Supplemental Data

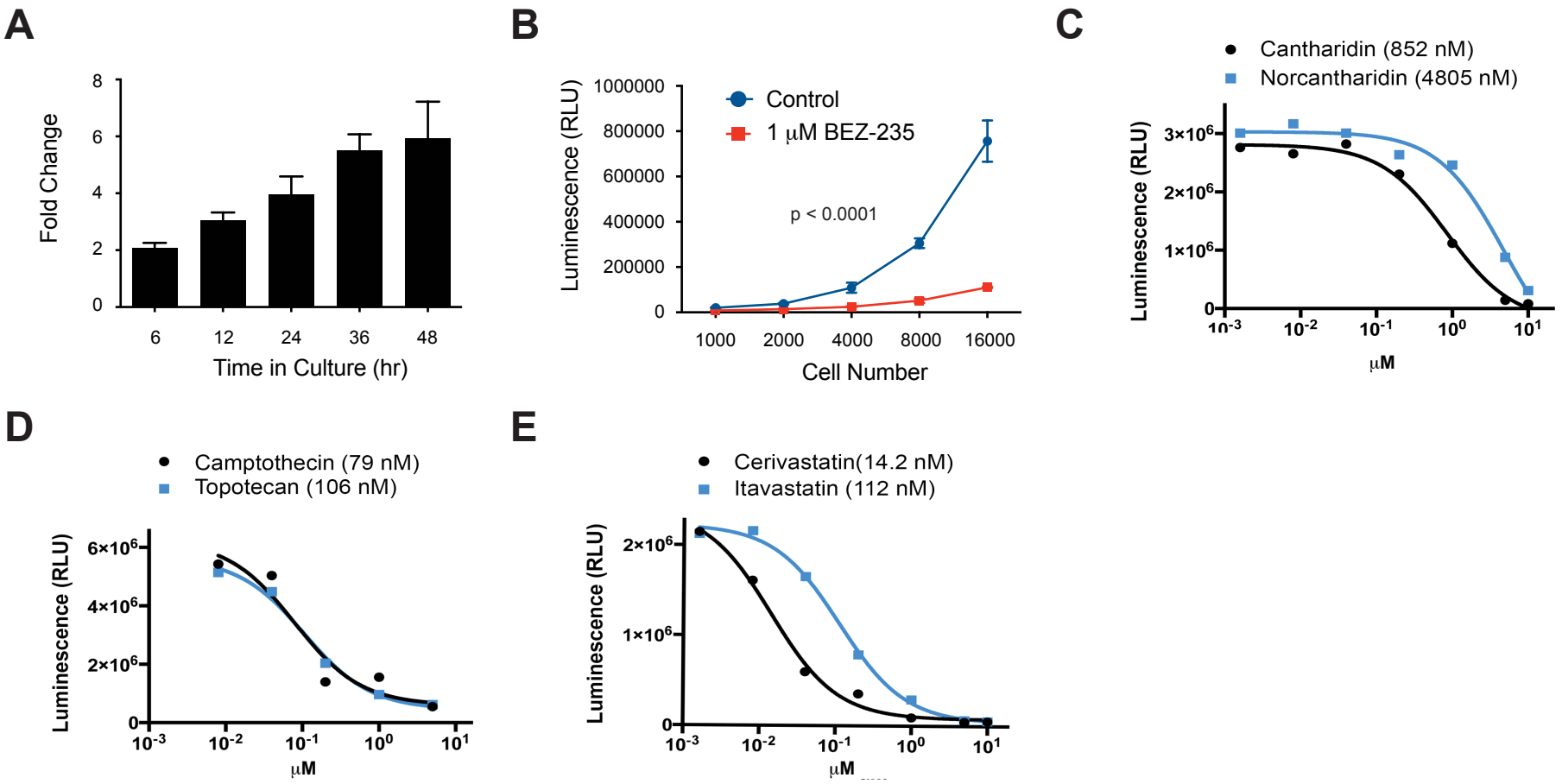

Figure S1, related to Figure 1. Optimization of conditions for high-throughput screening and response of MP tumor cells to representative hit compounds identified in the screen.

(A) MP tumor cells were cultured in 384-well plates (8000 cells/well) and treated with DMSO (0.05%) or BEZ-235 (1 μM) for the indicated time periods. Fold change was compared with the viability of control cells to BEZ-235 treated cells. Error bars represent mean ± SD. (B) The indicated number of MP tumor cells was treated with DMSO or BEZ-235 for 48 hr. Viability was measured using the CellTiter-Glo assay. P value was determined by two-way ANOVA analysis. Error bars represent mean ± SD. (C-E) MP tumor cells were treated with protein phosphatase inhibitors (C),HMG-CoA reductase inhibitors (D) or DNA topoisomerase inhibitors (E) at the indicated doses for 48 hr. Cell viability was assessed using the CellTiter-Glo assay. IC_{50} values for each compound are noted in parentheses.

Table S1, related to Table 1. Provided as an Excel file.

