, and imaged with the IVIS Spectrum Imaging System (Xenogen).

Compounds. I-BET151, JQ1, I-BET762, and TMZ were purchased from Tocris; resuspended in DMSO at a 10 mM concentration; and stored at –80 °C. DOX was purchased from SIGMA (cat. no. D9891) dissolved in H_2O (2 mg/mL) and stored at –80 °C. The Epigenetic library used in this project (Figs. S6 and S7) has been previously described (5, 6).

ChIP. LN18 cells were collected, cross-linked with formaldehyde, and processed using the EZ-Magna ChIP (Millipore cat. no. 17–408) according to the manufacturer's instructions. The chromatin was immunoprecipitated with antibodies for BRD4 (Bethyl Laboratories Inc. cat. no. A301-985A50) and negative control antibody IgG (Millipore, cat. no. 12–370). DNA–protein cross-links were reversed and DNA purified to be used in the quantitative amplification of HOTAIR's promoter with SYBR Green (forward primer, ACCTAATCCGACCAGCAAGA; reverse primer, GGGTGAAGGGAAGTGTCTGA).

1. Kapranov P, et al. (2010) The majority of total nuclear-encoded non-ribosomal RNA in a human cell is 'dark matter' un-annotated RNA. *BMC Biol* 8:149.
2. St Laurent G, et al. (2013) Genome-wide analysis of A-to-I RNA editing by single-molecule sequencing in Drosophila. *Nat Struct Mol Biol* 20(11):1333–1339.
3. Rinn JL, et al. (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* 129(7):1311–1323.
4. Ozawa T, James CD (2010) Establishing intracranial brain tumor xenografts with subsequent analysis of tumor growth and response to therapy using bioluminescence imaging. *J Vis Exp* 41:1986.
5. Plotkin A, Volmar CH, Wahlestedt C, Ayad N, El-Ashry D (2014) Transcriptional repression of ER through hMAPK dependent histone deacetylation by class I HDACs. *Breast Cancer Res Treat* 147(2):249–263.
6. Long J, et al. (2014) The BET bromodomain inhibitor I-BET151 acts downstream of smoothened protein to abrogate the growth of hedgehog protein-driven cancers. *J Biol Chem* 289(51):35494–35502.

