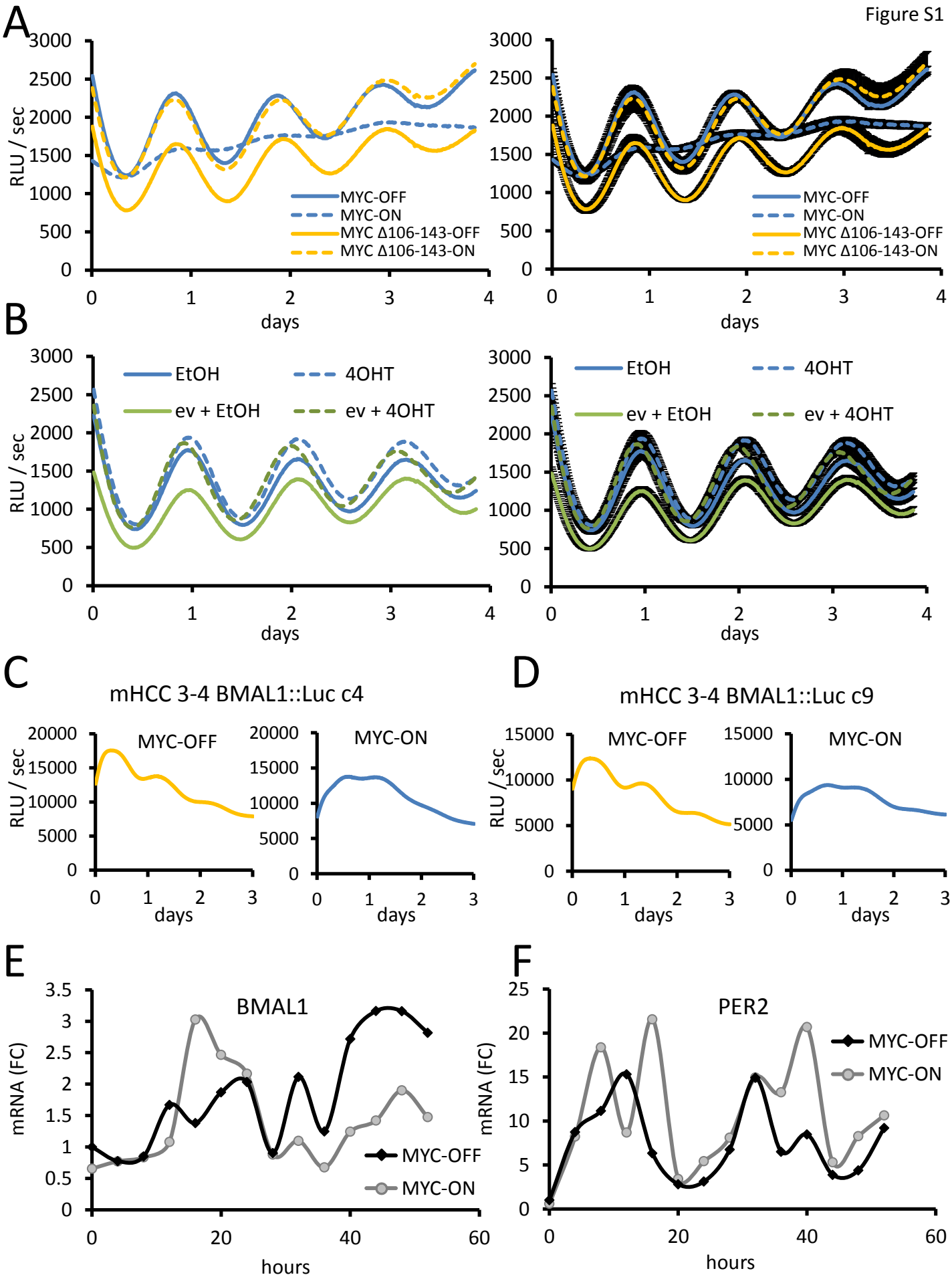
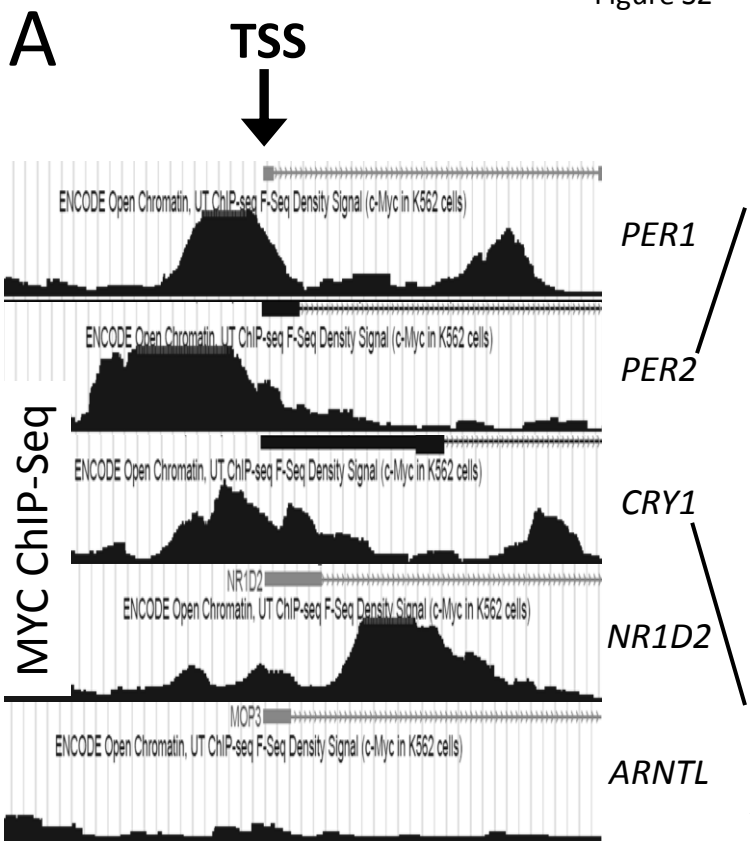

A
RLU / sec
days
MYC-OFF
MYC-ON
MYC Δ106-143-OFF
MYC Δ106-143-ON
B
EtOH
4OHT
ev + EtOH
ev + 4OHT
C
mHCC 3-4 BMAL1::Luc c4
MYC-OFF
MYC-ON
D
mHCC 3-4 BMAL1::Luc c9
E
BMAL1
mRNA (FC)
hours
F
PER2

