Supplemental Figure Legends

Figure S1. Related to Figures 1 and 3: Circadian study design for (A) in vivo mouse, and (B) Cell-autonomous U2 OS cell line (C) Cycling of the top 12 metabolites in mouse liver with JTK q-values < 4 x 10^{-10}. The y-axis is the intensity following run-order correction as described in the methods. Data was derived from two biological replicates and two analytical replicates. Error bars represent standard error of the mean. D) Temporal acrophases of glycogen (from literature, see text), glucose, citrate, and malate. E) Juxtaposition of metabolites indicative of oxidative status and methylation. Numbers in red indicate acrophase in CT. Data for all time-series plots was derived from two biological replicates and two analytical replicates. Error bars represent standard error of the mean.

Figure S2. Related to Figure 4: Effect of oscillations following knockdown of circadian clock components in U2 OS cells. U2 OS cells were transfected with siRNAs and relative expression is normalized to GAPDH. (A) Relative expression of *BMAL1* (left panel), *CRY1* (middle panel), *CRY2* (right panel). (B) Distribution of periods with each metabolite plotted as points. Size indicates JTK BH.Q –log value, and the line indicates density of periods. (C) *BMAL1* knockdown dampens oxidative cycling as measured by the NAD+/NADH raw ion count ratio (top panel) as compared to *CRY1* (middle) and *CRY2* (bottom). (D) Cycling of the top 13 metabolites under control conditions in the cell-autonomous model with JTK q-values < 0.05. The y-axis is the intensity following run-order correction as described in the methods. Data for all cycling plots was derived from two analytical replicates, and error bars represent standard error of the mean.

Figure S3. Related to Figure 4: Metabolic oscillations and pathway analysis under *CRY1* and *CRY2* knockdown conditions in U2OS cells. *CRY1* (left panel) and *CRY2* (right panel) knockdown increases the number of cycling metabolites, eliminates circadian oscillations. 69 metabolites (~50%) in *CRY1* knock down cells and 64 metabolites (47%) in *CRY2* knockdown cells show cycling.

Figure S4. Related to Figure 5: A) Differential analysis between U2 OS cells with Cry1 KD (green bar) or scrambled control (blue bar). Samples were considered from all time points

to understand global Cry1 knockdown effect on metabolism. B) Frozen liver samples from mice of the indicated genotype (WT or $CRY1^{-/-}$) and circadian timepoint (ZT 10 and ZT 22) were suspended in lysis buffer, lysed by Tissue Lyser II from Qiagen and further homogenized by passage through a Qiashredder column. Lysates were processed for expression by immunoblot for Cry1 and mTOR pathway proteins, with tubulin serving as a loading control.

Figure S5. Related to Figure 7: A) Cultured hepatocytes were synchronized with 0.1 μM dexamethasone, and mRNA was collected at the indicated timepoints after synchronization. Expression of *Cry1* and *Cry2* were determined by qPCR, normalized to expression of 36B4 (*Rplp0*) as has been described (Gréchez-Cassiau, 2008) previously. Data are from two biological replicates (ie, *Cry1-1* is the first replicate, *Cry1-2* is the second replicate). (B) Heatmap of significantly cycling metabolite from primary hepatocyte metabolite profiling.

Table S1. Related to Figures 1 and 3: Compilation of characteristics of published circadian metabolomics studies.

Table S2. Related to STAR methods: Oligonucleotides used in this study.

