Supplementary Information for

Integrative Analysis of Multi-omics Data Expands Druggable Targets for Circadian Medicine

Ying Chen, Chengxuan Chen, Dishu Zhou, Panpan Liu, Roberto E López-Valiente, Yuan Liu, Cam Mong La, Isabella Beraldo Xavier, Pradip Saha, Zheng Sun, Leng Han, Dongyin Guan

Corresponding author:

Leng Han, Ph.D. (lenghan@iu.edu)
Dongyin Guan, Ph.D. (dongyin.guan@bcm.edu)

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Supplementary Figures 1 to 6

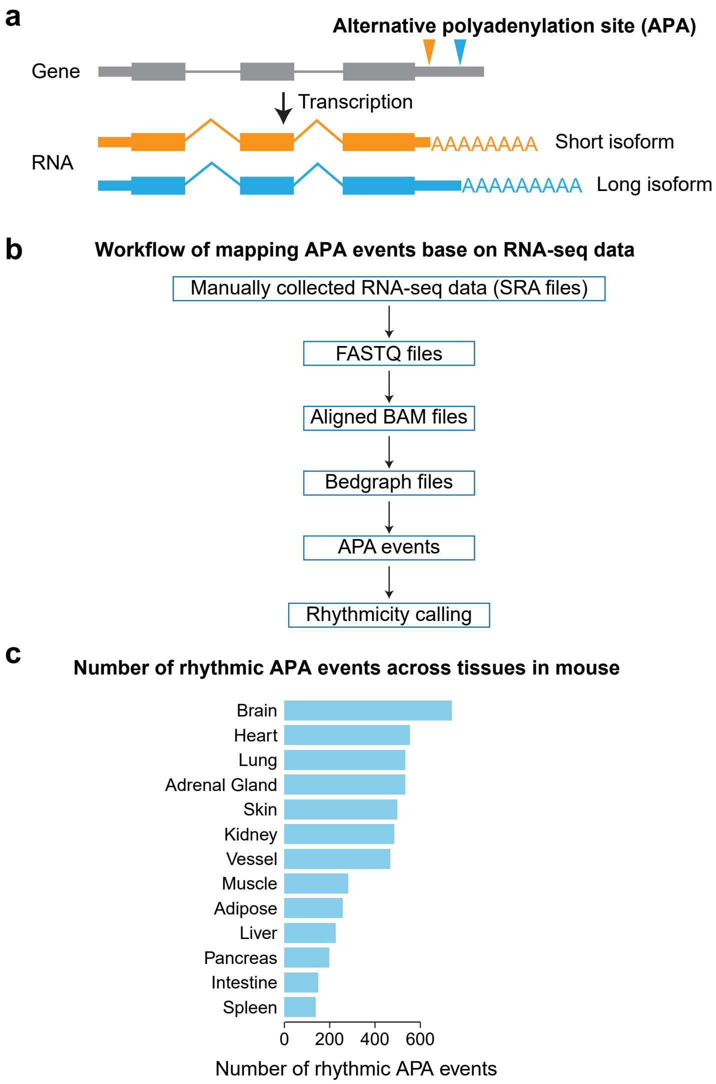


Figure S1. Mapping and characterization of rhythmic alternative polyadenylation (APA) events from RNA-seq data. a Schematic illustration of alternative APA. A gene can produce multiple transcript isoforms by utilizing different polyadenylation (polyA) sites, resulting in short and long isoforms with distinct 3' ends. **b** Workflow for mapping APA events and detecting rhythmicity based on RNA-seq data. Publicly available RNA-seq datasets were manually collected and processed through a pipeline including read alignment, coverage profiling, APA event detection, and rhythmic APA events identification. **c** Number of rhythmic APA events detected across different tissues in mouse.