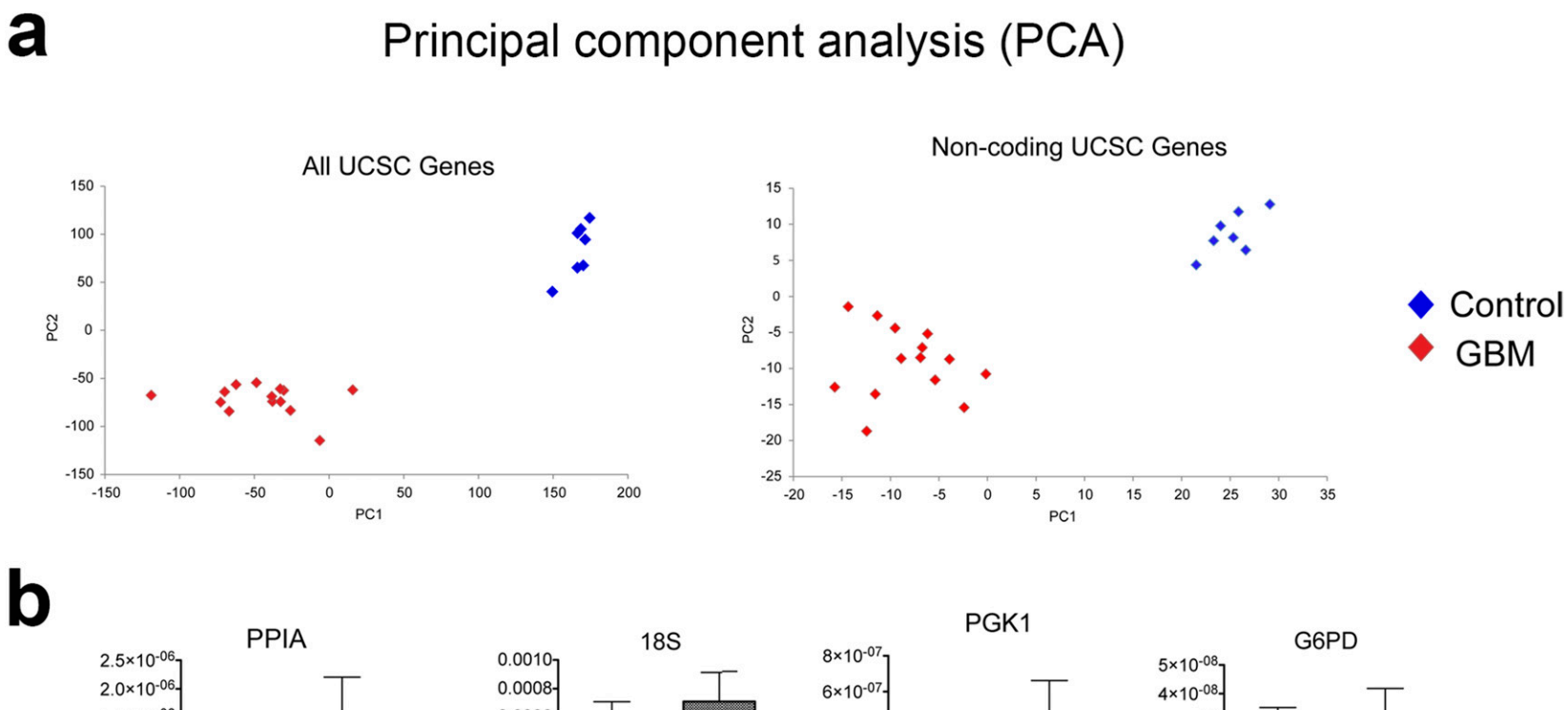


Fig. S1. (*A*) PCA on control and glioblastoma human samples. Separation of normal (blue) and GBM (red) samples in PCA performed on single-molecule expression values from various types of transcripts. The two top dimensions of PC1 and PC2 are shown, (*Left*) exons of UCSC Genes and (*Right*) exons of UCSC genes that have no annotated ORFs (lncRNAs). (*B*) The mRNA expression level of four different housekeeping genes (PPIA, 18S, PGK1, and G6PD) has been tested in control and GBM specimens. (*C*) The expression of MEG3, HOTAIRM1, and DGCR5 has been measured by RT-qPCR, normalizing the values on the housekeeping gene G6PD as alternative to 18S, which is reported in Fig. 1*B*. (*D*) RNA extracted from human insula, pons, frontal lobe, temporal lobe, hippocampus, or cerebellum was purchased from Clontech and used to test the expression of HOTAIR that was revealed to be undetectable (*Left*). Amplification curves (quantification of cycles, Cq) of GAPDH are shown (*Right*) to prove RNA integrity.