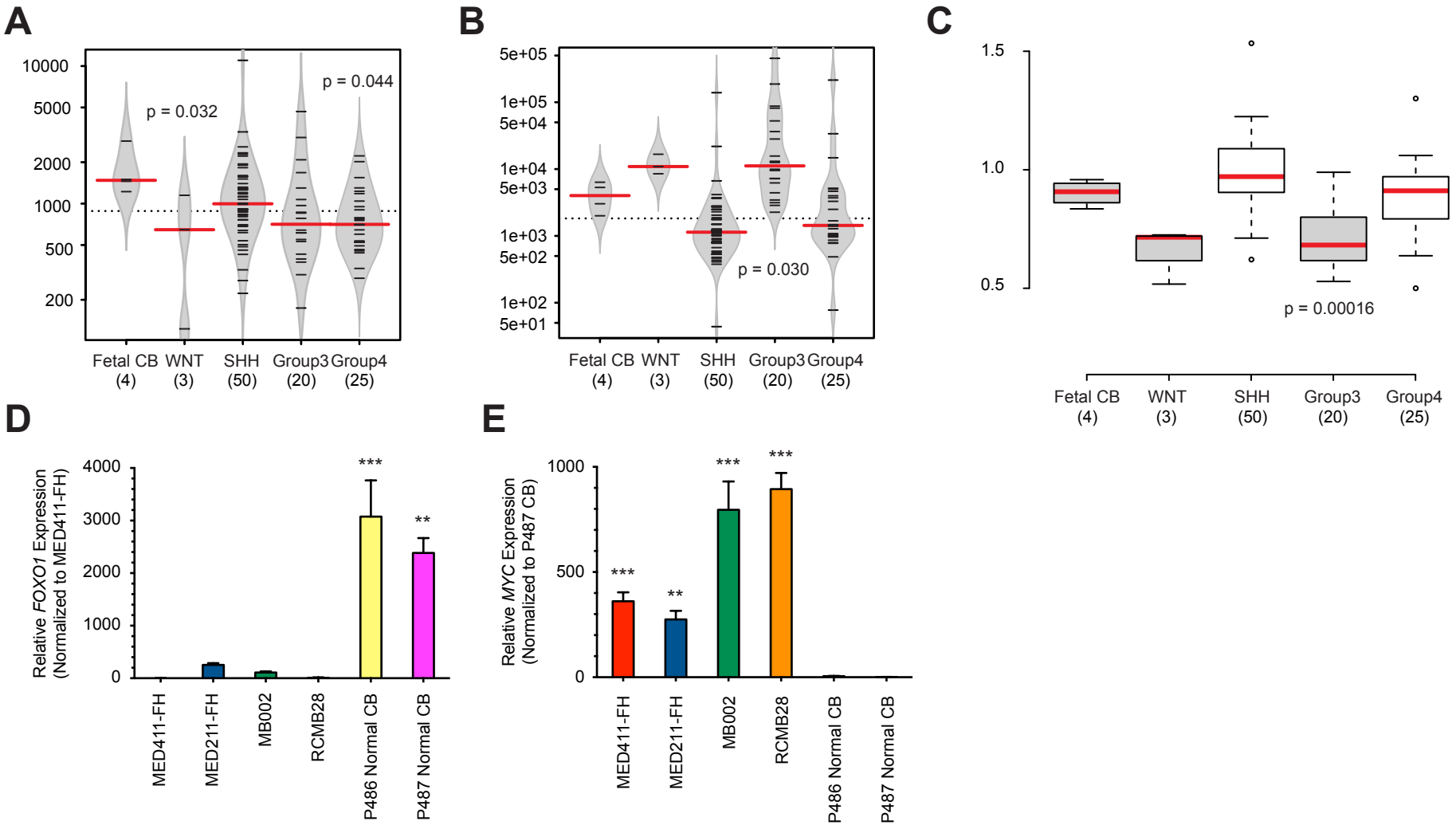

Figure S2, related to Figure 3. Expression of *FOXO1* and *MYC* in human medulloblastomas and patient-derived xenografts.
(A, B) Distribution of *FOXO1* (A) and *MYC* (B) expression from RNA sequencing of tumor samples across the four MB subgroups and normal fetal cerebella. Individual observations are shown as black lines; the grey silhouette widths represent the proportion of observations; red lines indicate the media; the dashed line is the median of all data points. The total number of samples in each group is indicated in the x-axis. One-tailed t test was used to compare expression in fetal cerebella to that of each subgroup of tumors. (C) Boxplots of log-ratio values of *FOXO1*:*MYC* expression across the four MB subgroups and in normal fetal cerebella. Center lines show the medians; box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by dots. The median log ratio in fetal cerebellum (0.91) is significantly higher than in Group 3 tumors (0.68; p value = 1.58e-04). The total number of samples in each group is indicated in the x-axis. One-tailed t test was used to compare log ratio in fetal cerebella to that of each subgroup of tumors. (D, E) Cells were isolated from orthotopic patient-derived xenografts (PDXs) representing Group 3 MB (MED411-FH, MED211-FH, MB002, RCMB28), or from normal human adult cerebellum (P486, P487). mRNA was purified and levels of *FOXO1* (D) and *MYC* (E) were determined by quantitative RT-PCR. CB, cerebellum. One-way ANOVA followed by Tukey post-hoc test was used to compare each of the normal cerebella to each of the PDX lines. Error bars represent mean ± SD. **, $p < 0.001$; ***, $p < 0.0001$.

Table S2, related to Figure 3. Provided as an Excel file.

Table S3, related to Figure 3. Provided as an Excel file.

Table S4, related to Figure 3. Provided as an Excel file.

Table S5, related to Figure 3. Provided as an Excel file.

Table S6, related to Figure 3. Provided as an Excel file.

Table S7, related to Figure 3. Provided as an Excel file.

Table S8, related to Figure 3. Provided as an Excel file.