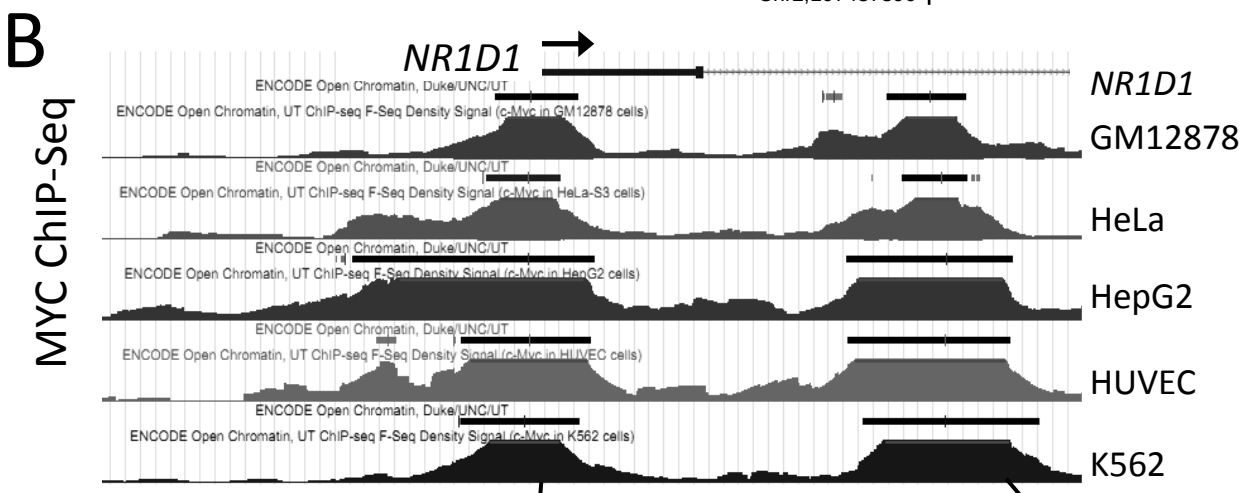
Figure S1, related to Figure 1. **Verification of MYC-inducible cell models, and disruption of circadian oscillation by MYC in mouse heptaocellular carcinoma cells. A.** U2OS BMAL1::Luciferase cells stably expressing human wild-type MYC-ER$^{TM}$ or human MYC-ER$^{TM}$ Δ106-143 were treated as described in **Figure 1A,** and measured as technical triplicates for luminescence every 10 minutes on a Lumicycle luminometer. Left panel is identical to **Figure 1A**, right panel represents standard error of technical triplicate samples collected at different times over the course of no more than 10 minutes. **B.** U2OS BMAL::Luc cells expressing pBabe-Zeo empty vector (ev) or mock infected were treated as described in **Figure 1D,** and measured as technical triplicates for luminescence every 10 minutes on a Lumicycle luminometer. Left panel is identical to **Figure 1D**, right panel represents standard error of technical triplicate samples collected at different times over the course of no more than 10 minutes. **C,D.** (**C**) mHCC 3-4 clone 4 or (**D**) mHCC 3-4 clone 9 cells were treated with tetracycline (tet) for 24 hours (MYC-OFF) or control (-tet, MYC-ON), then cultured ± tet and 100 mM luciferin, and synchronized with 0.1 μM dexamethasone. Temporal luminescence over 3 days representing BMAL1 promoter activity was visualized in real time using a LumiCycle™ luminometer. **E,F.** The parental line of mHCC 3-4 cells with conditional Tet-OFF c-Myc expression was cultured with 20ng/ml tetracycline (+tet, MYC-OFF) to ablate MYC expression or media (-tet, MYC-ON) control for 24 hours, then cultured ± tet and synchronized with dexamethasone. mRNA was collected every four hours for 52 hours, reverse-transcribed to cDNA, and (**E**) BMAL1 (*Arntl)* expression or (**F**) *Per2* expression was determined by quantitative real-time PCR, normalized to expression of β2M. Data are representative of two or more experiments. mRNA (FC) = Fold Change.

Figure S2




| Species | *PER2* promoter E-Box | |
|---|---|---|
| Human | …A G T T C | **C A T G T G** C G T C T … |
| Chimp | …A G T T C | **C A T G T G** C G T C T … |
| Rhesus | …A G T T C | **C A T G T G** C G T C T … |
| Mouse | …A G T T C | **C A T G T G** C G T C T … |
| Rat | …A G T T C | **C A T G T G** C G T C T … |
| Pig | …A G T T C | **C A T G T G** C G T C T … |
| Cow | …A G T T C | **C A T G T G** C G T C T … |
| Dog | …A G T T C | **C A T G T G** C G T C T … |
| Aardvark | …A G T T C | **C A T G T G** C G T C T … |
| Opossum | …A G T T C | **C A T G T G** C G T C T … |
| Wallaby | …A G T T C | **C A T G T G** C G G C T … |
| | Chr2,239197590 \| | 239197580 \| |

| Species | *CRY1* promoter E-Box | |
|---|---|---|
| Human | … C T C C C | **C T C G T G** A C C A C… |
| Chimp | … C T C C C | **C T C G T G** A C C A C… |
| Rhesus | … C T C C C | **C A C G T G** A C C A C… |
| Mouse | … C A C C C | **C A C G T G** A C C A C… |
| Rat | … C T C C C | **C A C G T G** A C C A C… |
| Pig | … C T C C C | **C A C G T G** A C C C C… |
| Cow | … T T C C C | **C A C G T G** A C C A C… |
| Dog | … C T C C C | **C A C G T G** A C C C C… |
| Aardvark | … T - T C C | **C A C G T G** A C C A T… |
| Opossum | … T C A G C | **C A C G T G** A C C C A… |
| Wallaby | … C C T G C | **C A C G T G** A C C G C… |
| | Chr2,107487390 \| | 107487400 \| |




| Species | *NR1D1* promoter E-Box | | *NR1D1* 1ST intron E-Box | |
|---|---|---|---|---|
| Human | … G C A A T | **C A C G T G** A A G C C… | …A G G C A | **C A C G T G** G A G C A … |
| Chimp | … G C A A T | **C A C G T G** A A G C C… | …A G G C A | **C A C G T G** G A G C A … |
| Rhesus | … G C A A T | **C A C G T G** A A G C C… | …A G G C A | **C A C G T G** G A G C G … |
| Mouse | … C C A A T | **C A C G T G** A A G C T… | …G G G C A | **C A C G T G** G A G T G … |
| Rat | … G C A A T | **C A C G T G** A A G C T… | …G G G C A | **C A C G T G** G A G C G … |
| Pig | … G C A A T | **C A C G T G** A A G C C… | …A G G C A | **C A C G T G** G A G C T … |
| Cow | … A C A A T | **C A C G T G** A A G C C… | … A G T C A | **C A C G T G** G A G C T … |
| Dog | … G C A A T | **C A C G T G** A A G C C… | …A G G C A | **C A C G T G** G A G T G … |
| Aardvark | … G C A A T | **C A C G T G** A A G C C… | … A G A C A | **C A C G T G** G A G C A … |
| Opossum | … G C A A T | **C A C G T G** A A C C C… | …C G G C A | **C A C G T G** G A G T G … |
| Wallaby | … G C A A T | **C A C G T G** A A C C C… | …C G G C A | **C A C G T G** G A G T G … |
| | Chr17,38257310 \| | 38257295 \| | 38255405 \| | 38255390 \| |

Figure S2, related to Figure 2. **MYC directly engages the E-box promoter elements of circadian rhythm genes**. **A.** The UCSC Genome Browser Human March 2006 and February 2009 assemblies, which contain the results of a MYC CHIP-Seq experiment from the ENCODE project, were queried for c-Myc binding to the regulatory elements of *PER1, PER2, CRY1, NR1D2* (REV-ERBβ), and *ARNTL* (BMAL1) in K562 chronic myelogenous leukemia cells. Arrow indicates transcriptional start site (TSS). Similar binding data were observed in GM12878, HeLa, HepG2, and HUVEC (Dunham et al., 2012). The canonical E-box sequence of the promoter MYC binding peak in *PER2 and CRY1* was identified (boxed sequence), and conservation was compared to other mammalian species. The sequence was not conserved in frog and chicken (Siepel et al., 2005). **B.** ENCODE data (Dunham et al., 2012; Kent et al., 2002) was queried for MYC binding to REV-ERBα (*NR1D1)* promoter in multiple cell lines. MYC showed strong binding to canonical E-Box element (Zeller et al., 2006) in the promoter region of *NR1D1* in GM12878 (lymphoblastoid), HeLa (cervical cancer), HepG2 (kidney cancer), K562 (chronic myelogenous leukemia lines), and HUVEC (Human Umbilical Vein Endothelial Cells) cells. The canonical E-box sequence of each MYC binding peak in *NR1D1* was identified (boxed sequence), and conservation was compared to other mammalian species. The sequence was not conserved in frog and chicken (Siepel et al., 2005).