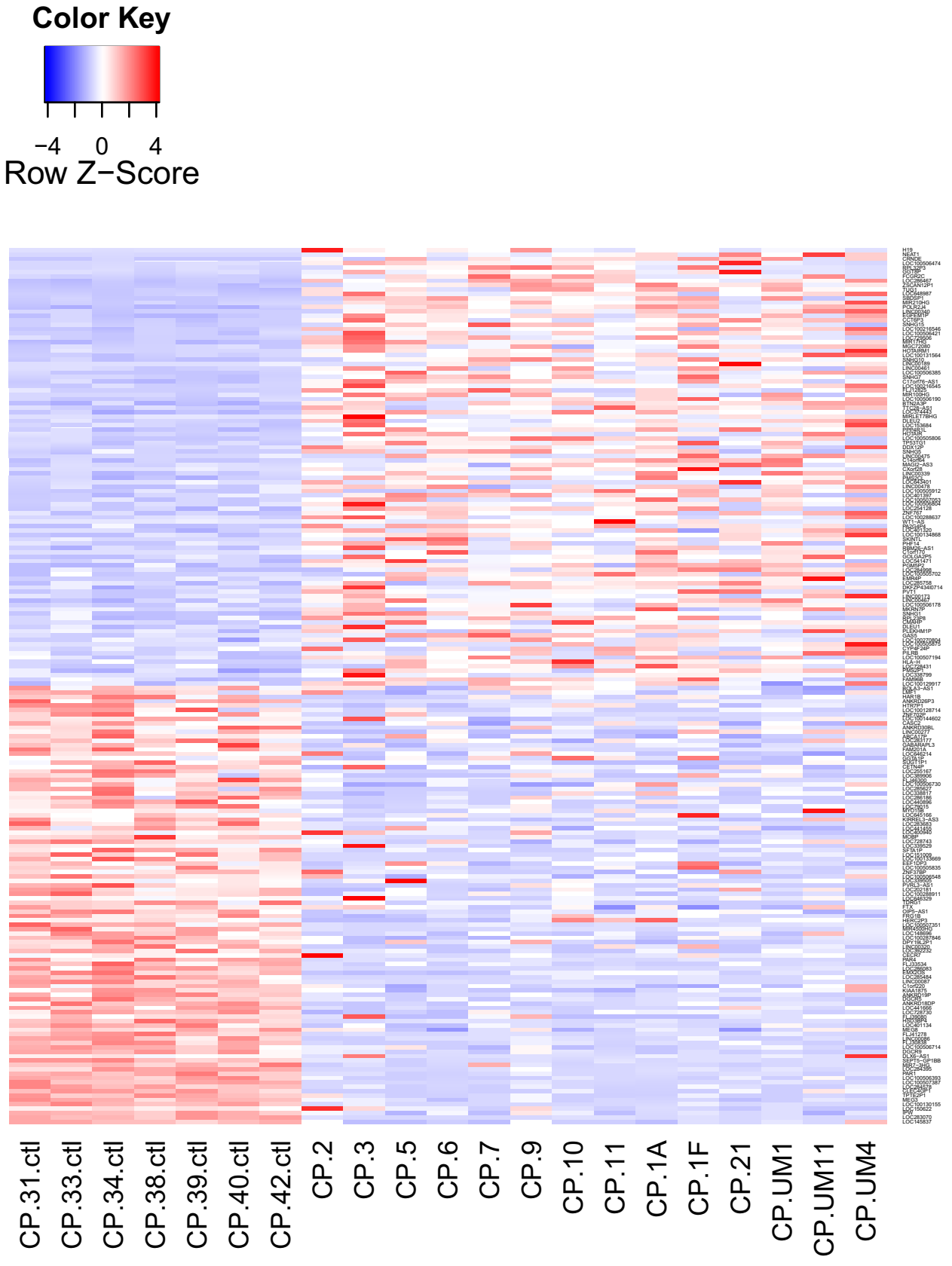


Fig. S2. High-quality figure for the heat map representing the 100 most up-/down-regulated lncRNAs in GBM versus control samples (Fig. 1*A*).