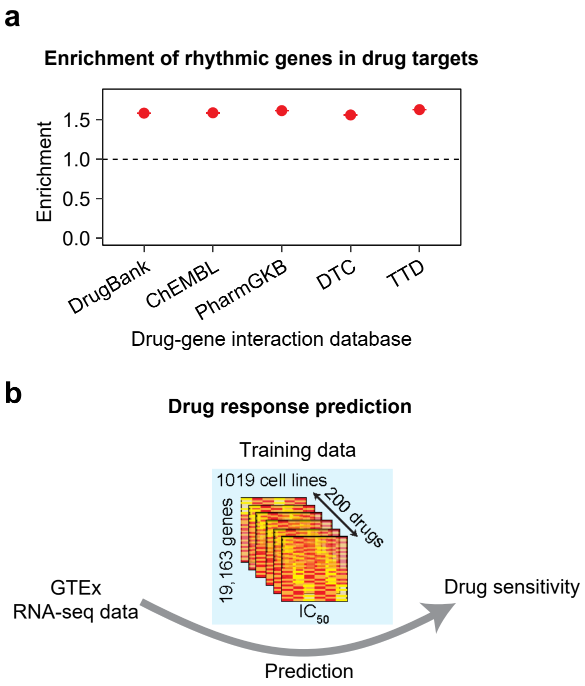


Figure S2. Enrichment of rhythmic genes in drug targets. a Analysis of rhythmic gene enrichment in drug targets. The enrichment is calculated as the ratio of observed rhythmic drug target genes to the expected number. The expected rhythmic gene set was generated by randomly selecting genes from all expressed genes. Random sampling was performed 1,000 times, and the median number of drug targets in the 1,000 expected rhythmic gene sets was used as the expectation. **b** Schematic illustrating the prediction of drug responses as described by Li et al.

