Supplementary Methods

Information of patient derived GSCs and other cells

The T387 GSCs was derived from a GBM from a 76-year old female patient. The T3565 GSCs was derived from a glioblastoma from a 32-year old male patient. The CW738 GSCs were derived from a 37-year old male patient. The CW839 GSCs were derived from a 47-year old female patient. The 1517 GSC were derived from a 54-year old female patient.

ENSA (ENSterm-A, Millipore, SCC003) are human embryonic stem derived neural progenitors. hNP1 Human Neural Progenitors (Neuromics, HN60001) are fully differentiated and derived as adherent cells from hESC WA09 line. Non-malignant brain cultures (NM263, NM290) were derived from excess surgical tissue from human epilepsy specimens. Non-malignant brain cells were cultured in mixed media composed of half Dulbecco's modified Eagle medium (DMEM) with 10% FBS and half Neurobasal-A medium. All NSCs and isolated glioblastoma stem cells (GSCs) were cultured in Neurobasal-A medium supplemented with B27 (Life Technology, 12587010), 20 ng/ml EGF (R&D system, 236-EG-01M) and 20 ng/ml bFGF (R&D system, 4114-TC-01M), 1 mM glutaMAX™ (Life Technology, 35050061) and 1 mM sodium pyruvate (Life Technology, 11360070) to maintain the expression of cell surface makers .

Plasmids and cloning

Lentivirus vector clones expressing sh*BMAL1* and sh*CLOCK* were from Sigma-Aldrich (Sigma). Lentivirus were produced as previously described (47). shRNAs used in this study are sh*BMAL1*: TRCN0000019097 and TRCN0000019096 (NM_001178.3-1536s1c1, NM_001178.3-689s1c1), sh*CLOCK*: TRCN0000018976 and TRCN0000018978 (NM_004898.2-1053s1c1, NM_004898.2-1494s1c1). sh*SDHA*: TRCN0000028085 and TRCN0000028043 (NM_004168.1-1523s1c1, NM_004168.1-619s1c1). shNPAS2: TRCN0000234494 and TRCN0000234492 (NM_002518.3-1083s21c1, NM_002518.3-469s21c1), A non-targeting shRNA that does not target any known human transcript (Sigma-Aldrich, SCH002), is the negative control. CRISPR/Cas9-gRNAs targeting BMAL1 or CLOCK were cloned by inserting the annealed oligos to lentiCRISPR v2 backbone (Addgene plasmid

#52961) and an empty backbone without insertion was used as negative control. Human BMAL1 and CLOCK cDNAs were obtained from Vectorbuilder (BMAL1: VB161019-1118qsc, CLOCK: VB170403-1071kfe). CRY1 coding sequences were amplified from pDONR223_CRY1_WT (addgene, 82264). GFP cDNA was amplified from pCDH-MSCV-MCS-EF1a-GFP (System Biosciences, CD711-B1). Then these sequences were cloned into mammalian expression vectors (pCDH-MCS-T2A-Puro-MSCV) by In-Fusion® HD Cloning Kit (Clontech, 638920). All plasmids were verified by Sanger sequencing.

Cell Viability Assay and Sphere Formation

For experiment measuring cell viability, cells were plated in 96-well plate at a density of 1,000 cells per well. Relative ATP level was measured following the instruction of CellTiter-Glo Luminescent Cell Viability Assay (Promega, G7571). To record sphere formation, cells were planted in 96-well plates with decreasing serial numbers per well (500, 200, 100, 50, 20, and 10) with 6 replicates. Presence and number of spheres in each well were recorded 8 days after plating. Extreme limiting dilution assay were analyzed using package “statmod” in R language.

Apoptosis Assay

Apoptosis was measured using FITC-Annexin V antibody and Propidium Iodide (PI) staining following manufacturer’s instruction (Life Technology, V13241). Cells were read using flow cytometry (BD Bioscience) and the results were analyzed via FlowJo.

Cell Cycle and Edu Assays

Cells were collected and fixed 48 hr after lentivirus infection, followed by PI staining for 30 min at room temperature. Then cells were spun down and suspended in PBS for flow cytometry. Results were analyzed using Modfit and FlowJo software.

Edu staining was performed by Click-iT Edu Alexa Fluor 488 imaging kit (Life Technology, C10632) following manufacturer's instruction.