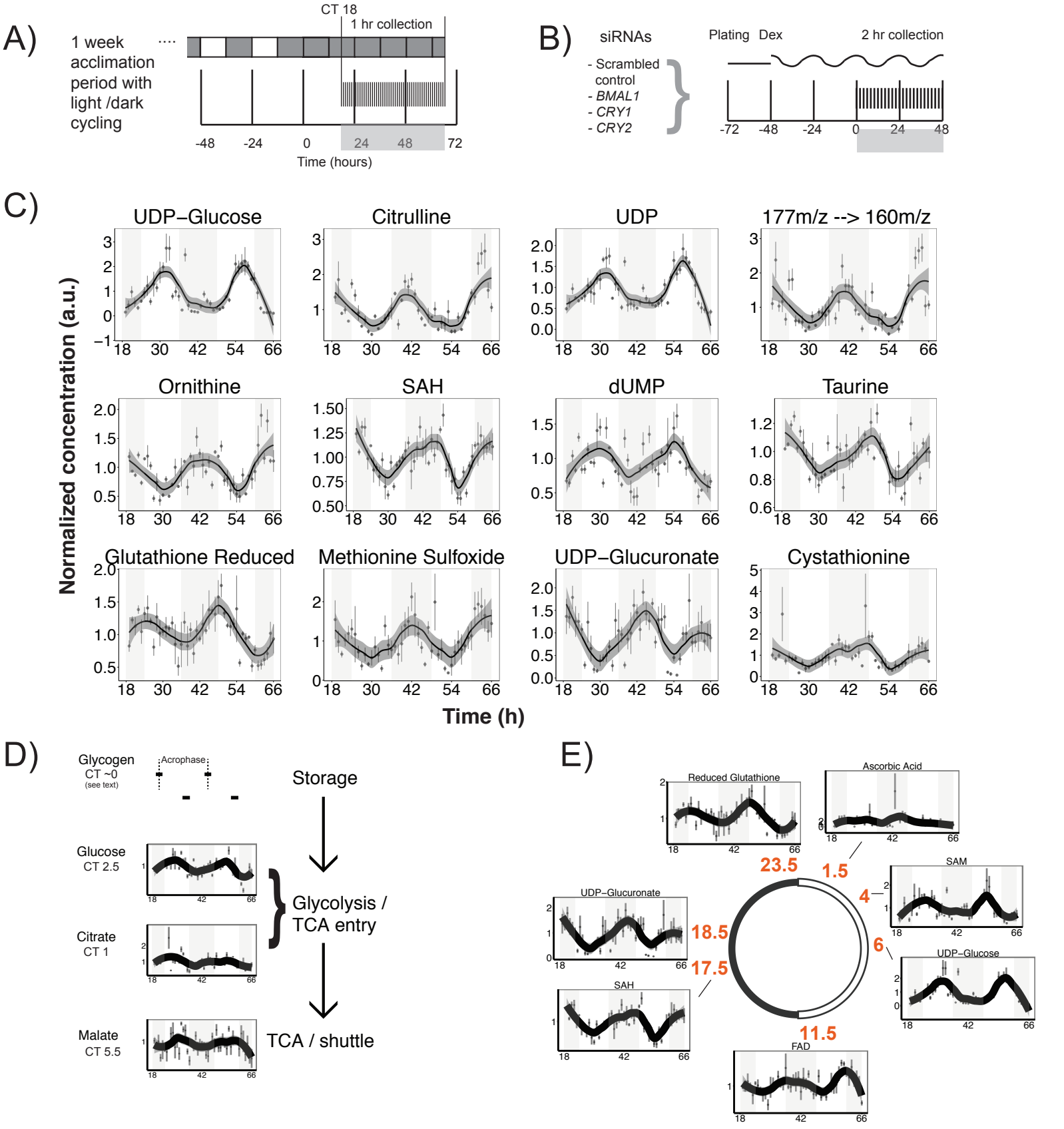
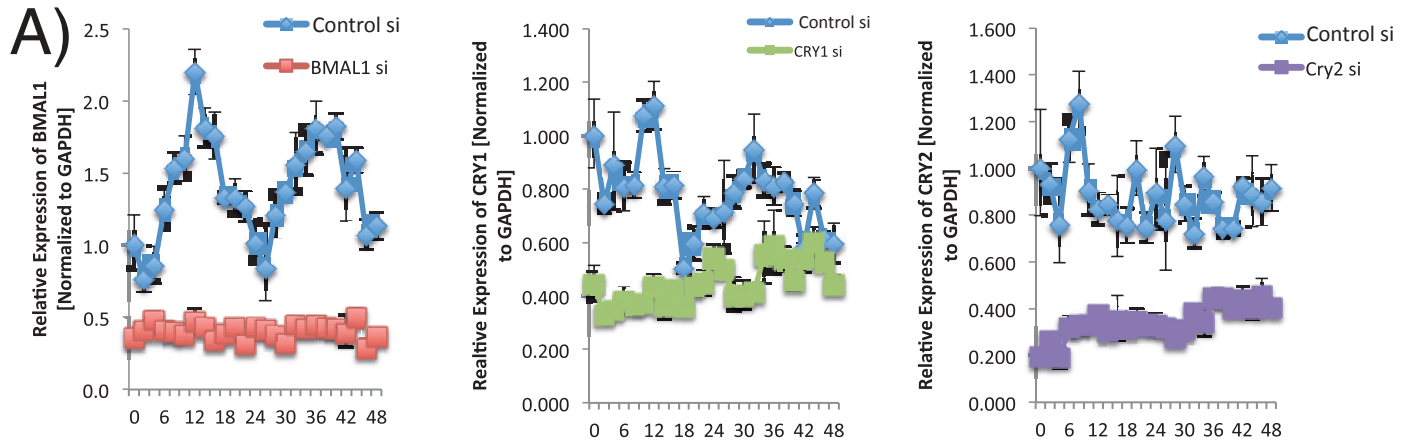