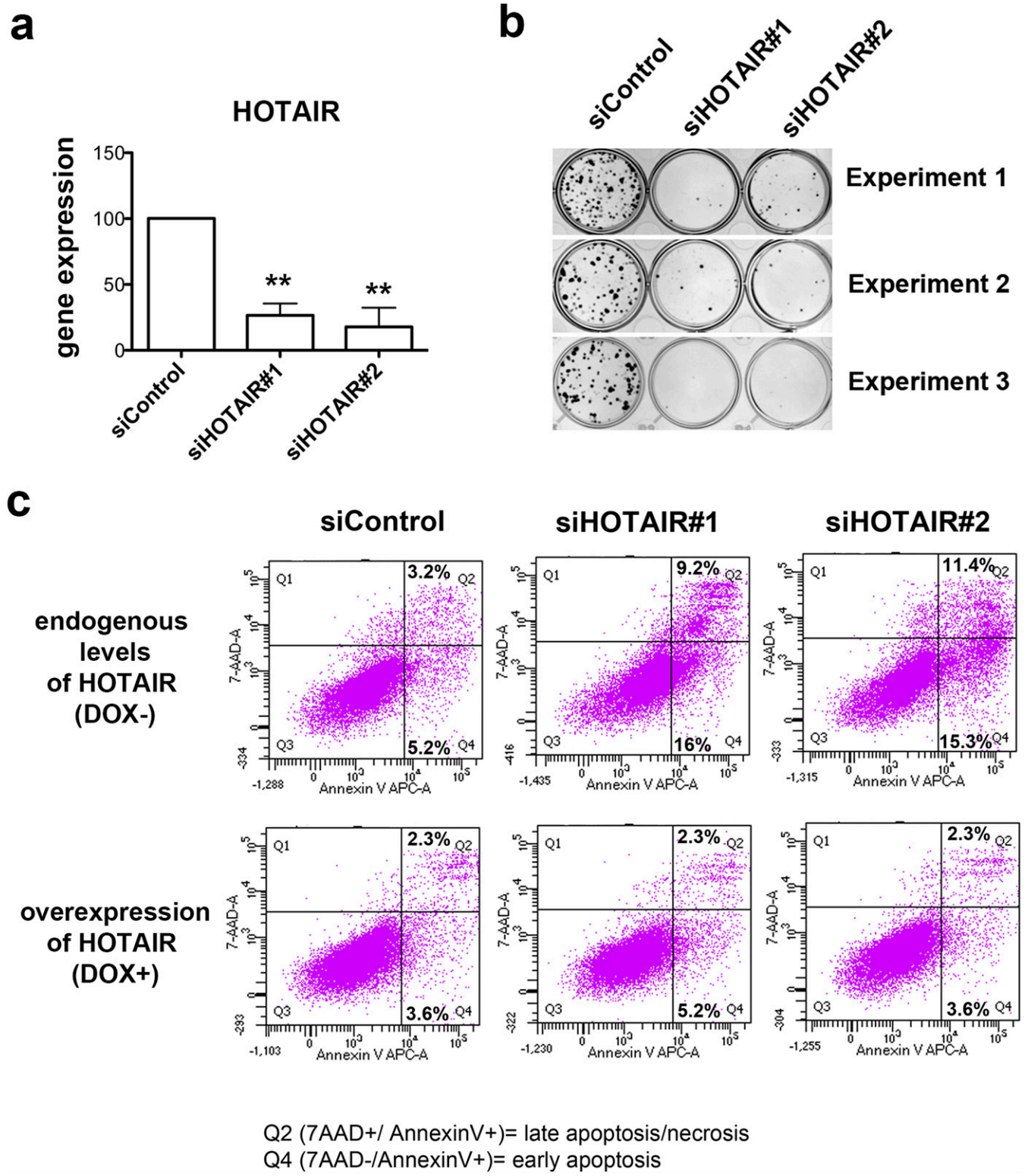


Fig. S3. (*A*) LN18 cells were transfected with siHOTAIR#1, siHOTAIR#2, and siControl (25 nM), and the RNA was extracted 96 h later to quantify the depletion of HOTAIR by RT-qPCR. (*B*) HOTAIR was depleted in LN18 with siHOTAIR#1 and siHOTAIR#2, and cells were plated at low confluence (500 per well) to be fixed and stained with crystal violet for a colony forming assay after 2 wk. Three experiments are represented. (*C*) U87MG cells were transduced with a HOTAIR-expressing vector and sorted to obtain a pure population of cells expressing HOTAIR. HOTAIR was induced by DOX. Control cells (–DOX) and HOTAIR-over-expressing cells (+DOX) were transfected with siControl, siHOTAIR#1, and siHOTAIR#2. After 5 d, cells were harvested and stained with annexinV and 7-AAD for viability assay. The percentage of early/late apoptotic cells was determined by flow cytometry.