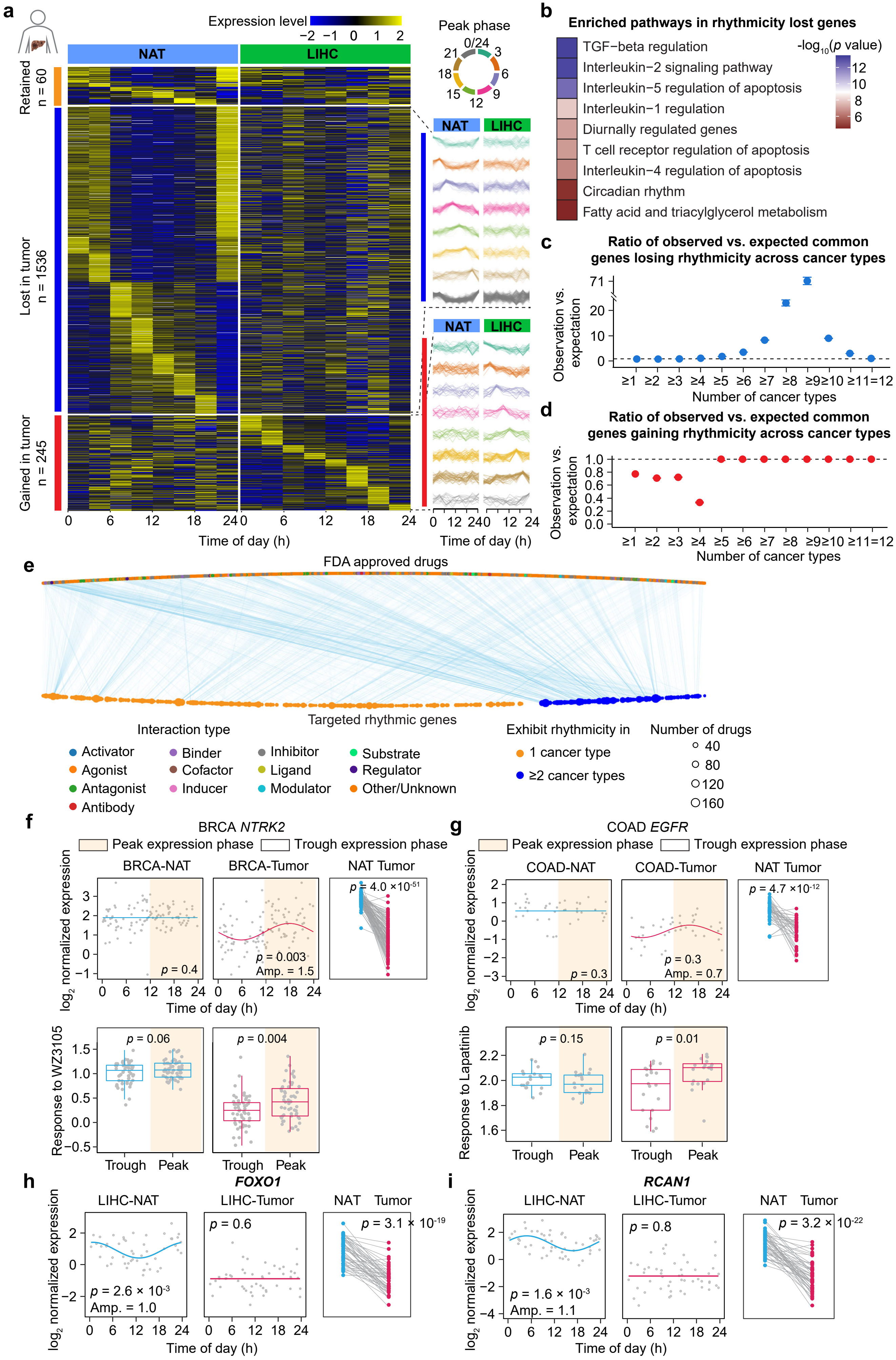
a
Expression level
−2 −1 0 1 2
NAT
LIHC
Retained n = 60
Lost in tumor n = 1536
Gained in tumor n = 245
0 6 12 18 24 0 6 12 18 24
Time of day (h)
Peak phase
0/24 3 6 9 12 15 18 21
NAT LIHC
NAT LIHC
0 12 24 0 12 24
Time of day (h)
b
Enriched pathways in rhythmicity lost genes
TGF−beta regulation
Interleukin−2 signaling pathway
Interleukin−5 regulation of apoptosis
Interleukin−1 regulation
Diurnally regulated genes
T cell receptor regulation of apoptosis
Interleukin−4 regulation of apoptosis
Circadian rhythm
Fatty acid and triacylglycerol metabolism
-log10(p value)
12 10 8 6
c
Ratio of observed vs. expected common genes losing rhythmicity across cancer types
Observation vs. expectation
71 20 10 0
≥1 ≥2 ≥3 ≥4 ≥5 ≥6 ≥7 ≥8 ≥9 ≥10 ≥11 =12
Number of cancer types
d
Ratio of observed vs. expected common genes gaining rhythmicity across cancer types
Observation vs. expectation
1.0 0.8 0.6 0.4 0.2 0.0
≥1 ≥2 ≥3 ≥4 ≥5 ≥6 ≥7 ≥8 ≥9 ≥10 ≥11 =12
Number of cancer types
e
FDA approved drugs
Targeted rhythmic genes
Interaction type
Activator
Agonist
Antagonist
Antibody
Binder
Cofactor
Inducer
Inhibitor
Ligand
Modulator
Substrate
Regulator
Other/Unknown
Exhibit rhythmicity in
1 cancer type
≥2 cancer types
Number of drugs
40
80
120
160
f
BRCA NTRK2
Peak expression phase
Trough expression phase
BRCA-NAT
BRCA-Tumor
NAT Tumor
p = 4.0 ×10-51
p = 0.4
p = 0.003
Amp. = 1.5
log2 normalized expression
3 2 1 0 −1
0 6 12 18 24 0 6 12 18 24
Time of day (h)
Response to WZ3105
1.5 1.0 0.5 0.0 −0.5
p = 0.06
p = 0.004
Trough Peak Trough Peak
g
COAD EGFR
Peak expression phase
Trough expression phase
COAD-NAT
COAD-Tumor
NAT Tumor
p = 4.7 ×10-12
p = 0.3
p = 0.3
Amp. = 0.7
log2 normalized expression
2 1 0 −1 −2 −3
0 6 12 18 24 0 6 12 18 24
Time of day (h)
Response to Lapatinib
2.2 2.0 1.8 1.6
p = 0.15
p = 0.01
Trough Peak Trough Peak
h
FOXO1
LIHC-NAT
LIHC-Tumor
NAT Tumor
p = 0.6
p = 3.1 × 10-19
p = 2.6 × 10-3
Amp. = 1.0
log2 normalized expression
2 0 −2
0 6 12 18 24 0 6 12 18 24
Time of day (h)
i
RCAN1
LIHC-NAT
LIHC-Tumor
NAT Tumor
p = 0.8
p = 3.2 × 10-22
p = 1.6 × 10-3
Amp. = 1.1
log2 normalized expression
2 0 −2 −4
0 6 12 18 24 0 6 12 18 24
Time of day (h)