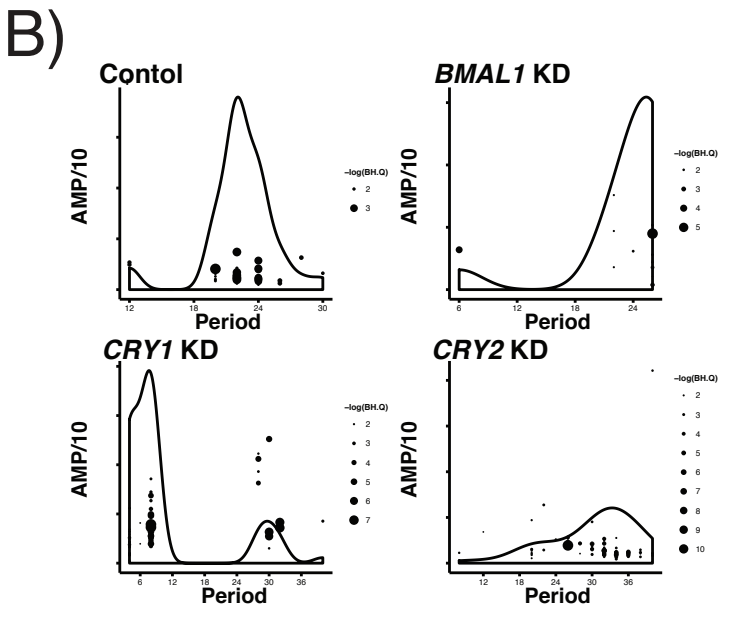
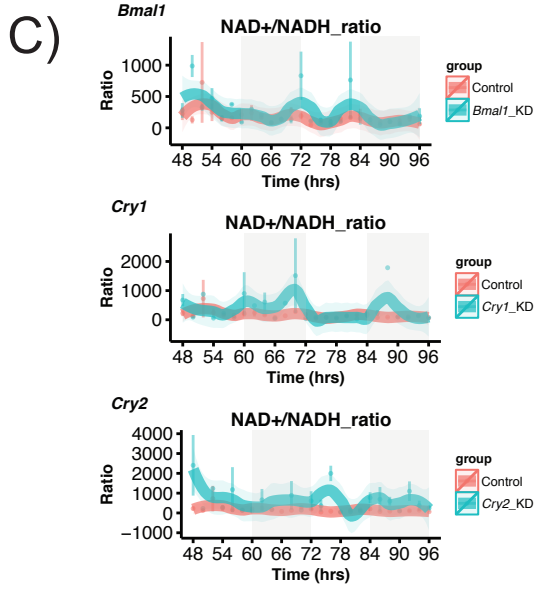