Figure S3

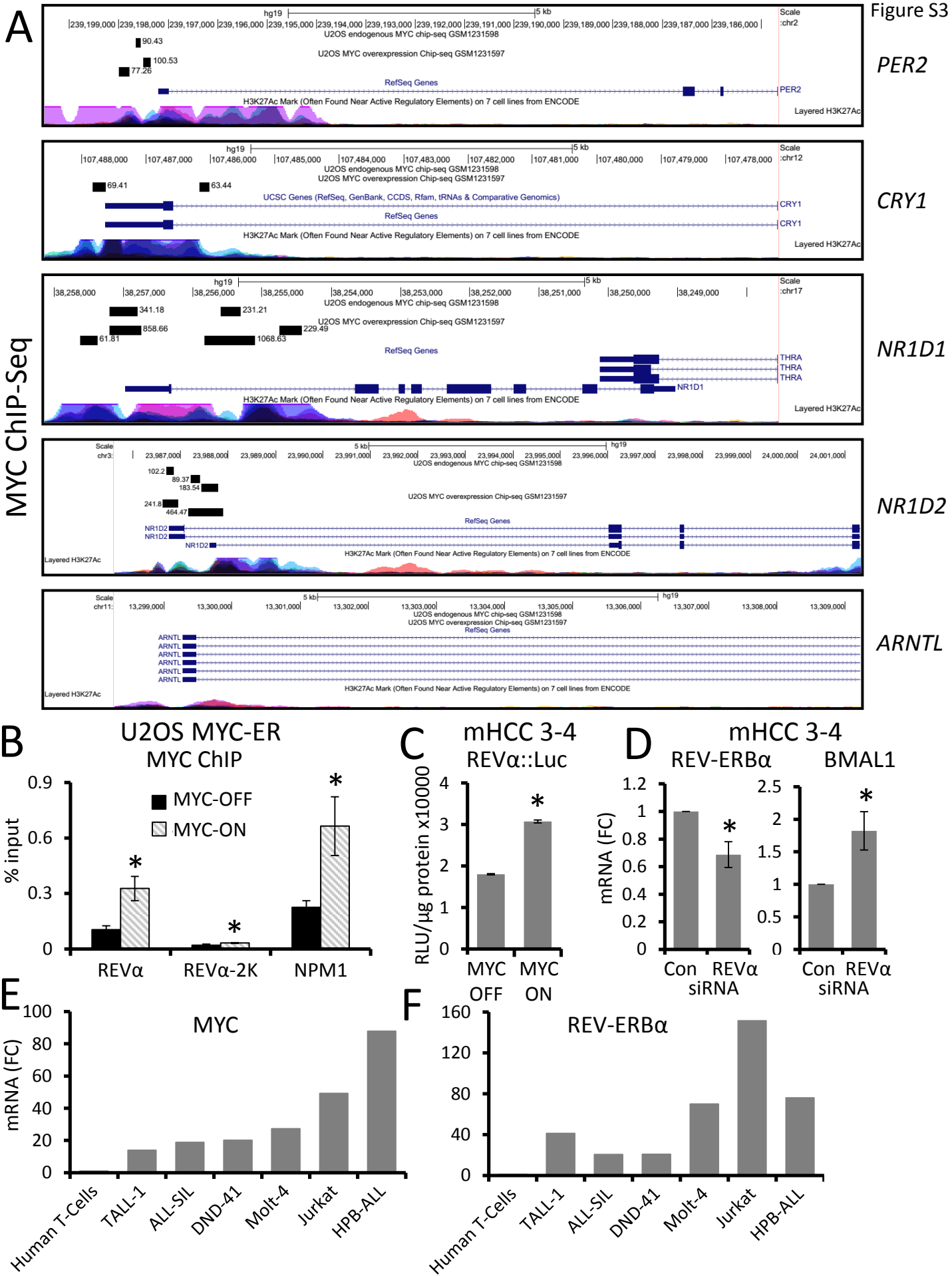

A
MYC ChIP-Seq
PER2
CRY1
NR1D1
NR1D2
ARNTL
U2OS endogenous MYC chip-seq GSM1231598
U2OS MYC overexpression Chip-seq GSM1231597
RefSeq Genes
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
Layered H3K27Ac
B
U2OS MYC-ER
MYC ChIP
MYC-OFF
MYC-ON
% input
REVα
REVα-2K
NPM1
C
mHCC 3-4
REVα::Luc
RLU/µg protein x10000
MYC OFF
MYC ON
D
mHCC 3-4
REV-ERBα
BMAL1
mRNA (FC)
Con siRNA
REVα siRNA
E
MYC
mRNA (FC)
Human T-Cells
TALL-1
ALL-SIL
DND-41
Molt-4
Jurkat
HPB-ALL
F
REV-ERBα

Figure S3, related to Figure 2. **Oncogenic MYC binds circadian gene promoters and regulates expression in U2OS and mouse hepatocellular carcinoma cells, and correlates with elevated REV-ERBα in human T-acute lymphoblastic leukemia (T-ALL). A.** The results of an endogenous MYC and doxycycline-inducible overexpressed MYC ChIP-Seq experiment (Walz et al., 2014) were loaded onto the February 2009 assembly of the USCS Genome Browser. Endogenous and inducible MYC binding to the promoters of *PER2, CRY1, NR1D1* (REV-ERBα)*, NR1D2* (REV-ERBβ)*,* and *ARNTL* (BMAL1) were assessed, indicated by black boxes above the gene, and numbers indicate quantified peak intensities. Below the gene, layered H3K27Ac peaks from the ENCODE project indicate sites of active transcription. **B.** U2OS MYC-ER expressing cells were treated with 4OHT (MYC-ON) or EtOH control (MYC-OFF) for 24 hours, and cells were fixed, lysed, sonicated, and chromatin was immunoprecipitated (ChIP) by anti-MYC according to manufacturer's instructions (Sigma Imprint Ultra Chromatin Immunoprecipitation Kit, Sigma). The following sites were targeted: the REV-ERBα promoter centered on the promoter E-box region (REVα, 81 bp fragment), 2Kb upstream of REV-ERBα transcription start site (REVα-2k, 99 bp fragment), and the promoter of the canonical MYC target nucleophosmin 1 (*NPM1*)(O'Donnell et al., 2005). Means and SDs from technical triplicates are shown. *$p < 0.05$ by Student's t test of 4OHT-treated samples relative to EtOH-treated samples. Data are representative of three or more experiments. **C.** mHCC 3-4 cells were stably transduced with a promoterless luciferase construct driven by the -1156 to +31 region of the REV-ERBα promoter (REVα::Luc). Cells were cultured with (MYC-OFF) or without tetracycline (MYC-ON) for 48 hours. Luminescence was recorded using a luminometer. RLU: relative light unit. Means and SDs from triplicate samples are shown, and *$p < 0.05$ by Student's t test for MYC-ON vs. MYC-OFF. Data are representative of three or more experiments. **D.** mHCC 3-4 cells cultured with media control (no tetracycline, MYC-ON) were transfected with either 60nM non-targeting siRNA (Con) or 60 nM siRNA against REV-ERBα (REVα) for 48 hours. mRNA was collected, reverse-transcribed to cDNA, and expression of REV-ERBα and BMAL1 were determined by quantitative real-time PCR, normalized to expression of *β2M*. Means and SDs from at least three experiments are shown. mRNA (FC) = Fold Change. **E,F.** Human primary T-cells were activated for 48 hours with 10µg / ml anti-CD3 and 2.5 µg / ml anti-CD28, and mRNA from the activated human T-cells along with six human T-cell acute lympoblastic leukemia (T-ALL) cell lines (T-ALL1, ALL-SIL, DND-41, Molt-4, Jurkat, and HPB-ALL) was extracted, reverse-transcribed to cDNA, and (**E**) *MYC* and (**F**) REV-ERBα expression were determined by real-time quantitative PCR, normalized to expression of *β2M*. Data are from a single experiment.