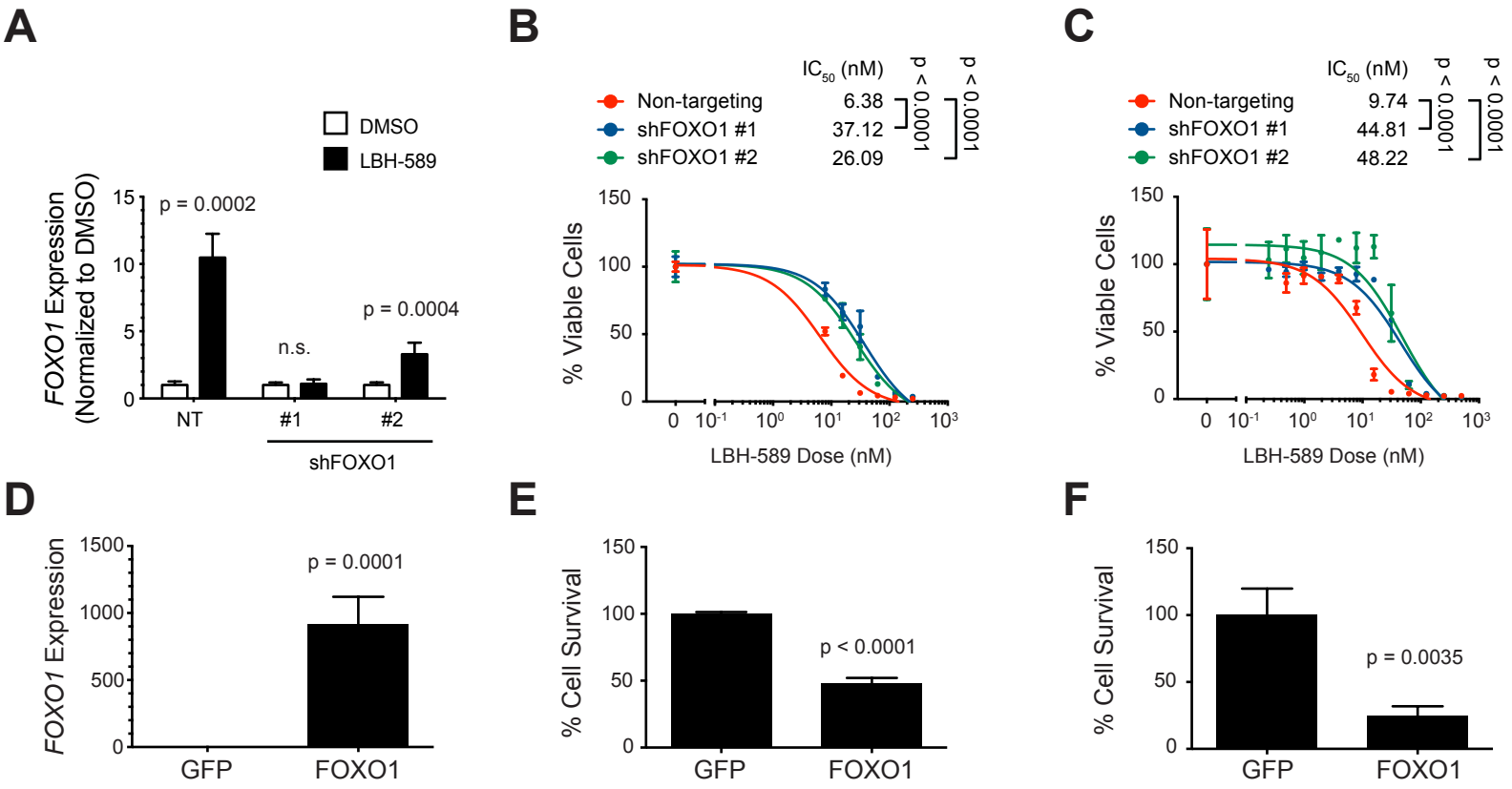

Figure S3, related to Figure 4. FOXO1 overexpression inhibits tumor growth and its knockdown renders human PDX tumor cells more resistant to HDACI.

(A) Human PDX MED411-FH tumor cells were infected with lentiviruses carrying non-targeting (NT) shRNA or two different shRNAs targeting FOXO1. Cells were treated with DMSO or LBH-589 for 6 hr and *FOXO1* expression levels were determined by qRT-PCR. Unpaired, one-tailed t tests were used to compare LBH-589-induced *FOXO1* expression in cells transduced with shFOXO1 #1 or #2 to expression in cells transduced with NT shRNA. (B) MED411-FH tumor cells expressing shRNAs were treated with indicated concentrations of LBH-589 for 48 hr and viability was measured. IC50 values for each condition are shown. P values were determined by sum-of-squares F test. (C) Human PDX MB002 tumor cells expressing shRNAs were treated with LBH-589 and viability was measured as in B. (D) MED411-FH tumor cells were infected with GFP or FOXO1-IRES-GFP retroviruses for 24 hr and FACS-sorted for analysis of *FOXO1* mRNA by qRT-PCR. P value was determined by unpaired t test. (E) GFP- or FOXO1-overexpressing MED411-FH tumor cells were sorted and plated for 48 hr before assaying viability. p value was determined by unpaired t test.(F) GFP- or FOXO1-overexpressing MB002 tumor cells were sorted and plated for 48 hr before assaying viability. p value was determined by unpaired t test. Error bars represent mean ± SD.