Figure S3. Rhythmic gene expression alterations in cancer. a Heatmap of rhythmic gene expression in human normal liver and liver tumor tissues. **b** Pathway enrichment analysis of rhythmic genes lost in human liver tumor tissue. **c-d** Ratio of observed to expected genes with rhythmicity commonly lost **(c)** and gained **(d)** across multiple cancer types. **e** Associations between drugs and rhythmic genes. **f-g** The predicted drug responses for the drugs of WZ3105 **(f)** and Lapatinb **(g)** in individuals with different genotypes and internal circadian phases. **(h-i)** The rhythmic expression of *FOXO1* **(h)** and *RCAN1* **(i)** in liver cancer.

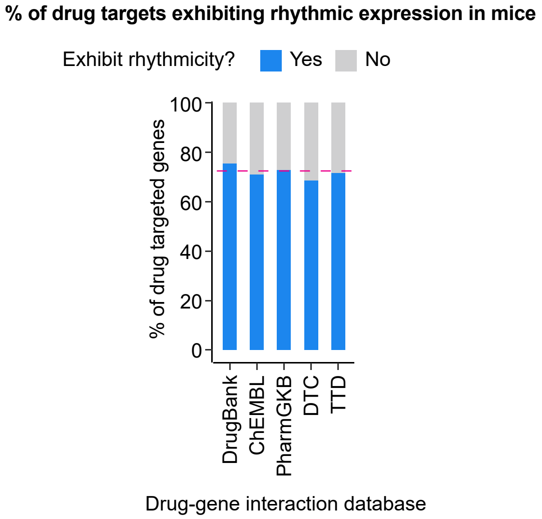
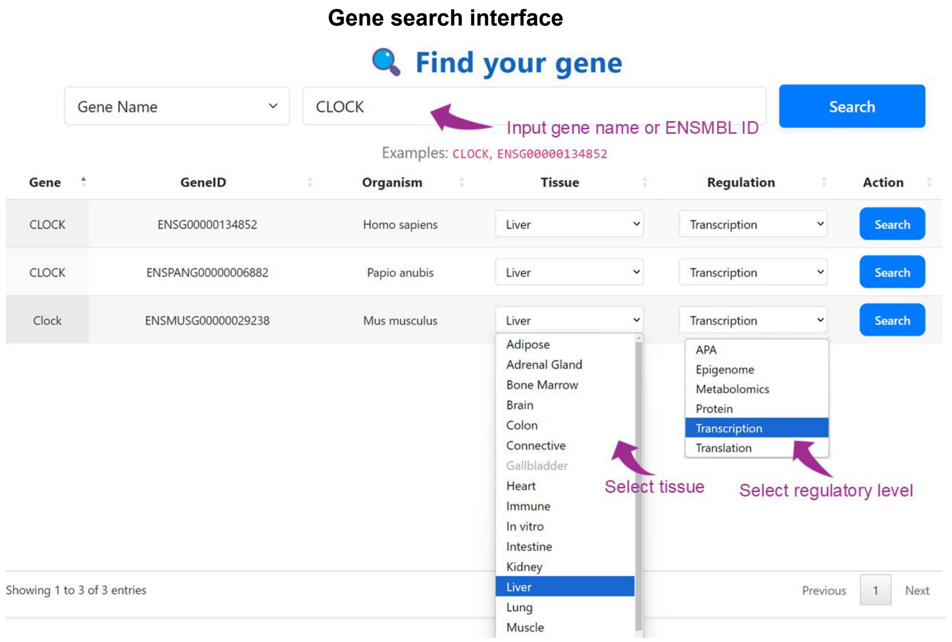


Figure S4. Percentage of drug targets exhibiting rhythmic expression in mice. Rhythmic drug targets are identified based on rhythmic transcriptomic profiles from various tissues under normal physiological conditions.