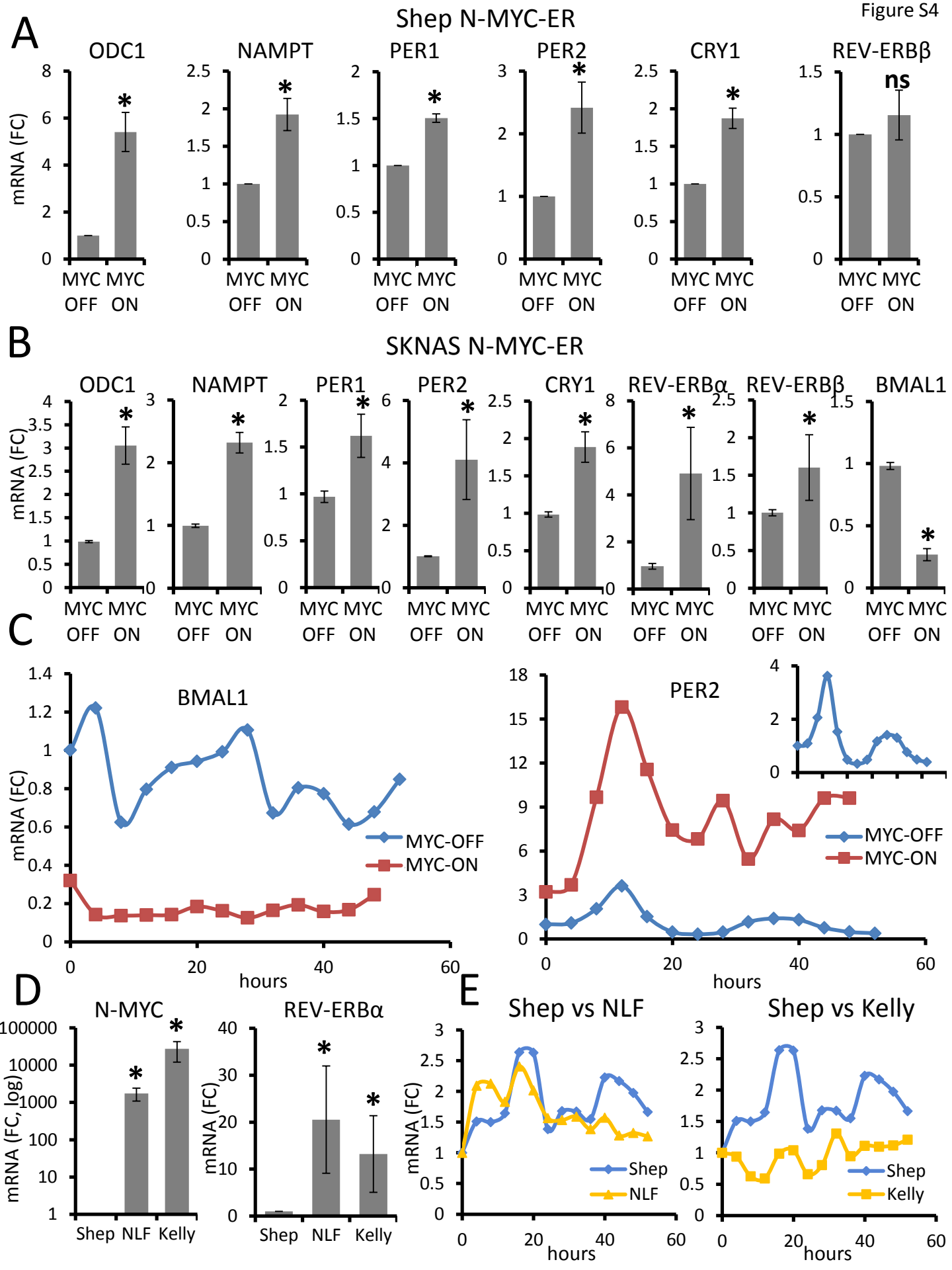

A
Shep N-MYC-ER
ODC1
NAMPT
PER1
PER2
CRY1
REV-ERBβ
mRNA (FC)
ns
MYC OFF
MYC ON
B
SKNAS N-MYC-ER
ODC1
NAMPT
PER1
PER2
CRY1
REV-ERBα
REV-ERBβ
BMAL1
mRNA (FC)
C
BMAL1
PER2
mRNA (FC)
MYC-OFF
MYC-ON
hours
D
N-MYC
REV-ERBα
mRNA (FC, log)
mRNA (FC)
Shep
NLF
Kelly
E
Shep vs NLF
Shep vs Kelly
mRNA (FC)
Shep
NLF
Kelly
hours

Figure S4, related to Figure 4. **Oncogenic N-MYC upregulates canonical MYC targets and circadian genes, and disrupts circadian oscillation. A.** Shep cells stably expressing N-MYC-ER were treated with 500 nM 4OHT to activate N-MYC-ER (MYC-ON) or EtOH control (MYC-OFF) for 24 hours. mRNA was harvested and reverse transcribed to cDNA and expression of *ODC1, NAMPT, PER1*, *PER2*, *CRY1*, and REV-ERBβ was determined by quantitative real-time PCR, normalized to expression of *β2M*. mRNA (FC) = Fold Change. **B.** SKNAS neuroblastoma cells stably expressing N-MYC-ER were treated with 500 nM 4OHT to activate N-MYC-ER (MYC-ON) or EtOH control (MYC-OFF) for 24 hours, mRNA was harvested and reverse transcribed to cDNA, and expression of *ODC1, NAMPT, PER1*, *PER2*, *CRY1*, REV-ERBα, REV-ERBβ, and BMAL1 was determined by quantitative real-time PCR, normalized to expression of *β2M*. **C.** SKNAS N-MYC-ER-expressing cells were cultured with 4OHT (MYC-ON) or EtOH (MYC-OFF) control for 24 hours, then cultured with EtOH or 4OHT and synchronized with dexamethasone. mRNA was collected every four hours for 52 hours, reverse-transcribed to cDNA, and endogenous BMAL1 expression (left panel) or *PER2* expression (right panel) was determined by quantitative real-time PCR, normalized to expression of the non-circadian gene *β2M*. **INSET** for *PER2* graph is EtOH (MYC-OFF) treated cells, with a smaller Y axis to demonstrate *PER2* oscillation. Data are from a single experiment. **D.** Shep neuroblastoma, with single copy *MYCN* (N-MYC), or NLF and Kelly neuroblastoma, with amplified *MYCN*, were harvested for mRNA, which was reverse-transcribed to cDNA. Expression of *MYCN* and REV-ERBα was determined by quantitative real-time PCR, normalized to expression of *β2M*. *Note log scale for MYCN expression.* For **A,B,** and **D**. means and SDs from at least three experiments are shown. *$p < 0.05$ by Student's t test of 4OHT samples (MYC-ON) relative to EtOH samples (MYC-OFF) for **A-B**, N-MYC amplified cells to Shep cells for **D.** NS: not statistically significant. **E.** Shep neuroblastoma, with single copy *MYCN* (N-MYC), or NLF and Kelly neuroblastoma, with amplified *MYCN*, were synchronized with 0.1 μM dexamethasone. mRNA was collected every four hours for 52 hours, reverse-transcribed to cDNA, and endogenous BMAL1 expression was determined by quantitative real-time PCR, normalized to expression of the non-circadian gene *β2M*. Shep cell BMAL1 expression was compared to NLF (left panel) or Kelly (right panel). Data are representative of two or more experiments.

