nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	N/A
Data analysis	We used Rstudio and packages: rstatix, ggpubr, pheatmap, venneuler, and ggplot2, Fastqc 0.12.1 from the trim galore suite 0.6.10, Bowtie2 2.5.3, samtools 1.6 and sambamba 1.0, MACS2 2.2.9.1, bedtools 2.31.1 , HOMER 4.11, deeptools 3.5.5. We also used web-based softwares: Clock correlation distance analysis (https://hugheylab.shinyapps.io/deltaccd/) and WEB-based GEne SeT AnaLysis Toolkit -WebGestalt (https://www.webgestalt.org/)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

RNA sequencing and CUT&RUN sequencing data were deposited into the Gene Expression Omnibus (GEO) database. We expect to have an accession number available by June 12, 2024, and the data will be made available prior to publication. (Token is now provided for review).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We report results for experiments performed in male and female mice. Data from human subjects analyzed in the manuscript includes both males and females (combined).
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Analyses of publicly available data includes all available samples. Luciferase assays routinely use six samples per condition in each experiment based on observed variability in outcomes, and we report the mean of three independently replicated experiments. RNA sequencing and CUT&RUN ChIP sequencing experiments include three samples per condition to enable robust statistical analysis while containing costs. Cell growth assays (colony formation) include three or four samples per condition for each experiment and the results of one representative experiment among at least three replicates is shown. For xenograft measurements, ten hosts were used for each cell line to enable detection of a 20% or greater difference in tumor growth with 80% power with an alpha of 0.05 based on previously observed variability in tumor growth.
Data exclusions	One BMAL1 IP in shBMAL1 expressing 786O cells was discarded from analysis because there were only 500,000 sequencing reads for this sample and all other samples had ~20M reads/sample. No other data points were excluded from this study.
Replication	All cell-based experiments were successfully replicated at least three times. Variability in outcomes is reflected in results where appropriate.
Randomization	N/A
Blinding	Investigators were blinded to sample identities where possible. Blind data collection for weekly Murine xenograft measurements were performed by pulling cages at random for measuring and not identifying mouse identity until after measurements were collected.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[ ]	[x] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used: Primary antibodies used for Western blotting were anti-HA polyclonal (Sigma #H6908), anti-Flag polyclonal (Sigma #F7425), anti-βActin (Sigma #A1978), anti-HIF2a polyclonal (Novus Biologicals #NB100-122), anti-BMAL1 polyclonal (Abcam #ab93806) and anti-Cry1-CT and anti-Cry2-CT as described (Lamia et. Al., 2011), and anti-BMAL1 monoclonal (VWR #102231-824). Secondary antibodies used were Goat Anti-Mouse IgG (H + L)-HRP Conjugate (Bio-Rad #1706516), Goat Anti-Rabbit IgG (H + L)-HRP Conjugate (Bio-Rad #1706515), Goat Anti-Guinea Pig IgG-HRP Conjugate (Sigma #A7289). The primary antibodies used for immunoprecipitation were 5 ug of rabbit mAb IgG isotype as a negative control (CST #66362), 2 ug of rabbit mAb tri-methyl-lys-4 (CST # C42D8), 1 ug of HIF-2α rabbit mAb (CST #59973), or 2 ug of BMAL1 rabbit mAb (CST #14020).

Validation: Antibodies were validated by included negative controls (shRNA knockdown or genetic deletion of the target) wherever possible.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): All cell lines were purchased from ATCC

Authentication: None of the cell lines used were independently authenticated.

Mycoplasma contamination: The cells were not tested for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register): None of the cell lines used are included in the ICLAC register of commonly misidentified cell lines.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: Eight weeks old NIH-III Nude mice were used as hosts for xenograft tumors.

Wild animals: N/A

Reporting on sex: Male and female mice were used as hosts and results are reported separately for each sex.

Field-collected samples: N/A

Ethics oversight: All murine husbandry and experiments were in regulation with the Institutional Animal Care and Use Committee at the Scripps Research Institute (La Jolla, California) under protocol #10-0019.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks: *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication: *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	A token has been created for reviewers to access this data set and has been sent to the editor for distribution to the reviewers.
Files in database submission	Raw paired-end sequencing files (fq.gz), bigWig (.bw), peak file (.bed) (except for negative control)
Genome browser session (e.g. UCSC)	https://genome-euro.ucsc.edu/s/rmello%40scripps.edu/Mello_2024

Methodology

Replicates	BMAL1 and HIF2a IPs were performed in technical triplicate in both 786O cells expressing shControl or shBMAL1. IgG IPs were performed in technical duplicate in both 786O cells expressing shControl or shBMAL1. One BMAL1 IP in shBMAL1 expressing 786O cells was discarded from analysis because there were only 500,000 sequencing reads for this sample and all other samples had ~20M reads/sample.
Sequencing depth	Paired end reads 20 million reads/sample.
Antibodies	The primary antibodies used for immunoprecipitation were 5 ug of rabbit mAb IgG isotype as a negative control (CST #66362), 2 ug of rabbit mAb tri-methyl-lys-4 (CST # C42D8), 1 ug of HIF-2α rabbit mAb (CST #59973), or 2 ug of BMAL1 rabbit mAb (CST #14020).
Peak calling parameters	Peak calling was performed using MACS2 2.2.9.1, specifying parameters --keep-dup all --max-gap 400 –p 1e-5
Data quality	peak calling used a stringent p-value threshold of 1e-5.
Software	reads underwent alignment to both the human genome hg19 and the yeast genome sacCer3 using Bowtie2 2.5.3, with the following parameters: --local --very-sensitive --fr --dovetail --no-mixed -I 10 -X 700 Alignment files (SAM) were then converted to BAM format, and subjected to filtering, and duplicate reads were removed using samtools 1.6 and sambamba 1.0 Peak calling was performed using MACS2 2.2.9.1, specifying parameters --keep-dup all --max-gap 400 –p 1e-5 Post-peak calling, filtering against the hg19 blacklist was executed using bedtools 2.31.1 with the intersect option annotation and motif analysis of the peaks was carried out using HOMER 4.11, using annotatePeaks.pl and findMotifsGenome.pl options with the human genome hg19 reference Peak functional annotation was directly done by Homer using -go option, or with WEB-based GEne SeT AnaLysis Toolkit -WebGestalt (https://www.webgestalt.org/) to identify gene ontologies and KEGG-related pathways after crossing peaks annotation with RNA-seq data. Spike-in normalization with the aligned reads was achieved against the yeast genome sacCer3 with deeptools 3.5.5 using bamCoverage –scaleFactor --smoothLength 60 --extendReads 150 –centerReads to produce BigWig files. Spike-in scale factor values were calculated as described in the manufacturer protocol (CST #86652). Profiles and heatmap were obtained by using computeMatrix –referencePoint center after spike-in normalization. BigWig files were uploaded to the UCSC genome browser (https://genome-euro.ucsc.edu/index.html) and tracks were visualized against the human genome hg19