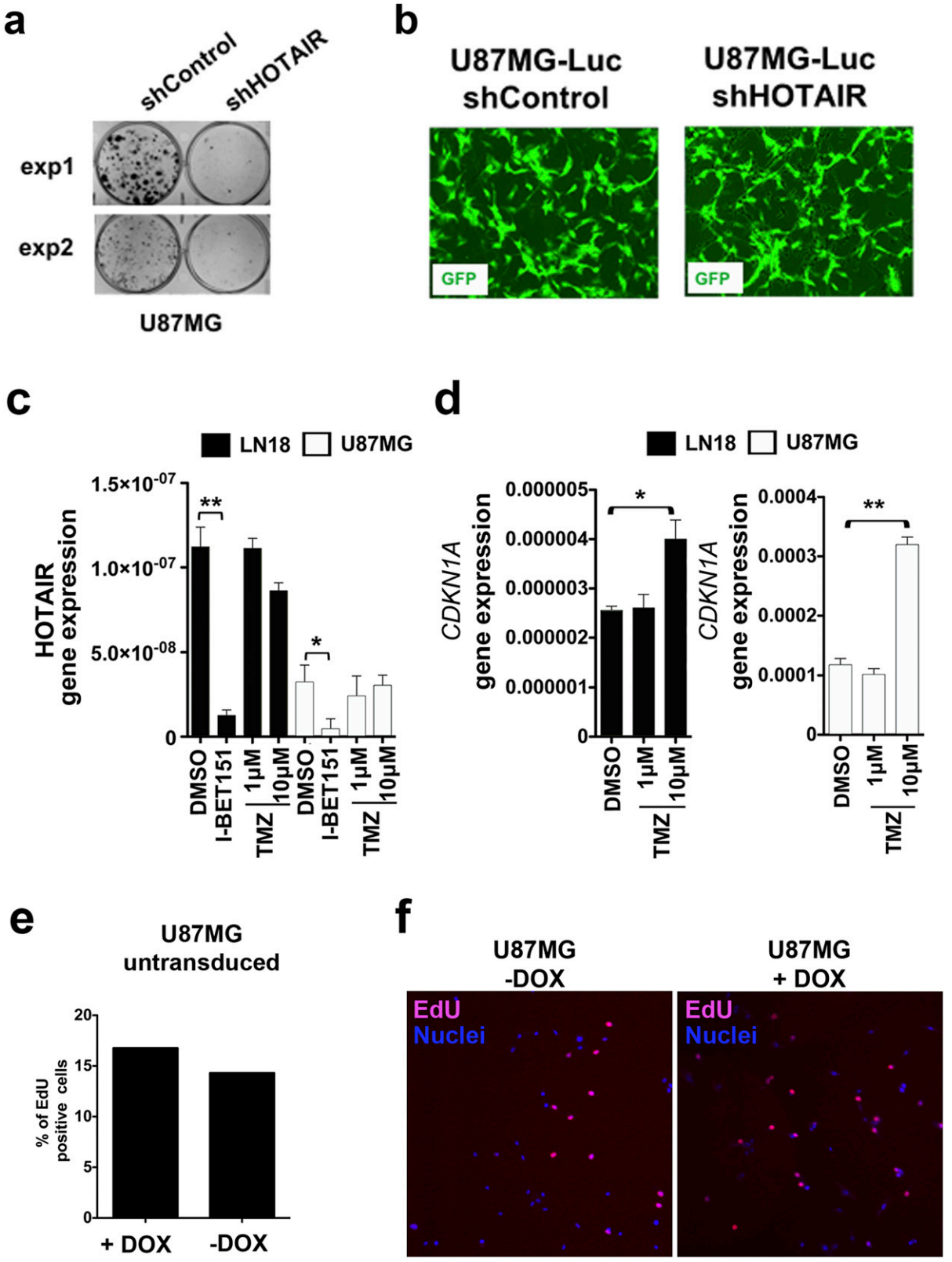
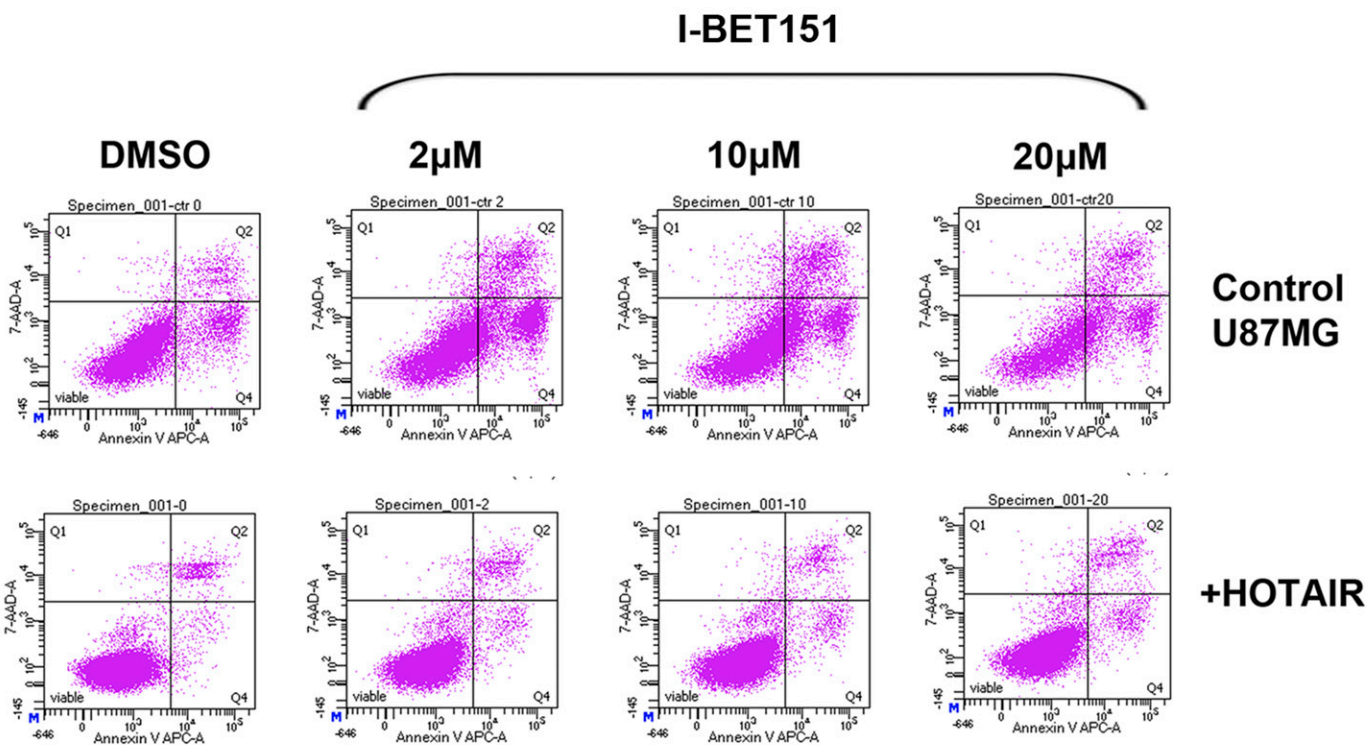