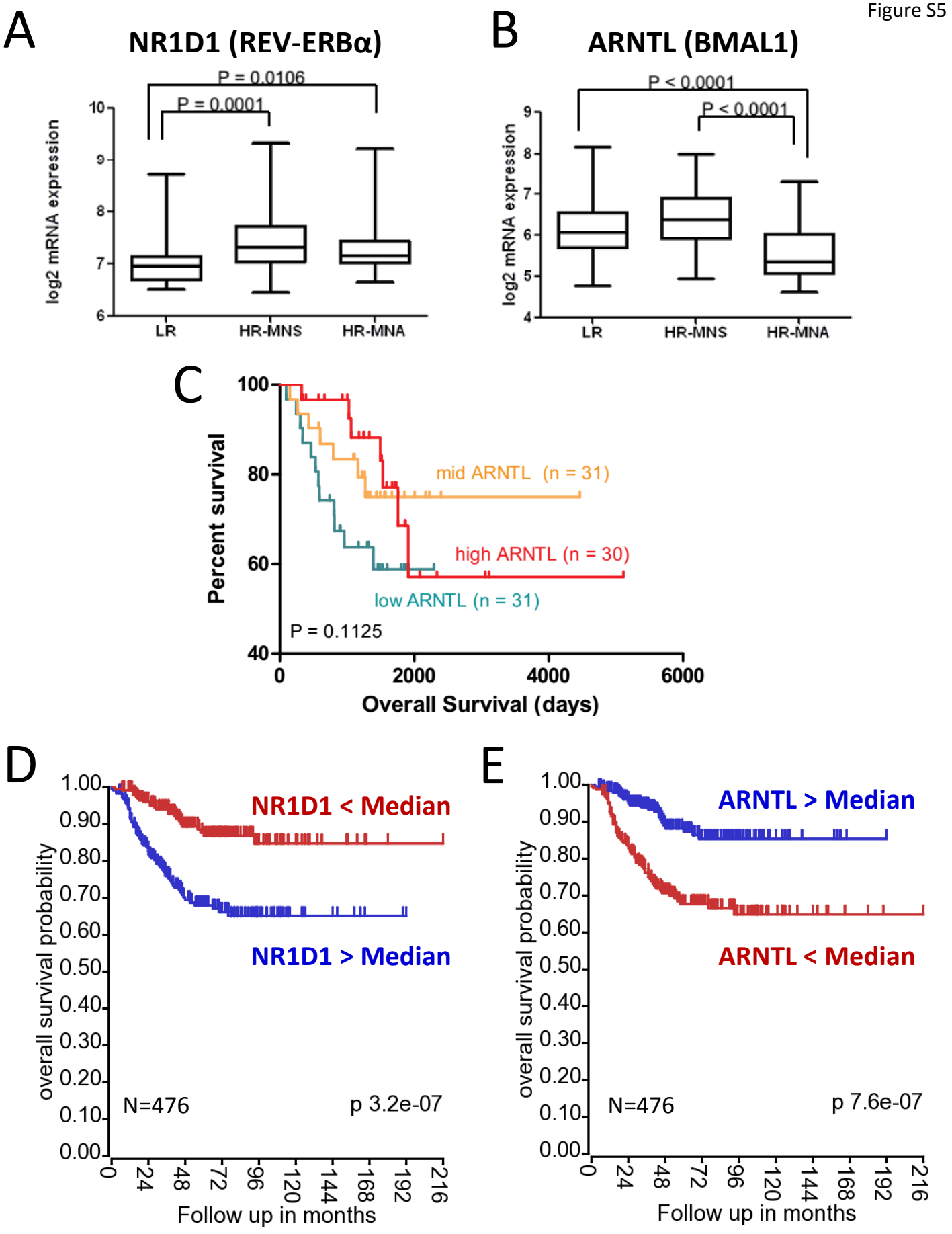

A
NR1D1 (REV-ERBα)
P = 0.0106
P = 0.0001
log2 mRNA expression
LR
HR-MNS
HR-MNA
B
ARNTL (BMAL1)
P < 0.0001
P < 0.0001
log2 mRNA expression
LR
HR-MNS
HR-MNA
C
Percent survival
mid ARNTL (n = 31)
high ARNTL (n = 30)
low ARNTL (n = 31)
P = 0.1125
Overall Survival (days)
D
NR1D1 < Median
NR1D1 > Median
overall survival probability
N=476
p 3.2e-07
Follow up in months
E
ARNTL > Median
ARNTL < Median
overall survival probability
N=476
p 7.6e-07
Follow up in months

Figure S5, related to Figure 4. **High-risk neuroblastoma exhibits increased *NR1D1* (REV-ERBα) and decreased *ARNTL* (BMAL1) correlating with amplified *MYCN* and these features correlate with poor prognosis. A, B.** (**A**) *NR1D1* is significantly overexpressed and (**B**) BMAL1 (*ARNTL*) is repressed in high-risk *MYCN*-amplified neuroblastoma compared to low- and intermediate-risk tumors (from a sample group of 240 tumors total). Box and whisker plot of *NR1D1* and *ARNTL* expression in primary neuroblastomas based on Children's Oncology Group (COG) risk groups and *MYCN* amplification status. LR, low-risk; HR-MNS, high-risk, non-amplified *MYCN*; HR-MNA, high risk, amplified *MYCN*. Whiskers represent 5$^{th}$ and 95$^{th}$ percentiles respectively. **C.** In the same patient group represented in **Figure 4E**, decreased *ARNTL* expression in primary tumors obtained at diagnosis was associated with worse overall survival. Kaplan Meier analysis shown; patients grouped by tertiles of *ARNTL* expression. Log rank P-values shown. **D, E.** In a separate cohort of 476 patients, (**D**) higher than median expression of *NR1D1* or (**E**) lower than median expression of *ARNTL* were both correlated with worse prognosis, as plotted and visualized by Kaplan-Meier analysis. Log rank p values shown.

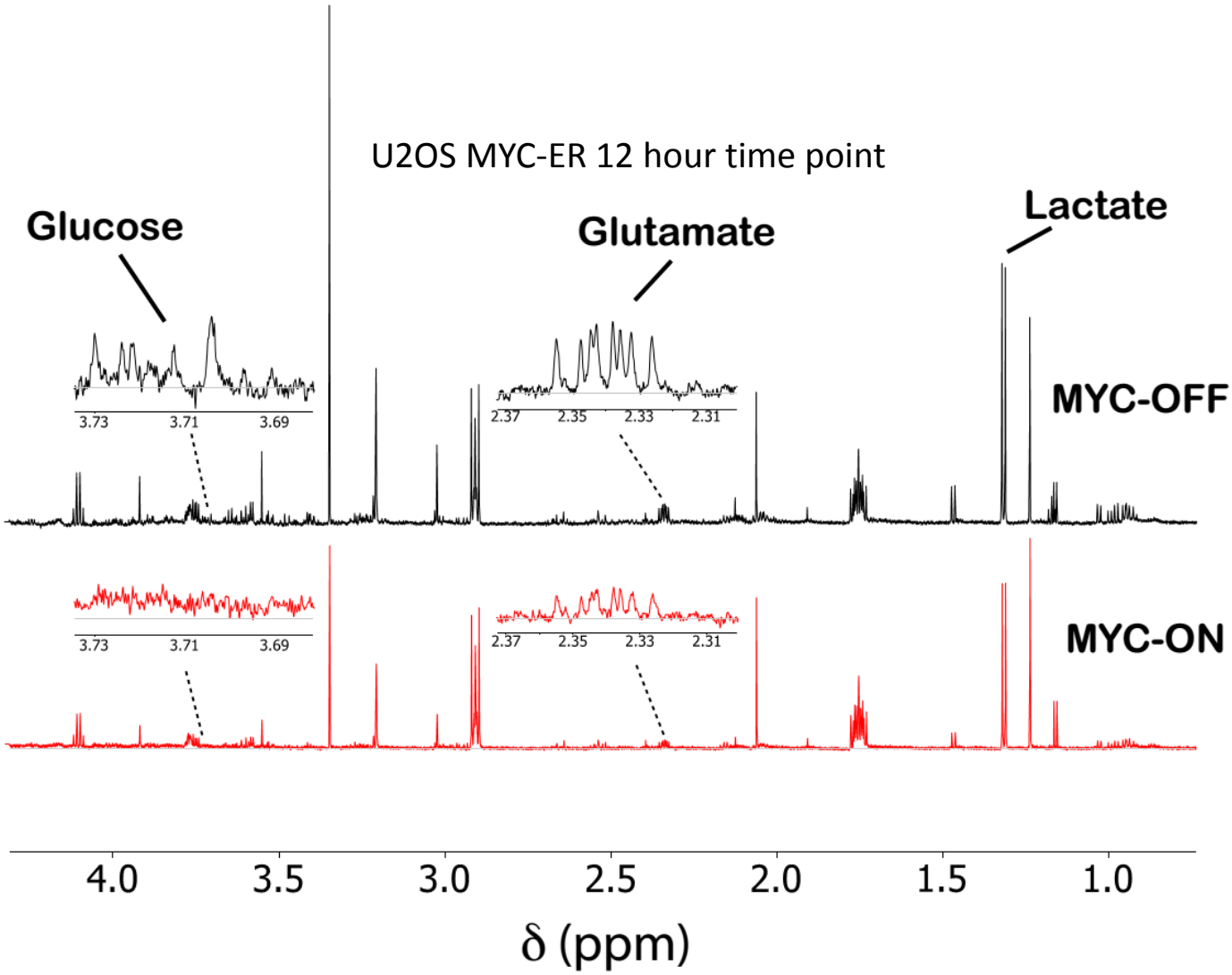

U2OS MYC-ER 12 hour time point
Glucose
Glutamate
Lactate
MYC-OFF
MYC-ON
3.73
3.71
3.69
2.37
2.35
2.33
2.31
4.0
3.5
3.0
2.5
2.0
1.5
1.0
δ (ppm)