In Vitro Drug Treatment

Cells were plated in a 96-well or 6-well plate (10^3 cells per well) with 4 replicates. The cells were treated with various concentration of SR9011 (Xcess Biosciences, M66049-2), SR9009 (Calbiochem, 5542) and KL001 (Sigma, SML1032) for 3 days. Cell viability was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, G7571). For gene expression analysis, transcript levels of *BMAL1, PER1, PER2, SOX2* and *OLIG2* were determined by quantitative RT-PCR as described above.

Western Blot and Immunofluorescence

Cells were collected and lysed with RIPA buffer. Specific antibodies used are BMAL1 (Cell Signaling Technology, 14020), CLOCK (Cell Signaling Technology, 5157), SOX2 (1:1000, Millipore, AB5603), OLIG2 (1:1000, Millipore, MABN50), MYC (Santa Cruz, sc-764), cleaved CASPASE 3 (Cell Signaling Technology, 9664), cleaved PARP (Cell Signaling Technology, 9532), TP73 (Proteintech, 66990-1-Ig), CRY1 (Abcam, ab104736), NPAS2 (Abcam, ab137601) and TUBULIN (Sigma, T6074). Afterward, membranes were incubated with HRP-linked secondary (Cell Signaling Technology, 7076 is anti-mouse IgG and 7074 is anti-rabbit IgG) for 1 hour at room temperature. Then chemical signal was detected by chemiluminescent ECL buffer (Thermo Fisher, PI34080).

To perform immunostaining, cells were plated on an 18-mm coverslip pre-coated with Matrigel (Corning, 354277) in 12-well plates. After transduction with shRNA, cells were rinsed with PBS twice and fixed with fresh 4% paraformaldehyde in PBS for 15 min at room temperature. Cells were subsequently rinsed twice with PBS followed by permeabilized in 0.05% Triton X-100 in PBS for 5 min. Coverslips with cells were then washed twice with PBS for 5 min each and blocked with normal 5% goat serum (Sigma, G9023) in PBS for 30 min, followed by incubated

with primary antibodies diluted in blocking buffer. Antibodies against cleaved CASPASE 3 and Ki67 (Dako, M7240) were incubated with cells overnight at 4°C, followed by incubation with the secondary antibody: Alexa Fluor 488 donkey anti-rabbit (Life Technology, 21202), Alexa Fluor 568 donkey anti-mouse (Life Technology, A-11057). Nuclei were stained with DAPI (Life Technology, D1306). Images were taken using Leica DM4000 upright microscope or confocal laser scanning microscope (Leica TCS SP5) with the same settings.

Chromatin Immunoprecipitation Assay

BMAL1 ChIP assays were performed with 10^7 cells using Magna ChIP-seq kit (Millipore, 17-10085) according to manufacturer's instruction. Briefly, cells were fixed with 1% formaldehyde at room temperature for 10 minutes, and subsequently quenched with 0.125M glycine. Then, the cells were washed with cold PBS and suspended with lysis buffer supplemented with Protease Inhibitor Cocktail II. Chromatin was sonicated to obtain DNA fragments with an average length of 200-500 bp for sequencing by Bioruptor Plus sonication device (Diagenode) followed by centrifugation for clearing. Soluble fraction of sheared chromatin was diluted 10-fold with ChIP dilution buffer and incubated with BMAL1 antibody (Cell Signaling Technology) and EZ-Magna ChIP A/G magnetic beads overnight at 4°C with rotation. Chromatin-captured beads were washed once with low salt wash buffer, once with high salt wash buffer, once with LiCl wash buffer and twice with TE buffer for 5 min each by rotation at 4°C. Beads were resuspended in elution buffer and removed by magnet. Proteinase K was added to the eluted DNA followed by incubation at 62°C for 4 hours with shaking. Then the eluted DNA was incubated at 95°C for 10 min to remove RNA. Reverse-crosslinked DNA was purified by using purification columns. Eluted DNA was used to do quantitative PCR (qPCR) or sequencing library preparation. ChIP quantitative RT-PCR was performed by using SYBR Green PCR Master Mix (ABI, 4309155). qPCR primers were listed as Table S1. Then sequencing libraries were prepared by using ThruPLEX® NGS Library Preparation Kits (Rubicon Genomics, R400427) according to manufacturer's instruction. ChIP-seq libraries were sequenced on Illumima HiSeq platform by Genewiz.