a


Gene search interface

CLOCK

Input gene name or ENSMBL ID

Examples: `CLOCK`, `ENSG00000134852`

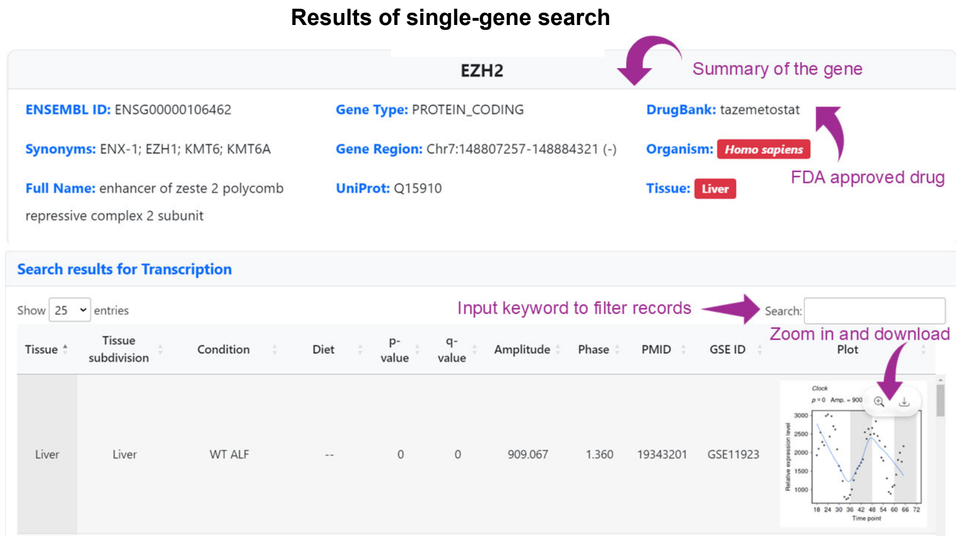
Gene	GeneID	Organism	Tissue	Regulation	Action
CLOCK	ENSG00000134852	Homo sapiens	Liver	Transcription	Search
CLOCK	ENSPANG00000006882	Papio anubis	Liver	Transcription	Search
Clock	ENSMUSG00000029238	Mus musculus	Liver	Transcription	Search

Select tissue

Select regulatory level

Showing 1 to 3 of 3 entries

b


Results of single-gene search

EZH2

Summary of the gene

ENSEMBL ID: ENSG00000106462
Gene Type: PROTEIN_CODING
DrugBank: tazemetostat
Synonyms: ENX-1; EZH1; KMT6; KMT6A
Gene Region: Chr7:148807257-148884321 (-)
Organism: *Homo sapiens*
Full Name: enhancer of zeste 2 polycomb repressive complex 2 subunit
UniProt: Q15910
Tissue: Liver