Figure S6, related to Figure 5. **Example NMR spectra of U2OS cell extracts.** Representative NMR spectra of MYC-ON (4OHT-treated) and MYC-OFF (EtOH-treated) cell pellet extracts taken from the 12 hour time point of sampling. Regions for glutamate and glucose have been expanded to show selected signals from these metabolites. The methyl peak from lactate has also been indicated.

## Supplemental Experimental Procedures

### Plasmids

c-MYC-ER™ wt and Δ106-143, which are responsive to tamoxifen and 4-hydroxytamoxifen but not to estrogen, were received in the pBabe-puro vector (courtesy of Dr, Linda Penn, Ontario Cancer Institute, University Health Network, University of Toronto, Canada) and were described previously (Littlewood et al., 1995). c-MYC-ER™ wt and Δ106-143 were subcloned into pBabe-Zeo (Morgenstern and Land, 1990) (courtesy of Dr. Robert Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA, USA, Addgene plasmid 1766, Addgene, Cambridge, MA, USA). Retrovirus was generated in 293T cells using the pCMV-VSVG and pUMVC vectors (Stewart et al., 2003) (courtesy of Dr. Robert Weinberg, Addgene plasmids 8449 and 8454).

The promoter region of human REV-ERBα (*NR1D1*) corresponding to bases -1156 to +31, where 0 is the ATG start site, was cloned from U2OS BMAL::Luc genomic DNA harvested by DNEasy Blood and Tissue Kit (Qiagen, Gaithersburg, MD, USA) using the Phusion ™ High-Fidelity DNA Polymerase (New England Biolabs, Ipswitch, MA), and inserted into the pGL4.15 promoterless luciferase construct (Promega, Madison, WI, USA). The insert in the resulting vector was sequenced by the University of Pennsylvania DNA Sequencing Facility and confirmed to not have mutations.

pCMV6-ARNTL (RC207870) and its empty vector pCMV-entry vector (PS100001) were purchased from Origene (Rockville, MD, USA).

### Cell Culture

U2OS cells with BMAL1::luciferase vector were kindly shared by Dr. John Hogenesch (Baggs et al., 2009). Murine hepatocellular carcinoma cell line (mHCC) 3-4, a primary culture tumor cell line, was derived by ring cloning from a liver tumor of the LAP-tTA/tet-OFF cMYC conditional transgenic mouse model (Shachaf et al., 2004; Xiang et al., 2015). U2OS cells, mHCC cells, Shep N-MYC-ER (Ushmorov et al., 2008), SKNAS N-MYC-ER (Valentijn et al., 2005), Shep, NLF, and Kelly were cultivated in Dulbecco's modified Eagle's medium (DMEM, Mediatech, Manassas, VA, USA) starting glucose concentration of 25 mM and starting glutamine concentration of 4 mM). Media was supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA or Life Technologies, Grand Island, NY, USA) and 1X Penicillin/Streptomycin (Mediatech) for all cells. mHCC cell media was additionally supplemented with 2 mM L-Glutamine (Mediatech), 1 mM Sodium pyruvate (Mediatech) and 1X

MEM non-essential amino acid (Life Technologies). Some U2OS MYC-ER™, Shep N-MYC-ER, and SKNAS N-MYC-ER cells were treated with 500 nM 4-hydroxytamoxifen (Sigma, St. Louis, MO, USA) or ethanol control. Some mHCC 3-4 cells were treated with 20ng/ml tetracycline (Sigma) or media control.

Primary human T-cells were cultured in RPMI 1640 medium (Mediatech) supplemented with 10% FBS (Gemini Bioproducts, West Sacramento, CA), 100 µ/mL Penicillin/Streptomycin (Life Technologies), 2 mM L-Glutamine (Life Technologies), and 55 µM β-mercaptoethanol (Life Technologies). Cells were activated for 48 hours on plates coated with 10µg / ml anti-CD3 and 2.5 µg / ml anti-CD28 (both from Ebioscience, San Diego, CA, USA).

Human T-ALL lines were cultured in the following medium: RPMI 1640 medium (Mediatech) supplemented with 100 µ/mL Penicillin/Streptomycin (Life Technologies), 2 mM L-Glutamine (Life Technologies), and 55 µM β-mercaptoethanol (Life Technologies). The following amounts of FBS were used: TALL-1: 15% FBS; ALL-SIL: 20% FBS; DND-41: 10% FBS; Molt-4: 10% FBS; Jurkat: 10% FBS; HPB-ALL: 20% FBS.

U2OS BMAL::Luc cells were stably transduced with MYC-ER™, MYC-ER™ Δ106-143, or pBabe-Zeo empty vector retrovirus using the plasmids and methods described above. Infected cells were selected for with 100 µg/ml Zeocin (Life Technologies) for two weeks, then continually cultured in Zeocin except during experiments. Similarly, Shep N-MYC-ER cells were continuously cultured with 10 µg/mL puromycin (Life Technologies) except during experiments.

To generate mHCC 3-4 and Shep N-MYC-ER stably expressing luciferase reporter constructs, plasmids (REV-ERBα::Luc described above or BMAL::Luc in pGL 4.27 [Promega (Baggs et al., 2009)] were linearized by digestion with Not1 (New England Biolabs) and purified by QIAquick PCR purification kit (Qaigen). Shep N-MYC-ER and mHCC 3-4 cells were then transfected with linearized plasmids using Lipofectamine 2000 (Life Technologies) and selected with Hygromycin (Mediatech) at the following concentrations: 0.4 mg / mL for Shep N-MYC-ER, and 0.8 mg / mL for mHCC 3-4.

To create stable single-cell mHCC 3-4 BMAL::Luc clones, cells were trypsinized and serially-diluted to 100 cells per 96 well plate. Clones were lysed and mixed with luciferase assay reagent (Promega) and luciferase activity was measured by luminometer. Only the clones with 10000 or above relative light units were selected. Selected clones were cultured with 20ng/ml tetracycline in regular media for mHCC cells (as described above) for 24 hours. The clones were then screened in Lumicycle™ luminometer (details described in Lumicycle section), and those that showed BMAL::Luciferase oscillation were used.

All cell culture, except that during live-cell luminometer experiments (see Lumicycle section below), was conducted in a 5% CO2 humidified atmosphere.

**siRNA**

Human or mouse SMARTpool® siRNAs were purchased from Dharmacon (GE Healthcare, Lafayette, CO, USA). Cells were transfected with siRNAs using Lipofectamine 2000 or RNAiMAX (Life Technologies). Non-targeting siRNAs with same molar amount were used as negative control. For all experiments, a total of 60 nM siRNA were used. For experiments in which REV-ERBα and REV-ERBβ were used together in **Figure 3**, 30 nM of each siRNA were used.