ChIP-Sequencing Data Analysis

Single-end BMAL1 ChIP-seq reads were trimmed using Trim Galore v0.4.3 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and cutadapt 1.14 (http://cutadapt.readthedocs.io/en/stable/guide.html). Reads were aligned to the hg19 human genome with BWA-MEM v0.7.17. BAM files were processed using SAMtools and PCR duplicates were removed with PicardTools (http://broadinstitute.github.io/picard/). BMAL1 peaks were called using MACS2 (v2.1.1) using a ChIP input file as a control using default settings. Replicate sample BAM files were merged using SAMBAMBA (v0.6.6, http://lomereiter.github.io/sambamba/). BIGWIG track coverage files were generated from merged BAM files using the DeepTools (v2.4.1) bamCoverage command with RPKM normalization (http://deeptools.readthedocs.io/en/latest/index.html). DiffBind was used to identify differential BMAL1 ChIP-seq peaks between GSC and NSC samples and to perform principal component analysis. Differential BMAL1 peaks (GSC or NSC specific BMAL1 peaks) were defined using the DESeq2 method within Diffbind, which selects differential regions based on an FDR-corrected q-value of 0.05. Motif enrichment analysis was performed using HOMER on the hg19 genome using default settings.

GSC and NSC “consensus” peak sets were defined by selecting peaks that overlapped in all 4 GSC or NSC samples respectively, using bedtools v2.24.0 (http://bedtools.readthedocs.io/en/latest/).

Gene Set Enrichment analysis was performed by selecting GSC and NSC specific BMAL1 peaks called using DiffBind and performing a preranked GSEA using the javaGSEA Desktop Application. Genes were ranked based on the BMAL1 signal fold-change as defined by DiffBind for each differential peak. Pathway enrichment bubble plots were generated using the javaGSEA Desktop application in conjunction with Cytoscape (v3.4.0). Coverage heatmaps and profiles were generated using the DeepTools (v2.4.1) computeMatrix and plotHeatmap functions with scaled regions. The canonical E-BOX motif was derived from Liver-Bmal1-ChIP-Seq (GSE39860) and genomic regions containing this sequence were identified using

HOMER. Genomic region annotation was performed using CEAS. The BMAL1 ChIP-seq in liver, heart and kidney were from GSE110604 (4).

Overlap among H3K27ac, H3K3me3 and BMAL1 peaks

Intersection and visualization of H3K27ac, H3K4me3, and BMAL1 ChIP-seq peak overlaps were performed using Intervene (v0.6.4) and the associated venn diagram and upset plot modules. Visualization of genomic regions was performed using the overlap region sets calculated with Intervene and plotted using the DeepTools (v2.4.1) “compute-matrix” function with the “scale-regions” flags and the “plotheatmap” function.

Bioluminescence Recording and Data Analysis

Plasmid expressing firefly luciferase under the control of mouse *Arntl* promoter (designed as *BMAL1*- LUC for pLV6-Bmal-luc) was obtained from Addgene (Addgene, 68833). Cells were infected with *BMAL1::Luc* lentivirus supplemented with polybrene (Sigma, H9268), followed by replacement with fresh media after 12 hours. Cells constitutively expressing *BMAL1::Luc* were obtained using blasticidin selection.

Cells were plated on 35-mm dishes in growth media and were synchronized as previously described by a dexamethasone or forskolin shock (1). For GSC, DGC and NSC cells, the dishes were pre-coated with Matrigel (Corning, 354277, 1:40-60 dilution in PBS, coated for 30 min at 37°C). In brief, after reaching 80-90% confluency, cell culture media was replaced with growth media containing 10 mM HEPES and 100 μM D-luciferin. Dishes were covered with 40 mm glass coverslips (Fisher Scientific) and sealed with vacuum grease to prevent evaporation. The luminescence signals of reporter cells were monitored every 10 min using a LumiCycle luminometer (Actimetrics) at 37°C without CO_2. After 12-24 hours, the media was replaced with growth media containing 10 mM HEPES, 100 μM D-luciferin, and 100 nM dexamethasone or 10 μM forskolin. And the recording was resumed.

Data were analyzed using the LumiCycle software (Actimetrics). The baseline drift was removed from data by applying “Running Average”, with “24 hours” selected. The Smooth control is used to smooth the small-amplitude, rapid fluctuations in the data. Baseline-subtracted data were then fitted to a sine wave and the cycle parameters were determined including phase, period, amplitude, damping rate, and goodness-of-fit (to the sine wave). Data of the first day post media change were excluded from the analysis.