Fig. S4. (*A*) U87MG cells were transduced with lentivirus (shControl and shHOTAIR) to test the proliferation with colony forming assay. (*B*) For the implantation of tumor cells in the mice brain, the luciferase-expressing U87MG cells (U87MGLuc) were transduced with lentivirus, and the efficiency of infection was confirmed by GFP expression visible at the fluorescence microscope. (*C* and *D*) LN18 and U87MG cells were treated with TMZ and I-BET151 for 72 h to collect RNA. HOTAIR (*C*), and *CDKN1A* (*D*) expression was measured by RT-qPCR. (*E*) U87MG cells not expressing the tet-inducible HOTAIR vector were treated with DOX (5 μg/mL) for 4 d, and then the effects on proliferation were assessed with an EdU imaging kit. The EdU percentage of positive cells was calculated with the Cellomics ArrayScan Reader. (*F*) Fluorescence microscope images of nontransduced U87MG cells treated with +/–DOX and stained with EdU (pink) and Hoechst (blue).

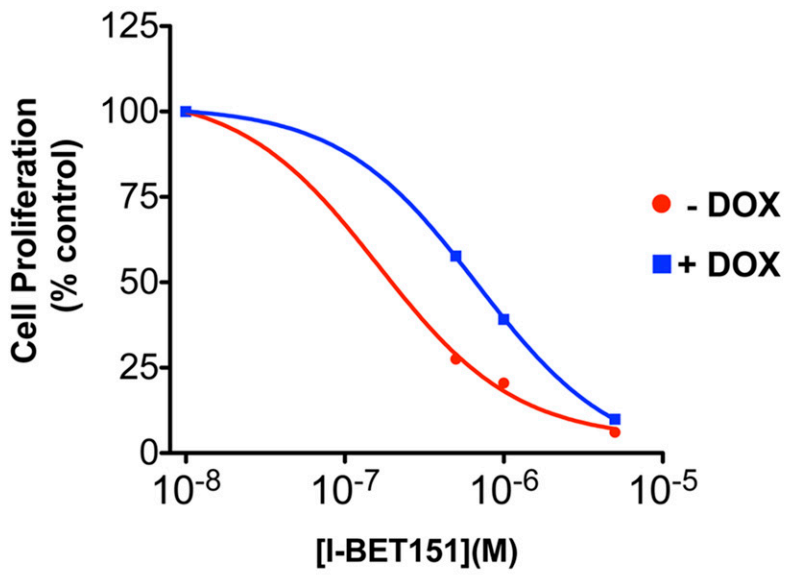
a



Q4= early apoptotic cells (Annexin+/7AAD-)
Q2= late apoptotic cells (Annexin+/7AAD+)

U87MG (5 days)	% Viable (control)	% Early apoptosis (control)	% Late apoptosis (control)	% Viable (+HOTAIR)	% Early apoptosis (+HOTAIR)	% Late apoptosis (+HOTAIR)
DMSO	82	12.5	4.7	95	2.1	1
2 μM	58	30	10	94	3.2	2.1
10 μM	61	26	10	93	2.9	2.7
20 μM	57	28	12	92	3.3	2.4