**ChIP**

Chromatin immunoprecipitation was done by using Imprint® Ultra Chromatin Immunoprecipitation Kit, according to manufacturer's instructions (Sigma). Briefly, cells were washed in PBS, crosslinked in 1% Formaldehyde (Sigma), lysed, sonicated and then immunoprecipitated with rabbit anti-MYC antibody (Abcam, Cambridge, MA, USA) or mouse anti-N-MYC antibody (Abcam). Myc-bound DNA fragments were reverse crosslinked, eluted and then amplified by quantitative RT-PCR. Primer pairs for ChIP assay were: REV-ERBα, 5'-GCTTTGCCAGGCAGAAAGGGTAAA-3' and 5'-GCAACGACAAGACTGTCGGGATTT-3'; REV-ERBα minus 2K, 5'- TGGCTTTCATGTTTCCCAGGCA-3' and 5'-TCCCAGTGCTTCTAGAGAGGGTTT-3'; *NPM1*, 5'-GCTACATCCGGGACTCACC-3' and 5'-GCTGCCATCACAGTACATGC-3' (O'Donnell et al., 2005).

**Luciferase**

Cells were washed with PBS, lysed with 1X Passive lysis buffer (Promega) and dislodged by cell scraper. Cell lysates were then collected, mixed with luciferase assay reagent (Promega) and luciferase activity was measured by luminometer.

**Lumicycle**

Lumicycle analysis of U2OS BMAL::Luc cells was described previously (Baggs et al., 2009). Briefly, three days prior to analysis, cells were plated in 35 mm dishes at a concentration of 250,000 cells / plate. 24 hours later, cells were transfected with siRNA as described above. 48 hours post transfection, cells were cultured atmospheric conditions, in 'Lumicycle media':

phenol red-free DMEM (Sigma) containing 5% FBS (Hyclone or Thermo Fisher, Grand Island, NY, USA), 25 mM D-glucose (Sigma), 35 mg/L sodium bicarbonate (Thermo Fisher), 10 mM HEPES (Thermo Fisher), Pen/Strep (Mediatech), 0.1 mM beetle-luciferin (Promega), and 0.1 μM dexamethasone (Sigma), and dishes were sealed with high vacuum grease (Dow Corning, Midland, MI, USA). Plates were measured for luminescence every 10 minutes for at least 4 days on a Lumicycle™ luminometer, and presented as relative light units (RLU) per second (Actimetrics, Wilmette, IL, USA). The experiment for mHCC 3-4 BMAL::Luc cells was performed in the same way, except that cells were treated with either tetracycline or mHCC media 24 hours before the plates were sealed. Where indicated, error bars represent standard error of three technical replicates collected at different times over the course of no more than 10 minutes resolution.

**Real-Time PCR**

All RNAs were extracted by RNeasy Plus Mini Kit (Qiagen) according to manufacturer's instruction and reversed to complementary DNA by using TaqMan Reverse Transcription Reagents (Life Technologies). cDNA was used as template to proceed to quantitative real time PCR (RT-PCR) with specific human or mouse primers. RT-PCRs were performed using the StepONE Plus system, 7900HT Real-time PCR System, or ViiA™ 7 Real-time PCR system (Life Technologies). Relative mRNA expression levels were normalized to *β2M* and analyzed using comparative delta-delta CT method. All RT-PCR primers are listed below:

| Gene Name | Sequence or Product Number | Source |
|---|---|---|
| | **Human Primers** | |
| *PER1* | ACTCCTGCGACCAGGTACTGGCTG, GGCCACCACGGATGCACGA | Primer Blast |
| *PER2* | GGATGCCCGCCAGAGTCCAGAT, TGTCCACTTTCGAAGACTGGTCGC | Primer Blast |
| REV-ERBα (*NR1D1*) | TGGACTCCAACAACAACACAG, GATGGTGGGAAGTAGGTGGG | Primer Bank (Spandidos et al., 2010) ID#300116298c1 |
| *ODC1* | Hs.PT.51.22750281.gs | IDT |
| REV-ERBβ (*NR1D2*) | Hs.PT.51.14785042 | IDT |
| *β2M* | GGCCGAGATGTCTCGCTCCG, TGGAGTACGCTGGATAGCCTCC | Primer Blast |
| BMAL1 (*ARNTL*) | Hs00154147_m1 | Taqman Gene Expression Assay |
| *NAMPT* | Hs00237184_m1 | Taqman Gene Expression |

| | | |
|---|---|---|
| | | Assay |
| *CRY1* | Hs01565974_m1 | Taqman Gene Expression Assay |
| *CRY2* | Hs.PT.53a.3983568 | Taqman Gene Expression Assay |
| | **Mouse Primers** | |
| *β2M* | ACCGGCCTGTATGCTATCCAGAAA, GGTGAATTCAGTGTGAGCCAGGAT | Previously Published (Altman et al., 2009) |
| REV-ERBβ (*Nr1d2*) | Mm.PT.51.12747673 | IDT |
| REV-ERBα (*Nr1d1*) | Mm00520708_m1 | Taqman Gene Expression Assay |
| *Per2* | Mm00478113_m1 | Taqman Gene Expression Assay |
| *Nampt* | Mm00451938_m1 | Taqman Gene Expression Assay |
| BMAL1 (*Arntl*) | Mm00500226_m1 | Taqman Gene Expression Assay |
| *Per1* | Mm00501813_m1 | Taqman Gene Expression Assay |
| *Cry1* | Mm00514392_m1 | Taqman Gene Expression Assay |
| *Odc1* | Mm.PT.53a.23589427 | Taqman Gene Expression Assay |

**Circadian mRNA Experiments**

Circadian mRNA experiments for U2OS MYC-ER™, mHCC 3-4, Shep N-MYC-ER, SKNAS N-MYC-ER, Shep, NLF, and Kelly were carried out in the media described above as a 'split timecourse.' 24 hours prior to the start of the experiment, half the cells were cultured in 0.1 μM dexamethasone (dex, Sigma), and at the start of the experiment, the other half of the cells were cultured in dexamethasone. At each time point, two plates were harvested representing 0 and 24 hours +dex, 4 and 28, etc, to arrive at a 52 hour timecourse. mRNA was processed and analyzed as described above.

**Immunoblots**

Cells were lysed in M-PER Mammalian Protein Extraction Reagent (Thermo Fisher Scientific) supplemented with protease inhibitor cocktail (BD Biosciences, San Jose, CA, or Promega) and phosphatase inhibitors (Sigma). Proteins were separated by SDS-PAGE using Criterion pre-cast gradient gels (Bio-Rad, Hercules, CA, USA). Primary antibodies used include: rabbit anti-MYC (Abcam); rabbit anti-phospho-AMPK Thr172 (Cell Signaling, Danvers, MA); rabbit anti-AMPK (Cell Signaling); rabbit anti-HK1 (Cell Signaling); rabbit anti-HK2 (Cell Signaling); rabbit anti-BMAL1 (Cell Signaling); rabbit anti-PER2 (Proteintech, Chicago, IL, USA) and mouse anti-α-Tubulin (EMD Millipore, Billerica, MA, USA). Secondary antibodies used include: Alexa-Flour 680 goat anti-rabbit IgG (Life Technologies, Grand Island, NY); Alexa-Flour 790 goat anti-mouse IgG (Life Technologies); and IRDye 80aCW Goat Anti-Mouse IgG (Licor, Lincoln, NE). Immunoblots were imaged with the Odyssey CLx infrared imaging system (Licor) and uniformly contrasted.

For U2OS MycER circadian immunoblots, cells were cultured in Lumicycle media as described above, treated with 4OHT or ethanol control two days prior to collection, synchronized with dexamethasone one day prior to collection, and then collected as a 'split timecourse', also described above.

**Colony Suppression Assay**

Kelly and NLF cells were seeded in 6-well plate and transfected with either pCMV6-*ARNTL* vector or pCMV6-entry empty vector (Origene) with Lipofectamine LTX with Plus (Life Technologies) in triplicates with concentrations of 0.2 or 0.8 µg/mL for NLF and Kelly cells, respectively. After one day of transfection, both Kelly and NLF cells were cultured in media containing 1 mg/ml G418 (Mediatech) for 7-10 days. G418-containing media was changed every 2 days. Colonies were stained with crystal violet solution for 20 min at room temperature and washed with water to remove extra crystal violet solution. Plates were scanned and quantified with ImageJ, and representative images were uniformly contrasted.