FDA approved drug

Search results for Transcription

Show 25 entries

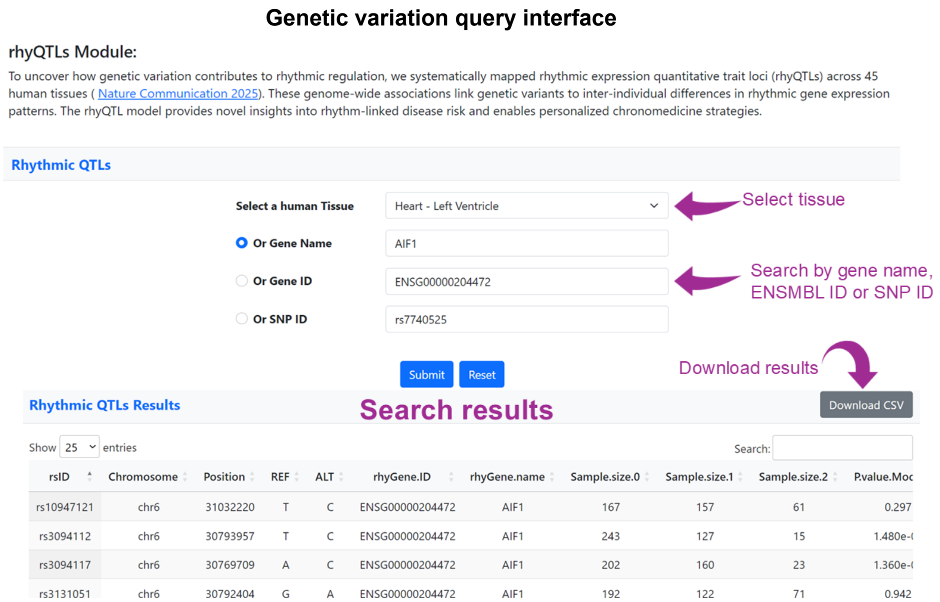
Input keyword to filter records

Search:

Zoom in and download

Tissue	Tissue subdivision	Condition	Diet	p-value	q-value	Amplitude	Phase	PMID	GSE ID	Plot
Liver	Liver	WT ALF	--	0	0	909.067	1.360	19343201	GSE11923	

c


Genetic variation query interface

rhyQTLs Module:

To uncover how genetic variation contributes to rhythmic regulation, we systematically mapped rhythmic expression quantitative trait loci (rhyQTLs) across 45 human tissues (Nature Communication 2025). These genome-wide associations link genetic variants to inter-individual differences in rhythmic gene expression patterns. The rhyQTL model provides novel insights into rhythm-linked disease risk and enables personalized chronomedicine strategies.

Rhythmic QTLs

Select a human Tissue Heart - Left Ventricle

● **Or Gene Name** AIF1

○ **Or Gene ID** ENSG00000204472

○ **Or SNP ID** rs7740525

Select tissue

Search by gene name, ENSMBL ID or SNP ID

Download results

Rhythmic QTLs Results

Search results

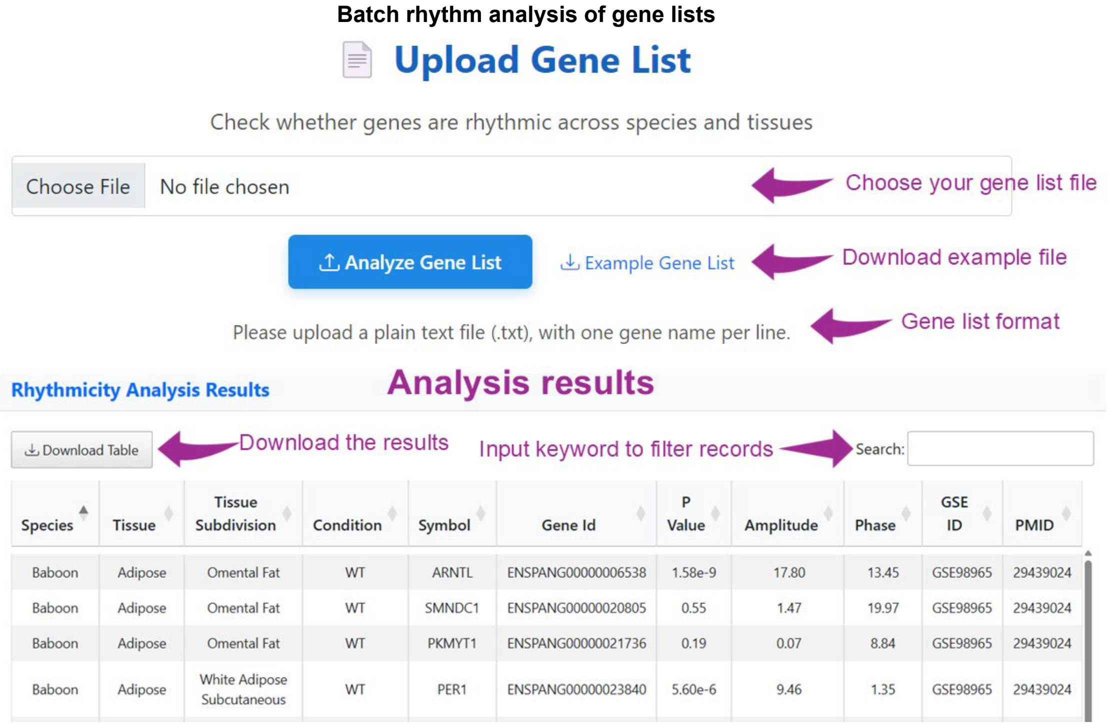
Show 25 entries

Search:

rsID	Chromosome	Position	REF	ALT	rhyGene.ID	rhyGene.name	Sample.size.0	Sample.size.1	Sample.size.2	P.value.Moc
rs10947121	chr6	31032220	T	C	ENSG00000204472	AIF1	167	157	61	0.297
rs3094112	chr6	30793957	T	C	ENSG00000204472	AIF1	243	127	15	1.480e-
rs3094117	chr6	30769709	A	C	ENSG00000204472	AIF1	202	160	23	1.360e-
rs3131051	chr6	30792404	G	A	ENSG00000204472	AIF1	192	122	71	0.942

Figure S5. Overview of query interfaces in the RHINO platform. a Gene search interface. Users can search for a gene of interest by name or ENSEMBL ID, and select the organism, tissue, and regulatory layer (e.g., transcription, translation, APA, etc.) to explore its rhythmicity. **b** Results of single-gene search. The output includes a gene summary and rhythmicity parameters such as amplitude, phase, and statistical significance, along with visualizations. **c** Genetic variation query interface. Users can search for genetic variations associated with rhythmic expression by gene name, ENSEMBL ID, or SNP ID across human tissues, and download the corresponding results.

a



Batch rhythm analysis of gene lists

Upload Gene List

Check whether genes are rhythmic across species and tissues

Choose your gene list file

Download example file

Please upload a plain text file (.txt), with one gene name per line.