b



	- DOX	+ DOX
IC50	1.625e-007	6.793e-007

c

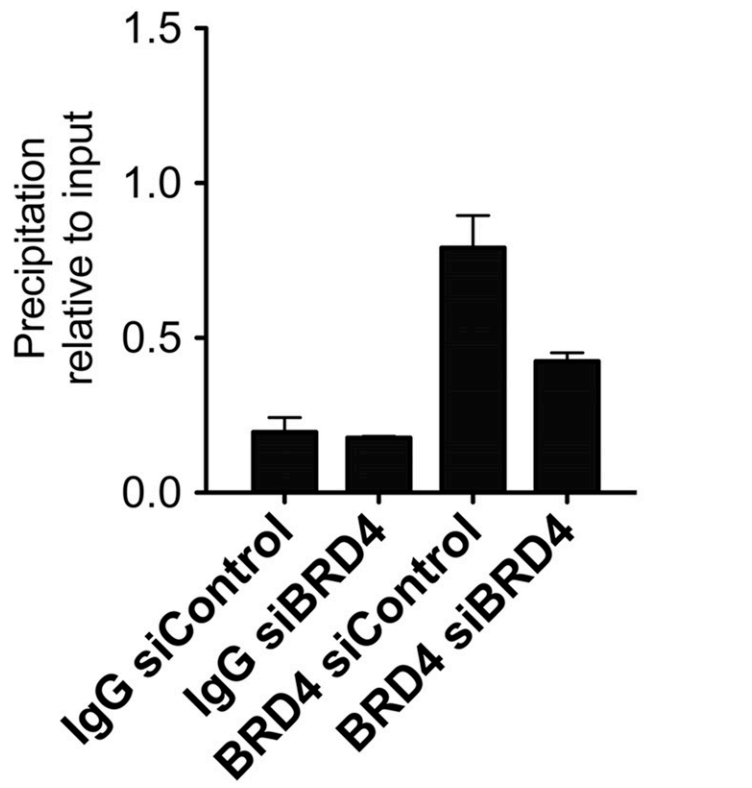


Fig. S5. (*A*) +/–DOX cells were treated with I-BET151 at 2, 10, and 20 μM for 5 d. To quantify the apoptosis rate by flow cytometry, the cells were incubated with annexinV and 7-AAD. The table reports the percentage of early apoptotic (Q4) and late apoptotic cells (Q2) for every sample. (*B*) U87MG cells transduced with HOTAIR-expressing lentivirus and treated with +/–DOX and different concentrations of I-BET151 (DMSO, 1 μM, 5 μM, and 10 μM) for 4 d. Proliferation was assessed by EdU staining, and the percentage of proliferating cells was calculated with the Cellomics ArrayScan reader. From these data, the IC_{50} was calculated for +/–DOX-treated cells. (*C*) ChIP was performed with BRD4 antibody and IgG as a negative control in LN18 cells 4 d after transfection with siControl and siBRD4. The chromatin obtained by the immunoprecipitation was used for qPCR to amplify the promoter region of HOTAIR located ~1 kb upstream from the transcription start site. The graph shows the fold enrichment of BRD4 on HOTAIR's promoter normalized to the IgG signal.

Epigenetic drugs library

Trichostatin A	HDAC inhibitor
2,4-Pyridinedicarboxylic Acid	Histone demethylase inhibitor
Garcinol	HAT inhibitor
Splitomicin	SIRT2 inhibitor
BML-210	HDAC inhibitor
Apicidin	HDAC inhibitor
Suberoyl bis-hydroxamic acid	HDAC inhibitor
Scriptaid	HDAC inhibitor
Nullscript	Scriptaid Neg control
5-Aza-2'-deoxycytidine (Decitabine)	DNA Me transferase inhibitor
Zebularine	DNA Me transferase inhibitor
Vorinostat (SAHA)	HDAC inhibitor
Isonicotinamide	nicotinamide antagonist
ITSA-1	Inhibitor of TSA activity
Phenylbutyrate·Na	HDAC inhibitor
Tranylcypromine hemisulfate	Lysine demethylase inhibitor
Valproic acid	HDAC inhibitor
EX-527	SIRT1 inhibitor
Resveratrol	SIRT1 activator
M-344	HDAC inhibitor
Nicotinamide	SIRT inhibitor
BML-266	SIRT2 inhibitor
Piceatannol	SIRT activator
Fluoro-SAHA	HDAC inhibitor
Valproic acid hydroxamate	HDAC inhibitor
AGK2	SIRT2 inhibitor
Salermide	SIRT inhibitor
MC-1293	HDAC inhibitor
Anacardic acid	HAT inhibitor
B2	SIRT2 inhibitor
BIX-01294·3HCl	Histone methyl transferase inhibitor
Butyrolactone 3	HAT inhibitor
CTPB	HAT inhibitor
Oxamflatin	HDAC inhibitor
Sirtinol	SIRT inhibitor
Suramin·6Na	SIRT1 inhibitor
BML-278	SIRT1 actvator
NCH-51	HDAC inhibitor
CI-994	HDAC inhibitor
NSC-3852	HDAC inhibitor
Aminoresveratrol sulfate	SIRT1 activator
BML-281	HDAC-6 inhibitor
Triacetylresveratrol	SIRT1 activator
I-BET151	BRD 2,3 4 and T inhibitor
GSK126	EZH2 Methyltransferase Inhibitor –
SGC0946	DOT1L inhibitor –
GSK-J1	H3K27 Histone Demethylases UTX and JMJD3 Inhibitor
GSK-J4	H3K27 Histone Demethylases UTX and JMJD3 Inhibitor
Daminozide	KDM2/7 Histone Demethylases Inhibitor
LSD1-C76	LSD1 Inhibitor
ACY-1215 (Rocilinostat)	HDAC6 inhibitor–
CUDC-907	PI3K/HDAC dual inhibitor
CUDC-101	HDAC/EGFR/HER2 Inhibitor
UNC1215	L3MBTL3 Domain Inhibitor
UNC669	L3MBTL1 Domain Inhibitor
AGI-5198 (IDH-C35)	Mutant IDH1 Inhibitor
AGI-6780	Mutant IDH2 Inhibitor
MI-2	Menin-MLL Inhibitor
IOX2	HIF Prolyl-Hydroxylases (PHD2) Inhibitor
WDR5-C47	WDR5-MLL (SET1) Antagonist

Fig. S6. Table of the epigenetic compound library.