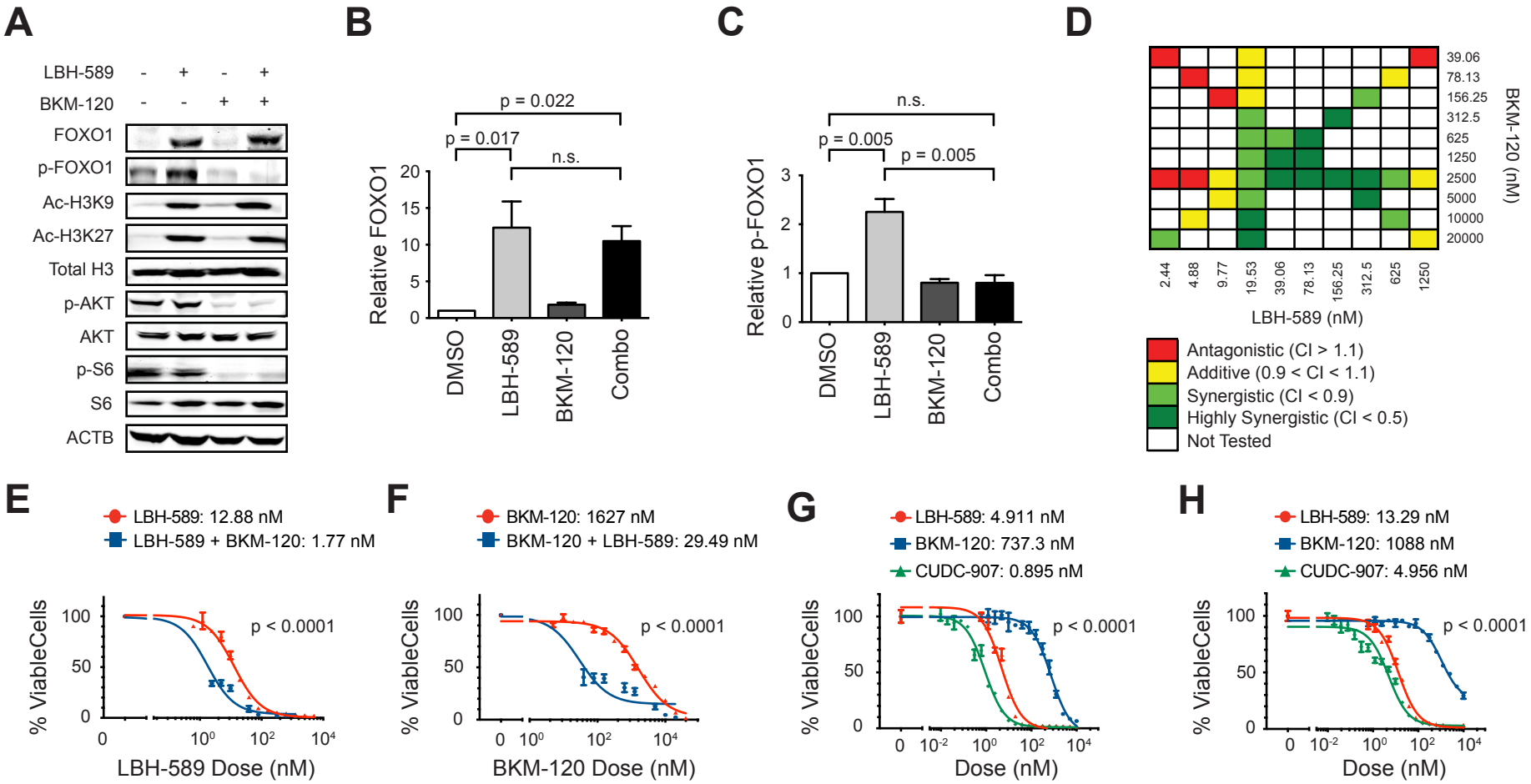

Figure S4, related to Figure 5. HDACI synergize with PI3KI to activate FOXO1 and suppress growth of human *MYC*-driven MB.
(A) Human Group 3 PDX (MED411-FH) cells were treated with 0.01% DMSO, 100 nM LBH-589, 2 μM BKM-120, or a combination of the two compounds for 12 hr. Cells were lysed for Western blot analysis. (B, C) Relative amounts of FOXO1 (B) and p-FOXO1 (C) were calculated by normalizing the levels of total FOXO1 and p-FOXO1 respectively to ACTB, and then comparing each treatment with the levels in DMSO-treated cells (set to 1), from three independent Western blots. Unpaired, one-tailed t test was used; p values < 0.05 is considered statistically significant for all comparisons. n.s., not significant. (D) MED411-FH cells were treated with the indicated concentrations of LBH-589 and BKM-120 for 48 hr in vitro before cell viability was evaluated using CellTiter-Glo assay. Compusyn software was used to calculate combination index (CI) for each treatment. The combination of LBH-589 and BKM-120 was deemed highly synergistic if the CI value was less than 0.5; synergistic if CI was less than 0.9 but more than 0.5; additive if CI was between 0.9 and 1.1; and antagonistic if CI was higher than 1.1. (E, F) MED411-FH cells were treated with the indicated concentrations of LBH-589 and 2500 nM BKM-120 (E) or BKM-120 and 19.53 nM LBH-589 (F) for 48 hr in vitro before cell viability was evaluated using CellTiter-Glo assay. IC_{50} values for each condition are shown. p values were determined by sum-of-squares F test. (G, H) The indicated concentrations of LBH-589, BKM-120, and the dual PI3K/HDAC inhibitor CUDC-907, were used to treat murine MP tumor cells (G) or MED411-FH cells (H) in vitro for 48 hr before cell viability was assessed with the CellTiter-Glo assay. IC_{50} values for each drug are shown. p values were determined by sum-of-squares F test. Error bars represent mean ± SD.