**UCSC Genome Browser, ENCODE, and Other Publicly Available Genomic Data**

The UCSC Genome Browser Human March 2006 and February 2009 assemblies (www.genome.ucsc.edu), which contain the results of a MYC CHIP-Seq experiment from the ENCODE project, were queried for MYC binding to REV-ERBα (*NR1D1),* REV-ERBβ *(NR1D1), PER1, PER2 CRY1,* and BMAL1 (*ARNTL)* promoters (Dunham et al., 2012; Kent et al., 2002). Location of E-boxes was also identified from the above experiments, and species homology was

based on previously published data (Siepel et al., 2005). The resulting images were exported using the Genome browser's website and cropped for display.

For U2OS ChIP-Seq for endogenous MYC peaks and overexpressed inducible MYC ChIP-Seq, publicly available data from Waltz et al was used [GEO accession GSE44672 (Walz et al., 2014) access date 6-27-2015]. MYC peak tracks from GEO accession numbers GSM1231597 and GSM1231598 were downloaded and analyzed on UCSC genome browser February 2009 assembly. The resulting images were exported using the Genome browser's website and cropped for display.

**Primary Neuroblastoma Data**

For survival data, two data sets were used. Previously published data (Wang et al., 2006) for ninety-two human tumors was assessed for overall survival based on *MYCN, NR1D1*, and *ARNTL* expression. Survival analyses were performed using the methods of Kaplan and Meier. Patients were divided into separate groups based on tertiles of individual mRNA expression. For overall-survival (OS), time was defined as the time from diagnosis until the time of death from disease or until the time of last contact if death did not occur. Patients who were alive were censored at the time last known alive. Log rank p-values $< 0.05$ were considered significant. A second data set representing a cohort of 476 patients (Kocak et al., 2013) were analyzed for overall survival, using the methods of Kaplan and Meier, divided into two groups based on greater than or less than median expression of either *NR1D1* or *ARNTL.* Expression data used to generate survival curves were from an Agilent-020382 Human Custom Microarray 44k, GEO accession # GPL16876. The *NR1D1* (REV-ERBα) probe ID used was UKv4_A_24_P250227, which corresponds to a region of the *NR1D1* coding region that does not coincide with the antisense and overlapping *THRA* gene. Significance was determined by log rank as described previously (Bewick et al., 2004). Data were generated using the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl)

For mRNA expression analyses of primary human neuroblastomas, we analyzed Affymetrix Exon ST array data of 240 tumors obtained at diagnosis (Pugh et al., 2013) as part of the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) project of the National Cancer Institute Office of Cancer Genomics. Data were normalized as described previously. A Student's t test was applied to assess expression differences across risk groups as defined by the Children's Oncology Group (COG), accounting for MYCN amplification status. $p < 0.05$ was considered significant.

**Cell and Media Extraction for NMR analysis**

For U2OS MYC-ER metabolomics, cells were plated three days prior to sample harvest and allowed to grow to confluence. Two days before harvest, cells were treated + 500 nM 4OHT (MYC-ON) or ethanol (MYC-OFF) (without changing media), and one day prior to harvest, cells were synchronized with 0.1 uM dexamethasone (without changing media). During the experiment, cells were collected every two hours by washing with cold PBS, scraping into a small volume of cold PBS, spinning down cells into a pellet, and snap-freezing. At each time point, 1 mL of media was spun down to clear it of debris, and also snap frozen. Pellets and media were extracted for NMR as described below.

U2OS cell pellets were thawed on ice and extracted using a modified Bligh-Dyer method (Bligh and Dyer, 1959; Tambellini et al., 2013). Briefly, a methanol:chloroform (2:1) mixture (300µl) was added to cell pellets, then vortexed and sonicated for 15 min. Chloroform and water (100µl each) was then added and samples were again vortexed. Organic and aqueous layers were partitioned by centrifugation at 13,300rpm, for 7mins at 4°C. 100µl of the aqueous layer was dried in a speed vacuum for 4hrs until dry and immediately prepared for NMR analysis as described below.

Media samples (50µl each) were centrifuged at 14000 rpm for 20 min, and the supernatant was prepared for NMR as described below. Liquid chromatography-mass spectrometry (LC-MS) - grade acetonitrile, methanol, chloroform was purchased from Thermo Fisher.

**NMR Spectroscopy and Quantitative Profiling**

The samples were dissolved in 200 µl phosphate buffer (final pH ~7.1) containing Sodium-2,2-Dimethyl2-Silapentane-5-Sulfonate (DSS, Cambridge Isotope Limited, Andover, MA) (Cambridge Isotope Limited, Tewksbury, MA, USA 0.175 mM for media samples and 0.255 mM for cell samples) and 10% D2O for field frequency lock purpose (Cambridge Isotope Limited). The samples were transferred to NMR tubes (3mm I.D, Bruker Biospin, Billerica, MA). All NMR spectra were acquired on Avance III HD 700 MHz NMR spectrometer from Bruker Biospin (Billerica, MA) equipped with a 3mm NMR triple resonance inverse probe and SampleJet cooled to $10^{o}$C for automated spectral acquisition.

For all 1-dimensional NMR spectra, the pulseprogram took the shape of first transient of a 2 dimensional NOESY and generally of the form RD-90-t-90-$t_m$-90-ACQ. Where RD = relaxation delay, t = small time delay between pulses, $t_m$ = mixing time and ACQ = acquisition (Beckonert et al., 2007). The water signal was saturated using continuous irradiation during RD

and $t_m$. The spectra were acquired using 76K data points and 14 ppm spectral width. 378 scans were performed and 1 second interscan (relaxation) delay and 0.1 second mixing time was allowed. The FIDs were zero filled to 128K; 0.1 Hz of linear broadening was applied followed by Fourier transformation.

NMR spectra were imported into Chenomx v 8.0. (Edmonton, Canada) for quantitative targeted profiling (Weljie et al., 2006). The processor module was used to phase and baseline correct the spectra followed by internal standard calibration and deletion of water region. The processed spectra were then imported to the profiler module for targeted profiling. Quantified data from this process were exported for further analysis.

**Quantitative Analysis of Metabolite Oscillations**

Output concentrations from the NMR analysis were imported into the R statistical framework (Team, 2015), and were noted to have significant temporal change in overall concentrations. Thus a detrending algorithm was applied from the pracma R package (Borchers, 2015) prior to assessment of rhythmicity in the metabolite data. Oscillations were assessed using the non-parametric JTK_CYCLE algorithm (Hughes et al., 2010), testing for period lengths between 22 and 26 hours corresponding to circadian events. The default cosine function was used to assess rhythmic patterns and tested against the experimental data as a function of period length, phase and amplitude. Rhythms were considered significant if the Benjamini-Hochberg corrected false discovery rate (BH.Q) was < 0.05 (not shown) (Benjamini and Hochberg, 1995).

**Statistical Analysis**

For all single time point mRNA expression data, error bars represent SD, and * or § represent $p < 0.05$ by Student's t test from three or more experiments. For Lumicycle error bars, bars represent SEM (standard error) technical triplicate samples collected at different times over the course of no more than 10 minutes. For ChIP and Rev-erbα::Luciferase reporter experiments, error bars represent SD, and * represents $p < 0.05$ by Student's t test from technical triplicates representing three or more experiments. For colony suppression assay image quantitation, error bars represent SD, and * represents $p < 0.05$ by Student's t test from three experiments. For primary human neuroblastoma expression data, whiskers represent $5^{th}$ and $95^{th}$ percentiles respectively, and p value is derived from Student's t test. For primary human neuroblastoma survival data, patients were evenly grouped by median expression into three groups (Wang et al., 2006) or two groups (Kocak et al., 2013), and p value was

determined by log rank.  For NMR metabolite data, the colored bands represent 95% confidence intervals from the locally weighted scatterplot smoothing function which generate the curves.  P-values indicate the significance of the cosine wave fit, as determined by the JTK algorithm, to experimental data.

## Supplemental References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