A)
1 week acclimation period with light /dark cycling
CT 18
1 hr collection
-48
-24
0
24
48
72
Time (hours)
B)
siRNAs
- Scrambled control
- BMAL1
- CRY1
- CRY2
Plating
Dex
2 hr collection
-72
-48
-24
0
24
48
C)
UDP−Glucose
Citrulline
UDP
177m/z --> 160m/z
Ornithine
SAH
dUMP
Taurine
Glutathione Reduced
Methionine Sulfoxide
UDP−Glucuronate
Cystathionine
Normalized concentration (a.u.)
Time (h)
D)
Glycogen
CT ~0
(see text)
Acrophase
Storage
Glucose
CT 2.5
Citrate
CT 1
Glycolysis / TCA entry
Malate
CT 5.5
TCA / shuttle
E)
Reduced Glutathione
Ascorbic Acid
SAM
UDP−Glucuronate
UDP−Glucose
SAH
FAD
23.5
1.5
4
6
18.5
17.5
11.5

Figure S1


A)
Control si
BMAL1 si
Relative Expression of BMAL1 [Normalized to GAPDH]
Control si
CRY1 si
Realtive Expression of CRY1 [Normalized to GAPDH]
Control si
Cry2 si
Relative Expression of CRY2 [Normalized to GAPDH]


B)
Contol
BMAL1 KD
CRY1 KD
CRY2 KD
AMP/10
Period
-log(BH.Q)


C)
Bmal1
Cry1
Cry2
NAD+/NADH_ratio
Ratio
Time (hrs)
group
Control
Bmal1_KD
Cry1_KD
Cry2_KD