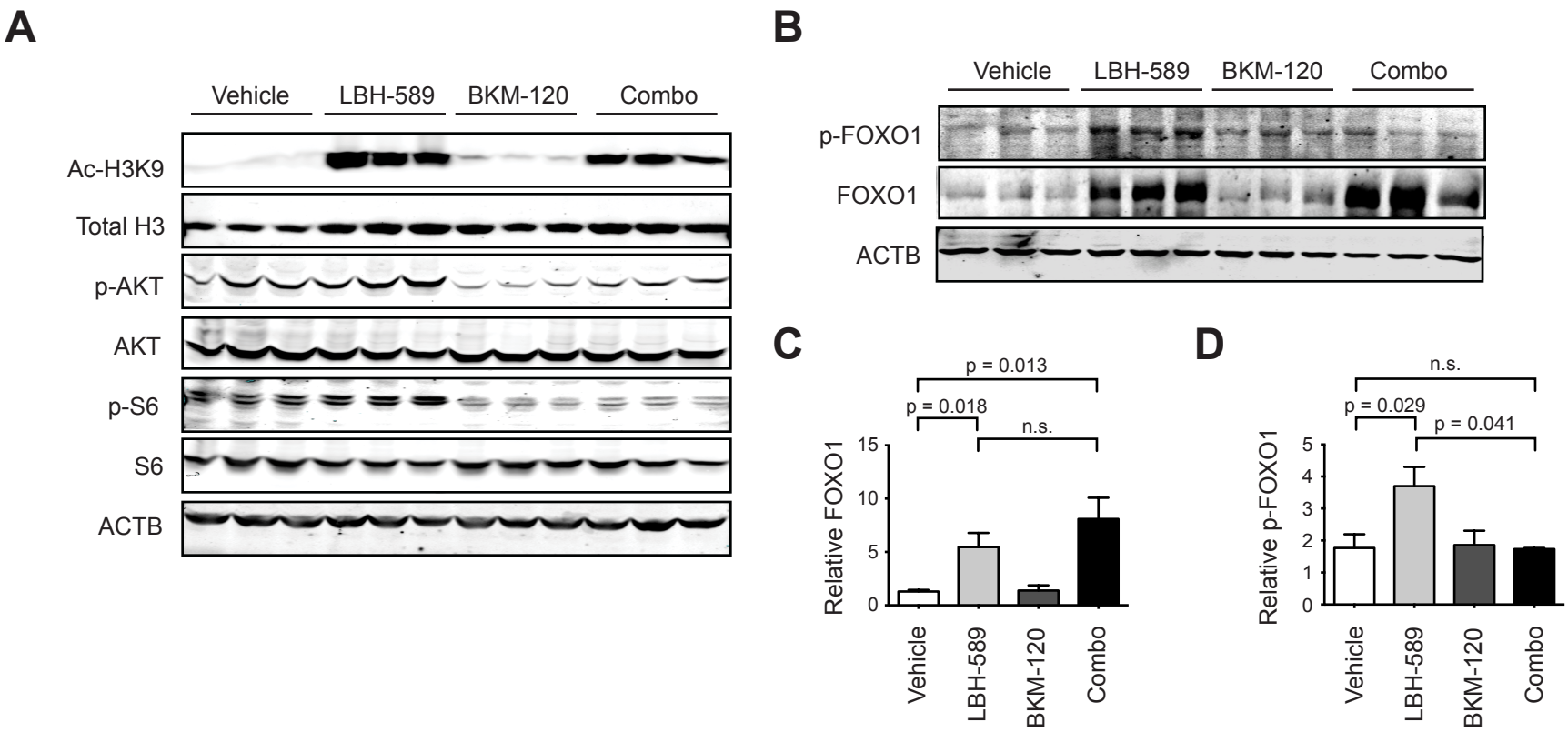

Figure S5, related to Figure 7. HDAC and PI3K antagonists synergize to inhibit growth of *MYC*-driven MB in vivo.
(A) Mice bearing MP tumor cells were treated with vehicle (0.5% methyl-cellulose + 5% dextrose), 30 mg/kg BKM-120 by oral gavage, 10 mg/kg LBH-589 by intraperitoneal injection, or a combination of LBH-589 and BKM-120 at the same doses 7 days post-transplantation. 6 hr after treatment, tumors were harvested and lysed for Western blot analysis using indicated antibodies. (B) Mice bearing MP tumor cells were treated with vehicle, 30 mg/kg BKM-120 by oral gavage, 20 mg/kg LBH-589 by intraperitoneal injection, or a combination of LBH-589 and BKM-120 10 days post-transplantation. Twelve hours after treatment, tumors were harvested and single-cell suspensions were prepared using Percoll density gradient centrifugation. Tumor cells were lysed for Western blot analysis using anti-FOXO1, p-FOXO1, and ACTB antibodies. (C, D) Quantification of total (C) and phosphorylated (D) FOXO1 protein levels in (B). Relative amounts of FOXO1 and p-FOXO1 were calculated from the 3 MP tumors in each treatment group by normalizing levels of total FOXO1 and pFOXO1 respectively to Actin, and then comparing each treatment with levels in vehicle-treated cells (set to 1). Unpaired, one-tailed t test was used; p values < 0.05 were considered significant. n.s., not significant. Error bars represent mean ± SD.