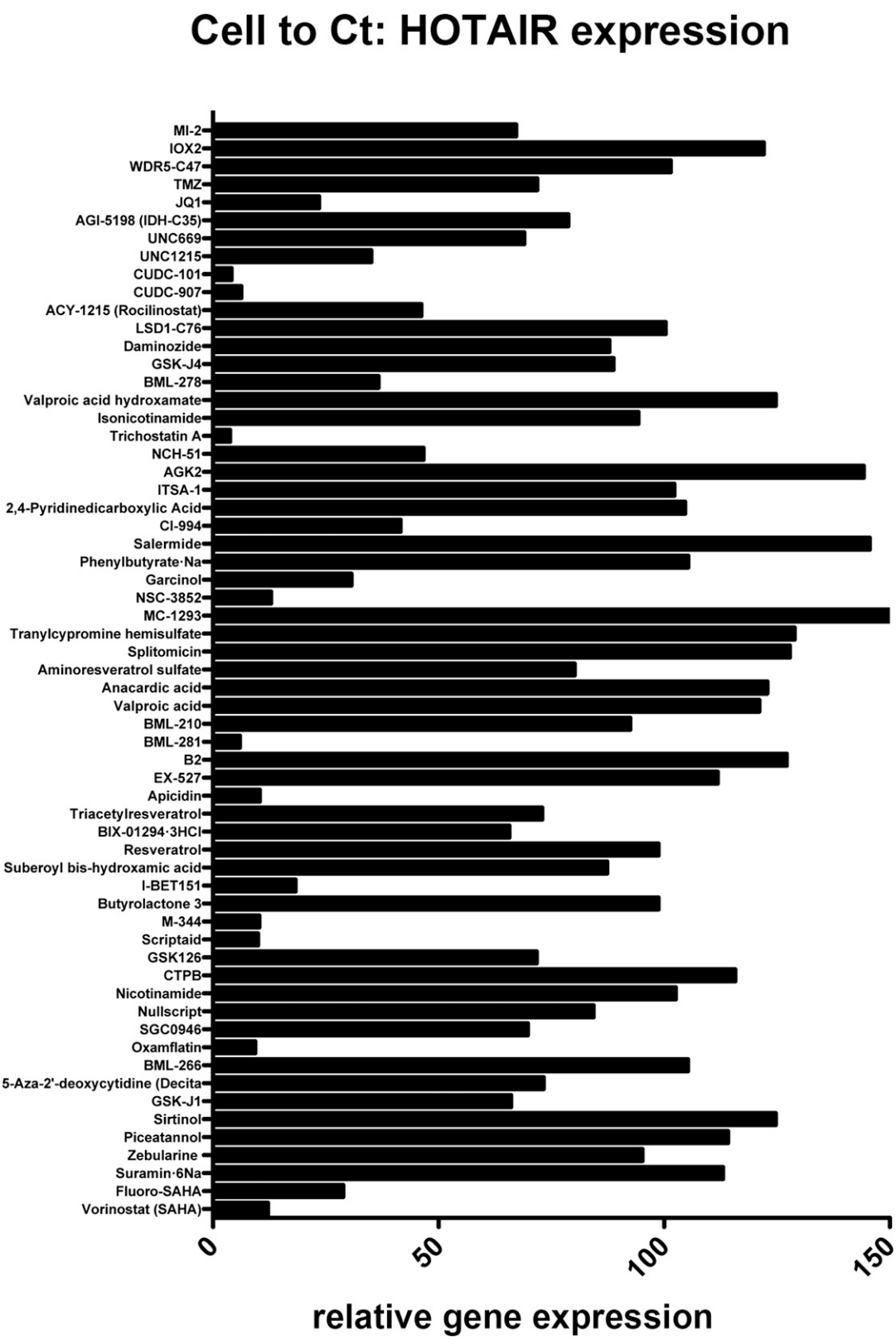


Fig. S7. We used the LN18 cell line to screen a library of 61 compounds (Fig. S6) acting as inhibitors of epigenetic enzymes such as HDACs, histones acetyl transferases (HATs), DNA methyl transferases, histone demethylase, histone methyl transferases, and histone mark "readers" such as the bromodomain proteins. BET Bromodomain inhibitors and HDAC inhibitors reduce the expression of HOTAIR in vitro. The compounds were applied at the dose of 10 μM for 24 h on LN18 cells, and the expression of HOTAIR was measured with the Cells-to-Ct Kit (Life Technologies).