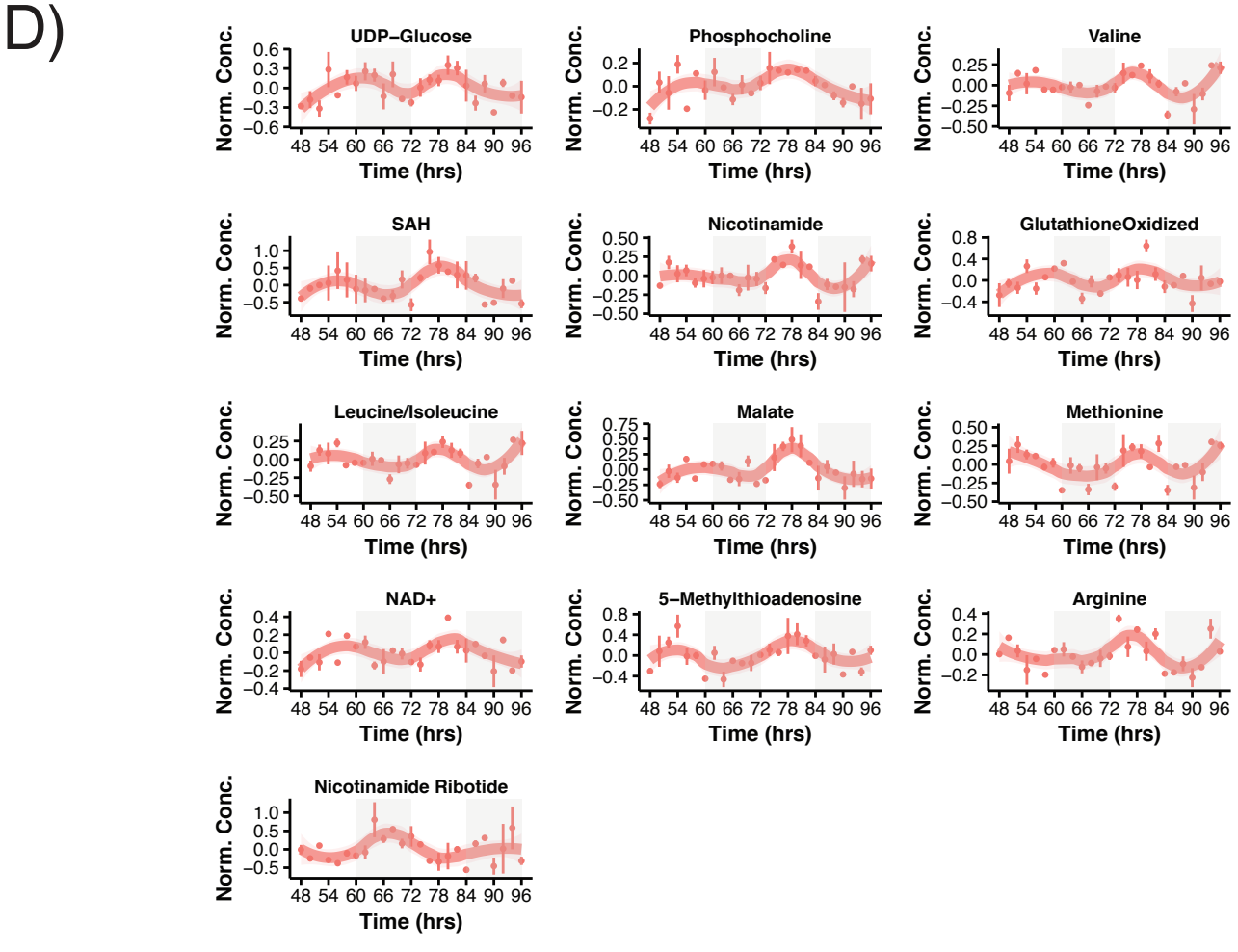

D)
UDP–Glucose
Phosphocholine
Valine
SAH
Nicotinamide
GlutathioneOxidized
Leucine/Isoleucine
Malate
Methionine
NAD+
5–Methylthioadenosine
Arginine
Nicotinamide Ribotide
Norm. Conc.
Time (hrs)