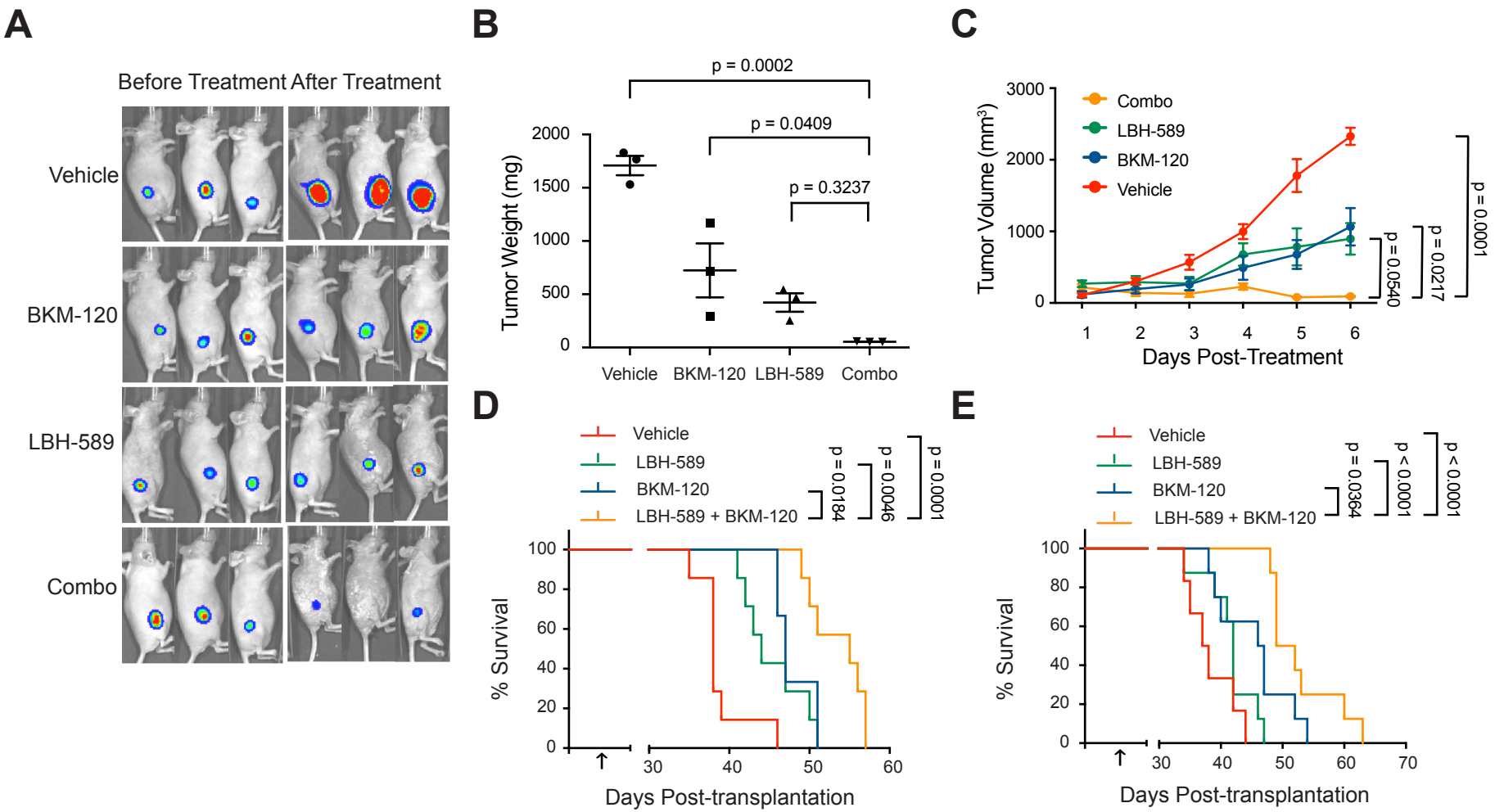

Figure S6, related to Figure 7. HDAC and PI3K antagonists synergize to inhibit growth of *MYC*-driven MB in vivo.
(A-C). The combination of HDACI and PI3KI inhibits growth of murine MP tumors growing subcutaneously. Cells from MP tumors were injected into the flanks of CD-1 nu/nu mice, and animals were treated with vehicle (5% Dextrose + 0.5% methylcellulose); 30 mg/kg BKM-120 by oral gavage; 10 mg/kg LBH-589 by intraperitoneal injection; or both drugs at the same doses. Tumor volume was measured using calipers. Animals were euthanized when their body weight decreased by ~8-10%. Tumor size (A) and weight (B) were determined at the time of sacrifice; tumor volume (C) was measured every 2 days during the course of treatment. Data are shown as mean ± SD for tumor weight and tumor volume. p values were determined using one-way ANOVA followed by Tukey post-hoc test. Tumor volumes on day 6 were used for statistical analysis. (D, E) Mice bearing intracranial xenografts of the Group 3 PDXs ICb-1572 (D) or MB002 (E) were treated with vehicle (0.5% methyl-cellulose + 5% dextrose), 30 mg/kg BKM-120 by oral gavage, 5 mg/kg LBH-589 by intra-peritoneal injection, or a combination of LBH-589 and BKM-120 at the same doses. Animals were treated with repeated cycles of 3 days on and 1 day off, and monitored until onset of symptoms. Survival curves were plotted as described. Arrows indicate the start of drug treatment, 10 days post-transplantation for both E and F. p values were determined by Log-rank (Mantel-Cox) test.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Libraries used for screening

For the screen, we tested compounds from 7 focused libraries – US Drug Collection (1040 compounds, http://www.msdiscovery.com/usdrugs.html), International Drug Collection (240, http://www.msdiscovery.com/index.html), LOPAC library of pharmacologically active compounds (1280, http://www.sigmaaldrich.com/chemistry/drug-discovery/validation-libraries.html), Kinase Inhibitor Collection (244, http://www.emdchemicals.com), the NIH Clinical Collection (446, http://www.nihclinicalcollection.com/), the StemSelect Collection (303, http://www.emdchemicals.com/life-science-research/stemselect-small-molecule-regulators-384-well-library-i/) and the NCI Oncology Collection (89, http://dtp.nci.nih.gov/branches/dscb/oncology_drugset_explanation.html).

Retroviruses and Lentiviruses

Retroviral vectors *pMSCV-IRES-GFP*, *pMSCV-Myc^{T58A}-IRES-Luciferase* (*Luc*), *pMSCV-DNp53-IRES-GFP*, *pMSCV-Myc^{T58A}-IRES-CD2*, and *pMSCV-DNp53-IRES-Luc* were described previously. MP tumors for all experiments were generated by using *pMSCV-Myc^{T58A}-IRES-Luc* and *pMSCV-DNp53-IRES-GFP*, except those used for FOXO1 overexpression experiments, in which *pMSCV-Myc^{T58A}-IRES-CD2* and *pMSCV-DNp53-IRES-Luc* were used instead.

To create *pMSCV-Foxo1-IRES-GFP*, *Foxo1* cDNA was PCR-amplified from *pCMV5-HA-Foxo1* (Addgene #14936), restriction digested with XhoI, then ligated into the *pMSCV-IRES-GFP* vector. The following primers were used to clone *Foxo1*: forward, 5'-ACTTCTCGAGTCTAGAGTCACCATGGC-3', and reverse, 5'-ACTTCTCGAGTTAGCCTGACA CCCAG-3'. All vectors were sequenced to confirm their sequence accuracy.

To create *pMSCV-DN-Foxo1-IRES-GFP* for mouse tumor cells, *Foxo1* cDNA was PCR-amplified from *pCMV5-Myc-Foxo1* D256 plasmid (Addgene #12145), restriction digested with XhoI, then ligated into the *pMSCV-IRES-GFP* vector. The following primers were used to clone *DN-Foxo1*: forward, 5'-AAACTCGAGATGGCCGAAGCGCCCCAG-3', and reverse, 5'- GGACTCGAGCTAGTCCATGGACGCAGC-3'. To create *pMSCV-DN-FOXO1-IRES-GFP* for human PDX tumor cells, *FOXO1* cDNA was PCR-amplified from *pCMV5-Myc-Foxo1* D256 plasmid (Addgene #45814), restriction digested by XhoI, then ligated into the *pMSCV-IRES-GFP* vector. The following primers were used to clone *DN-Foxo1*: forward, 5'-TATCTCGAGATGGCCGAGGCG CCTCAG-3', and reverse, 5'-GCGCTCGAGTTAAGCGTAGTCTGGGAC-3'. All vectors were sequenced to confirm their sequence accuracy.

Lentiviral vectors *pGIPZ-GFP* expressing a non-targeting shRNA, or shRNAs targeting FOXO1 were purchased from Thermo Scientific, Inc. The targeting sequences for mouse shRNAs were as following: shFOXO1 #1: 5'-TTTACTGTTGTTGTCCATG-3'; shFOXO1 #2: 5'-TTTACTGTTGTTGTCCATG-3'; for human: shFOXO1 #1: 5'-AGTCTGTCTGAGATAAGCA-3'; shFOXO1 #2: 5'-TGGAGGTATGAGTCAGTAT-3'.

In Vivo Bioluminescence Imaging

Mice injected with cells from MP tumors were subjected to weekly bioluminescence imaging. Briefly, mice were given intraperitoneal injections of 150 ng/g D-Luciferin (Caliper Life Sciences, cat#12279) and anesthetized with 2.5% isoflurane in an induction chamber. At 7–8 min after injection, animals were imaged using the Xenogen Spectrum (IVIS-200) imaging system.

Microarray Analysis

The quality of total RNA was assessed by the Agilent Bioanalyzer Nano chip (Agilent Technologies). The single-stranded cDNA was generated using the 400 ng of total RNA according to Whole Transcript (WT) Sense Target Labeling Assay protocol using Ambion's WT Expression kit (p/n 4411974). The 5.5 mg of sscDNA was then fragmented and labeled using GeneChip WT Terminal Labeling kit (PN 900671). The labeled sscDNA was then hybridized onto GeneChip Mouse Gene 2.0 ST Array (Affymetrix), which analyzes ~30,000 coding and non-coding transcripts using ~ 760,000 probe sets (on average 22 probes per transcripts). The staining and washing of the arrays were conducted using a Fluidics 450 station, and scanned using Affymetrix GeneChip Command Console Software (AGCC) and GeneChip® Scanner 3000 7G. All these procedures were conducted according to the manufacturer's instructions.