Gene list format

Rhythmicity Analysis Results

Analysis results

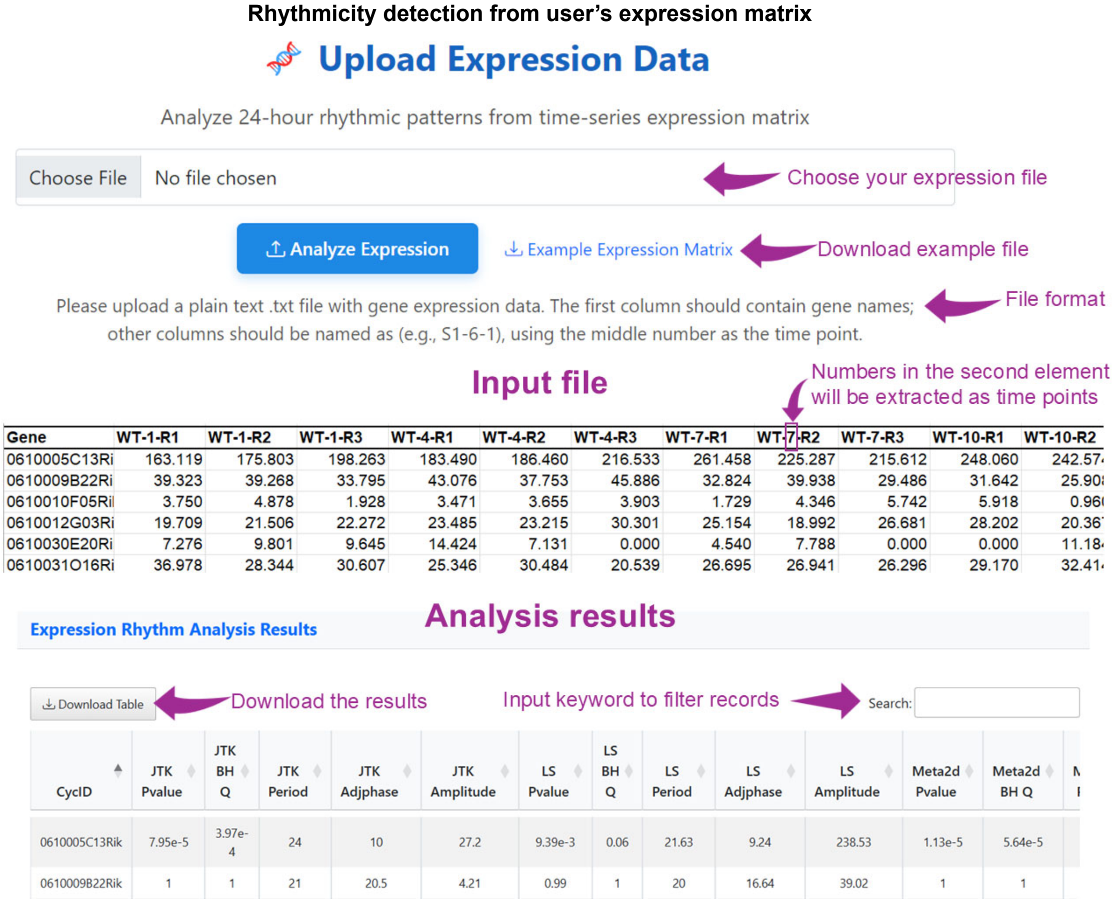
Download the results

Input keyword to filter records

Search:

Species	Tissue	Tissue Subdivision	Condition	Symbol	Gene Id	P Value	Amplitude	Phase	GSE ID	PMID
Baboon	Adipose	Omental Fat	WT	ARNTL	ENSPANG00000006538	1.58e-9	17.80	13.45	GSE98965	29439024
Baboon	Adipose	Omental Fat	WT	SMNDC1	ENSPANG00000020805	0.55	1.47	19.97	GSE98965	29439024
Baboon	Adipose	Omental Fat	WT	PKMYT1	ENSPANG00000021736	0.19	0.07	8.84	GSE98965	29439024
Baboon	Adipose	White Adipose Subcutaneous	WT	PER1	ENSPANG00000023840	5.60e-6	9.46	1.35	GSE98965	29439024

b



Rhythmicity detection from user's expression matrix

Upload Expression Data

Analyze 24-hour rhythmic patterns from time-series expression matrix

Choose your expression file

Download example file

Please upload a plain text .txt file with gene expression data. The first column should contain gene names; other columns should be named as (e.g., S1-6-1), using the middle number as the time point.

File format

Input file

Numbers in the second element will be extracted as time points

Gene	WT-1-R1	WT-1-R2	WT-1-R3	WT-4-R1	WT-4-R2	WT-4-R3	WT-7-R1	WT-7-R2	WT-7-R3	WT-10-R1	WT-10-R2
0610005C13Ri	163.119	175.803	198.263	183.490	186.460	216.533	261.458	225.287	215.612	248.060	242.57
0610009B22Ri	39.323	39.268	33.795	43.076	37.753	45.886	32.824	39.938	29.486	31.642	25.90
0610010F05Ri	3.750	4.878	1.928	3.471	3.655	3.903	1.729	4.346	5.742	5.918	0.96
0610012G03Ri	19.709	21.506	22.272	23.485	23.215	30.301	25.154	18.992	26.681	28.202	20.36
0610030E20Ri	7.276	9.801	9.645	14.424	7.131	0.000	4.540	7.788	0.000	0.000	11.18
0610031O16Ri	36.978	28.344	30.607	25.346	30.484	20.539	26.695	26.941	26.296	29.170	32.41

Expression Rhythm Analysis Results

Analysis results

Download the results

Input keyword to filter records

Search:

CycID	JTK Pvalue	JTK BH Q	JTK Period	JTK Adjphase	JTK Amplitude	LS Pvalue	LS BH Q	LS Period	LS Adjphase	LS Amplitude	Meta2d Pvalue	Meta2d BH Q
0610005C13Rik	7.95e-5	3.97e-4	24	10	27.2	9.39e-3	0.06	21.63	9.24	238.53	1.13e-5	5.64e-5
0610009B22Rik	1	1	21	20.5	4.21	0.99	1	20	16.64	39.02	1	1

Figure S6. Web interfaces for user-submitted rhythmicity analysis. a Batch rhythm analysis of gene lists. Users can upload a list of gene names to assess whether these genes exhibit rhythmic expression across species, tissues, and conditions. The output includes rhythmicity statistics such as *p* value, amplitude, and phase. **b** Rhythmicity detection from user-submitted expression matrices. Users can upload time-series gene expression data to identify 24-hour rhythmic patterns, allowing direct evaluation of rhythmicity in their own datasets.