Metabolic flux measurement

The extracellular acidification rate (ECAR) and oxygen consumption rate were measured using Seahorse Extracellular Flux Analyzer XF24 (Agilent Technology) and Mito Stress Test Kit (Agilent Technology, 103015-100) as described previously (2). Briefly, cells were plated 12 hours before the experiment at a density of $8x10^4$ cells per well in a Matrigel pre-coated special 24 well plate (Agilent Technology, 100867-100). Cells were equilibrated with un-buffered XF basal media (Agilent Technology, 102353) supplement with 1mM pyruvate at 37°C for 1 hours in the incubator without CO2. During this time, Seahorse cartridge containing oxygen and pH probes were filled with metabolic inhibitors including glucose, oligomycin, rotenone and 2-deoxyglucose (2-DG) (50mM), and loaded in Seahorse Analyzer for calibration. The results were calculated according to protein level. For OCR, the sequential inhibitors for oxidative phosphorylation are oligomycin (1 μM), FCCP (2 μM), rotenone (1 μM) and antimycin A (1 μM). For ECAR, the cells were sequentially treated with glucose (100 mM), oligomycin (oligo, 1 μM) and 2- deoxyglucose (2- DG, 50 mM). ECAR and OCR was subsequently monitored in real-time via a typical 8-min cycle of mix (2–4 min), dwell (2 min) and measure (2–4 min) with sequential injection of these inhibitors. There were 3 cycle measurements after each inhibitor treatment, and each data point is the average of 4 individual wells for each treatment. The ECAR and OCR curve were obtained. Different metabolic parameters were extracted from the results: (1) ATP uncoupled OCR by oligomycin, an inhibitor of ATP synthase, representing mitochondrial ATP capacity; (2) maximal respiration rate determined by FCCP, an un-coupler maximizing mitochondrial reserve capacity to adapt to the respiration stress; (3) basal ECAR using glucose injection and basal OCR were obtained before inhibitor treatment, (4) maximal

glycolysis capacity was induced by oligomycin during ECAR measurement, representing the ability of the cell to utilize glycolysis for ATP production with the electron transport chain inhibition.

RNA Isolation and Quantitative RT-PCR

Cellular RNA was isolated using RNeasy kit and reverse-transcribed to cDNA using cDNA synthesis kit (ABI, 4387406). Then real-time PCR was measured using SYBR Green Mastermix (ABI, 4309155) on an Applied Biosystems 7900HT cycler. mRNA levels were normalized to 18sRNA level (Table S1). RNA-seq library were sequenced on Illumima HiSeq platform by IGM at University of California, San Diego.

RNA-sequencing Data Analysis

The Fastq files of the RNA-seq data were processed to read quality control by running 'FastQC' (Galaxy version 0.72) in Galaxy (usegalaxy.org) and then to remove the adapters by running 'Trim Galore!' (Galaxy version 0.4.3.1). The trimmed reads were mapped to human genome 38 by 'HISAT2' (Galaxy version 2.1.0+galaxy 4) and the mapped reads were then counted with 'featureCounts' (Galaxy version 1.6.3+galaxy 2). The counts were processed to differentially expressed gene analysis with R package 'DESeq2' (Version 1.14.1). Pre-ranked genes by fold change were processed to GSEA (Gene Set Enrichment Analysis; Board institute) with either previously identified gene sets of cell cycle phase G2M and phase M (3) or metabolic targets of gained BMAL1 binding in GSCs (GSC specific metabolic genes).

Metabolic gene list was obtained by searching C2CP_KEGG collection in MSigDB (Molecular Signatures database, Board Institute) with keyword 'metabolism' and integrated all 43 gene sets into one gene list called 'metabolic genes'. 'GSC specific metabolic genes' were obtained by overlapping the metabolic gene list with GSC specific BMAL1 targets identified by gained BMAL1 binding.

Data Analysis and Statistical Methods

For quantitative real-time PCR, mRNA levels were determined using the Ct method with normalization to the housekeeping gene 18S ribosomal RNA. For all statistical tests, the exact "n" values can be found in figure legends. All quantitative data are presented as mean ± standard deviation from at least three samples or experiments, unless otherwise stated. The significance was determined by two-tailed un-paired Student's t-test, one-way ANOVA with Tukey's multiple comparison, or two-way ANOVA with Tukey's multiple comparison. Survival of tumor-bearing mice underwent Kaplan-Meier estimator survival analysis. Significance was determined by Mantel-Cox log-rank test. Methods were stated in figure legends. Statistical significance is shown as: *, $P < 0.5$; **, $P < 0.01$; and ***, $P < 0.001$. Statistical analysis was performed using Graphpad PRISM software.

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