The intensities of the scanned fluorescence images were extracted with Agilent Feature Extraction software Ver 10.7.3.1. The mean signals were background corrected and transformed to the log2 scale. Then the data were normalized between arrays by quantile approach. The empirical Bayes moderated t-statistics, which is implemented in the LIMMA Bioconductor package (Smyth, 2004), were used for differential expression detection. The Benjamini and Hochberg's approach (Hochberg and Benjamini, 1995) was used to control for false discovery. Genes with at least a 2-fold changes at the 95% confidence level were considered as significant. The hierarchical clustering and other statistical analyses were performed using R/Bioconductor

(Gentleman et al., 2004). Gene expression microarray data discussed in this study have been deposited in the NCBI's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) with accession number GSE69410.

Principal Component Analysis (PCA), Pathway, and Nextbio Analysis

PCA was performed with Partek Genomics Suite (Partek Inc. St. Louis, MO). Ingenuity Pathway Analysis was conducted with the gene expression data sets to identify regulated pathways, biological processes, and possible upstream regulators of the differentially expressed genes, using the Ingenuity Pathway Analysis software (Qiagen, Inc.). Nextbio analysis was performed on the differentially expressed gene lists using Nextbio online tool (Illumina, Inc.).

Gene Set Enrichment Analysis (GSEA)

Motif gene set collection in MSigDB (Broad Institute, c3.tft.v4.0) was used for GSEA. This collection includes gene sets containing genes that share cis-regulatory motifs that are conserved across the human, mouse, rat, and dog genomes. The motifs are catalogued and represent known or likely regulatory elements in promoters and 3'-UTR. These gene sets make it possible to link changes in a microarray experiment to conserved, putative cis-regulatory elements.

Messenger RNA library construction and sequencing for RNA sequencing (RNA-seq)

Two micrograms of total RNA samples were arrayed into a 96-well plate and polyadenylated (PolyA$^+$) messenger RNA (mRNA) was purified using the 96-well MultiMACS mRNA isolation kit on the MultiMACS 96 separator (Miltenyi Biotec, Germany) with on-column DNaseI-treatment as per the manufacturer's instructions. The eluted polyA$^+$ mRNA was ethanol precipitated and resuspended in 10µL of DEPC treated water with 1:20 SuperaseIN (Life Technologies, USA). First-strand cDNA was synthesized from the purified polyA$^+$ mRNA using the Superscript cDNA Synthesis kit (Life Technologies, USA) and random hexamer primers at a concentration of 5µM along with a final concentration of 1ug/uL Actinomycin D, followed by Ampure XP SPRI beads on a Biomek FX robot (Beckman-Coulter, USA). The second strand cDNA was synthesized following the Superscript cDNA Synthesis protocol by replacing the dTTP with dUTP in dNTP mix, allowing the second strand to be digested using UNG (Uracil-N-Glycosylase, Life Technologies, USA) in the post-adapter ligation reaction and thus achieving strand specificity. The cDNA was quantified in a 96-well format using PicoGreen (Life Technologies, USA) and VICTOR3V Spectrophotometer (PerkinElmer, Inc. USA). The quality was checked on a random sampling using the High Sensitivity DNA chip Assay (Agilent). The cDNA was fragmented by Covaris E210 (Covaris, USA) sonication for 55 seconds, using a Duty cycle of 20% and Intensity of 5. Plate-based libraries were prepared following the BC Cancer Agency's Michael Smith Genome Sciences Centre (BCGSC) paired-end (PE) protocol on a Biomek FX robot (Beckman-Coulter, USA). Briefly, the cDNA was purified in 96-well format using Ampure XP SPRI beads, and was subject to end-repair and phosphorylation by T4 DNA polymerase, Klenow DNA Polymerase, and T4 polynucleotide kinase respectively in a single reaction, followed by cleanup using Ampure XP SPRI beads and 3' A-tailing by Klenow fragment (3' to 5' exo minus). After cleanup using Ampure XP SPRI beads, picogreen quantification was performed to determine the amount of Illumina PE adapters used in the next step of adapter ligation reaction. The adapter-ligated products were purified using Ampure XP SPRI beads, then PCR-amplified with Phusion DNA Polymerase (Thermo Fisher Scientific Inc. USA) using Illumina's PE primer set, with cycle conditions of 98°C 30sec followed by 10-15 cycles of 98°C 10sec, 65°C 30sec and 72°C 30sec, and then 72°C 5min. The PCR products were purified using Ampure XP SPRI beads, and checked with a Caliper LabChip GX for DNA samples using the High Sensitivity Assay (PerkinElmer, Inc. USA). PCR products with a desired size range were purified using a 96-channel size selection robot developed at the BCGSC, and the DNA quality was assessed and quantified using an Agilent DNA 1000 series II assay and Quant-iT dsDNA HS Assay Kit using Qubit fluorometer (Invitrogen), then diluted to 8nM. The final concentration was verified by Quant-iT dsDNA HS Assay .The libraries, 2 per 100 PE lane, were sequenced on the Illumina HiSeq 2000/2500 platform using v3 chemistry and HiSeq Control Software version 2.0.10.

Alignment of strand-specific RNA-seq data

Illumina paired-end RNA sequencing data was aligned to GRCh37-lite genome-plus-junctions reference (Butterfield et al., 2014) using BWA (version 0.5.7) (Li and Durbin, 2009). This reference is a combination of GRCh37-lite assembly and exon-exon junction sequences with coordinates defined based on transcripts in Ensembl (v61), Refseq and known genes from the UCSC genome browser (both were downloaded from UCSC in November 2011; The GRCh37-lite assembly is available at http://www.bcgsc.ca/downloads/genomes/9606/hg19/1000genomes/bwa_ind/genome. BWA "aln" and "sampe" were run with default parameters, except for the inclusion of the (-s) option to disable the Smith-Waterman alignment, which is unsuitable for insert size distribution in paired-end RNA-Seq data. Finally, reads failing the Illumina chastity filter are flagged with a custom script, and duplicated reads were flagged with Picard Tools (version 1.31). After the alignment, the junction-aligned reads

that mapped to exon-exon junctions were repositioned to the genome as large-gapped alignments and tagged with "ZJ:Z" by JAGuaR (version 2.0).

Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

Cells were treated with DMSO, BKM-120, LBH-589 or the combination of these two drugs at the indicated doses for 6 or 12 hr. Cells were washed twice with cold PBS and lysed for total RNA extraction using a Qiagen RNeasy Kit. Extracted RNAs were then used to generate cDNA using iScript cDNA synthesis kit (Bio-Rad). Real-time PCR was carried out in triplicate using SYBR Green PCR Master Mix (Bio-Rad) on a Bio-Rad CFX384 Real-Time System. The following primers were used in this study: *Foxo1* forward: 5'-ATGCTCAATCCAGAGGGAGG-3', reverse: 5'-ACTCGCAGGCCACTTAGAAAA-3'; *Foxo3a* forward: 5'-CTGGGGGAACCTGTCCTATG-3', reverse: 5'-TCATTCTGAACGCGCATGAAG-3'; *Foxo4* forward: 5'-CTTCCTCGACCAGACCTCG-3', reverse: 5'-ACAGGATCGGTTCGGAGTGT-3'.

Subcellular Fractionation

Cells were treated with DMSO, BKM-120, LBH-589 or the combination of these two drugs at the indicated doses for 6 hr. For whole cell lysates, cells were washed and lysed as described above. Subcellular fractionation was performed using the Cell Fractionation Kit (#9038, Cell Signaling) according to manufacturer's instructions. Briefly, after wash, cell pellets were re-suspended in Cytoplasmic Isolation Buffer (CIB), vortexed, and incubated on ice for 5 min. Cells were then centrifuged at 500x g for 5 min. The supernatant was collected as the "cytoplasmic" fraction. The pellet was washed with ice-cold PBS twice and re-dissolved in Nuclear Isolation Buffer (NIB). The lysate was then vortexed, incubated on ice for 10 min, sonicated, and collected as the "nuclear" fraction. Proteins in all lysates were quantified and Western Blotting was performed as described.

Cell Lysis and Western Blotting

Cells were treated with DMSO, BKM-120, LBH-589 or the combination of these two drugs at the indicated doses for 6 or 12 hr. Cells were washed twice with cold PBS and lysed in RIPA buffer with protease and phosphatase inhibitor cocktail (Cell Signaling, Cat# 5872) for 30 min on ice. The cell lysates were sonicated using an ultrasonicator (Misonix) at amplitude 2 for 5 seconds, and then centrifuged at 13,000 rpm for 10 min at 4°C. Protein concentrations were measured using a Pierce BCA Protein Assay Kit (Thermo Scientific #23225). Equal amounts of protein were separated by 8% (for p-FOXO1, FOXO1, pAKT, AKT, pS6, S6, nuclear LMNA, GAPDH, or ACTB) or 12% (for Ac-H3K9, Ac-H3K27, and total histone H3) SDS-PAGE and transferred to nitrocellulose membranes (Invitrogen, Cat# LC2006). The membranes were blocked with 5% nonfat dried milk (Bio-Rad) for 1 hr at room temperature and incubated in primary antibodies diluted in 5% BSA (Cell Signaling #9998) overnight at 4°C with gentle shaking. The membranes were then washed with Tris-buffered saline with 0.1% Tween-20 (TBS-T) and incubated with Li-Cor IRDye secondary antibodies for 45 min at room temperature. The membranes were washed again as described above, imaged, and quantified using Li-Cor Odyssey CLx imager. The primary antibodies were purchased from Cell Signaling Technology and diluted 1:1000 in 5% BSA/TBS-T; the secondary antibodies were from Li-Cor Biosciences and diluted 1:5000 in 5% BSA/TBS-T.

Generation of Normal Neurons and Astrocytes

Cerebellar granule neuron precursors (GNPs) were isolated as described previously (Lee et al., 2005). Briefly, cerebella were collected from postnatal day 5-6 (P5-6) C57BL/6J mice, digested in papain solution (Worthington Biochemical Corporation, Lakewood, NJ) to obtain a single-cell suspension, and centrifuged through a 35%-65% Percoll gradient (Amersham Biosciences, Piscataway, NJ). GNPs were isolated from the 35%–65% interface. To generate post-mitotic granule neurons, GNPs were cultured for 5 days in NB-B27 (Neurobasal medium with 1 mM sodium pyruvate, 2 mM L-glutamine, penicillin/streptomycin, and B27 supplement, all from Invitrogen) plus 1% FBS (Invitrogen), 1mM KCl and 1 mM glucose. Differentiated cells were plated on 1% growth-factor-reduced Matrigel (BD BioScience)-coated 384-well plates, treated with compounds at 1 μM concentration in triplicate wells for 48 hr, and then assessed for survival using the CellTiter-Glo assay.

Mouse cerebellar astrocytes (ScienCell Cat. No. M1810) were cultured in astrocyte medium (Cat. No. 1831) in poly-D-lysine-coated 384-well plates. At ~90% confluence, cells were treated with compounds at 1 μM concentration for 48 hr and cell viability was assessed as described.

Drug Synergy Analysis

To determine whether the anti-tumor effects of LBH-589 and BKM-120 were synergistic, tumor cells were treated with various concentrations of LBH-589 and BKM-120, alone or in combination. Cell viability was measured using CellTiter-Glo assay. Dose-response curves of single compounds and combinations were generated by Graphpad Prism software (GraphPad Sofware, Inc., La Jolla, CA). The combination index (CI) was

calculated according to standard isobologram analysis using the CompuSyn software (ComboSyn, Inc., Paramus, NJ) following developer's instruction. Briefly, drug synergism was quantified by determining the combination index (CI), where CI < 1, CI = 1, and CI > 1 indicate synergistic, additive, and antagonistic effects, respectively.

Treatment of Mice with Flank Tumors

To create flank tumors, 10^5 cells of mouse MP tumor or human Group 3 xenograft were suspended in growth-factor-reduced Matrigel (BD BioSciences) at a ratio 1:1 and injected subcutaneously into the flanks of 6-8 weeks old CD-1 nu/nu immunocompromised mice. Tumors were measured with calipers every two days, and tumor volumes were calculated as length × width^2 × 0.52 with all measurements in millimeters. When tumor volume reached ~100mm^3 (usually ~10 days after inoculation), mice were randomized into the following treatment groups: vehicle (5% Dextrose + 0.5% methylcellulose); BKM-120 at 30 mg/kg by gavage; LBH-589 at 10mg/kg by intraperitoneal injection; or both drugs at the same doses for 8 days consecutively. 24 hr after the last dose, animals were euthanized and tumors were harvested and weighed. The preparation of drugs was the same as described in the Experimental Procedures.

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