Figure S2

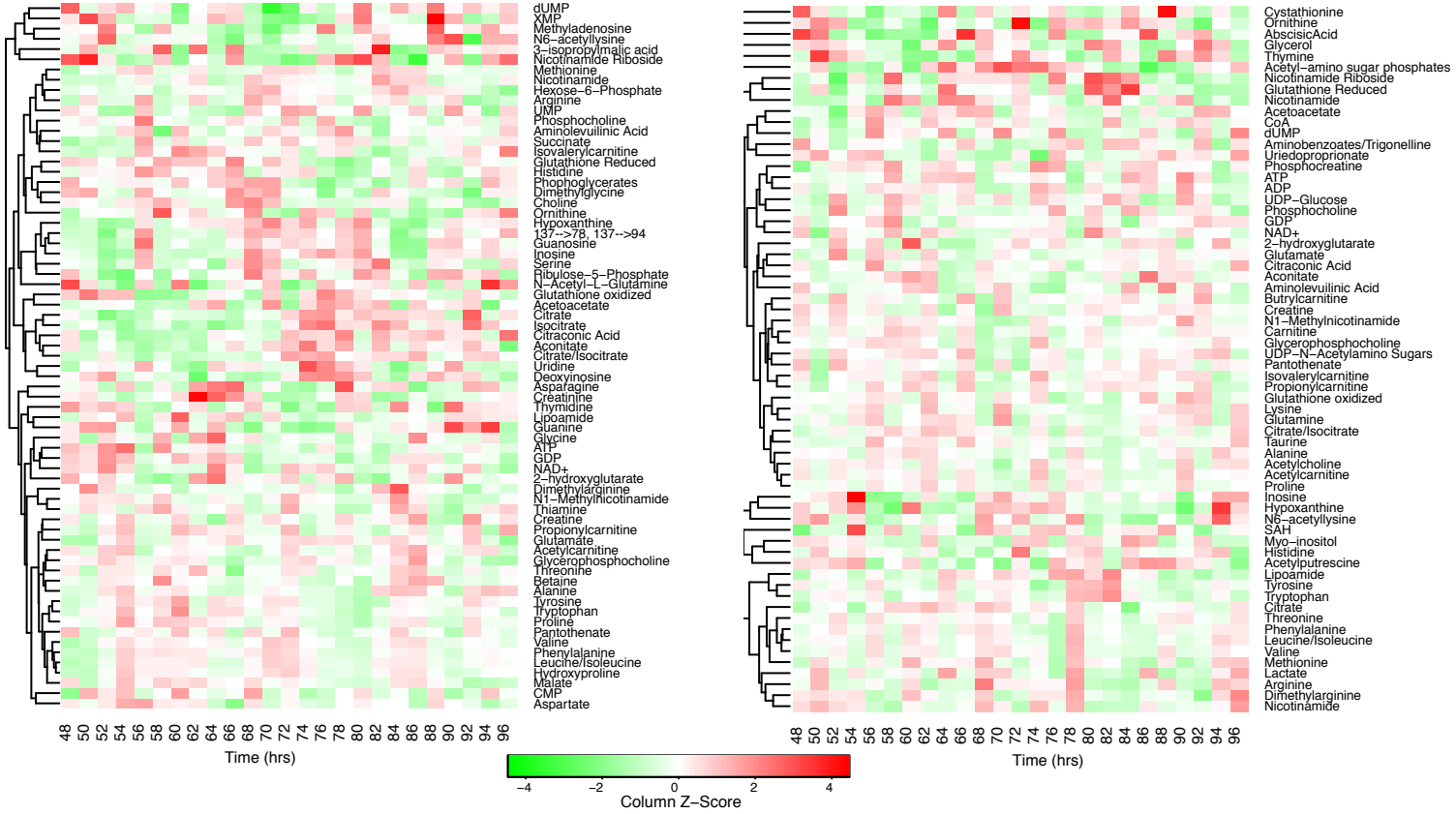

dUMP
XMP
Methyladenosine
N6−acetyllysine
3−isopropylmalic acid
Nicotinamide Riboside
Methionine
Nicotinamide
Hexose−6−Phosphate
Arginine
UMP
Phosphocholine
Aminolevulinic Acid
Succinate
Isovalerylcarnitine
Glutathione Reduced
Histidine
Phosphoglycerates
Dimethylglycine
Choline
Ornithine
Hypoxanthine
137−>78, 137−>94
Guanosine
Inosine
Serine
Ribulose−5−Phosphate
N−Acetyl−L−Glutamine
Glutathione oxidized
Acetoacetate
Citrate
Isocitrate
Citraconic Acid
Aconitate
Citrate/Isocitrate
Uridine
Deoxyinosine
Aspafagine
Creatinine
Thymidine
Lipoamide
Guanine
Glycine
ATP
GDP
NAD+
2−hydroxyglutarate
Dimethylarginine
N1−Methylnicotinamide
Thiamine
Creatine
Propionylcarnitine
Glutamate
Acetylcarnitine
Glycerophosphocholine
Threonine
Betaine
Alanine
Tyrosine
Tryptophan
Proline
Pantothenate
Valine
Phenylalanine
Leucine/Isoleucine
Hydroxyproline
Malate
CMP
Aspartate
48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96
Time (hrs)
Cystathionine
Ornithine
AbscisicAcid
Glycerol
Thymine
Acetyl−amino sugar phosphates
Nicotinamide Riboside
Glutathione Reduced
Nicotinamide
Acetoacetate
CoA
dUMP
Aminobenzoates/Trigonelline
Uriedoproprionate
Phosphocreatine
ATP
ADP
UDP−Glucose
Phosphocholine
GDP
NAD+
2−hydroxyglutarate
Glutamate
Citraconic Acid
Aconitate
Aminolevulinic Acid
Butrylcarnitine
Creatine
N1−Methylnicotinamide
Carnitine
Glycerophosphocholine
UDP−N−Acetylamino Sugars
Pantothenate
Isovalerylcarnitine
Propionylcarnitine
Glutathione oxidized
Lysine
Glutamine
Citrate/Isocitrate
Taurine
Alanine
Acetylcholine
Acetylcarnitine
Proline
Inosine
Hypoxanthine
N6−acetyllysine
SAH
Myo−inositol
Histidine
Acetylputrescine
Lipoamide
Tyrosine
Tryptophan
Citrate
Threonine
Phenylalanine
Leucine/Isoleucine
Valine
Methionine
Lactate
Arginine
Dimethylarginine
Nicotinamide
48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96
Time (hrs)
−4 −2 0 2 4
Column Z−Score

Figure S3

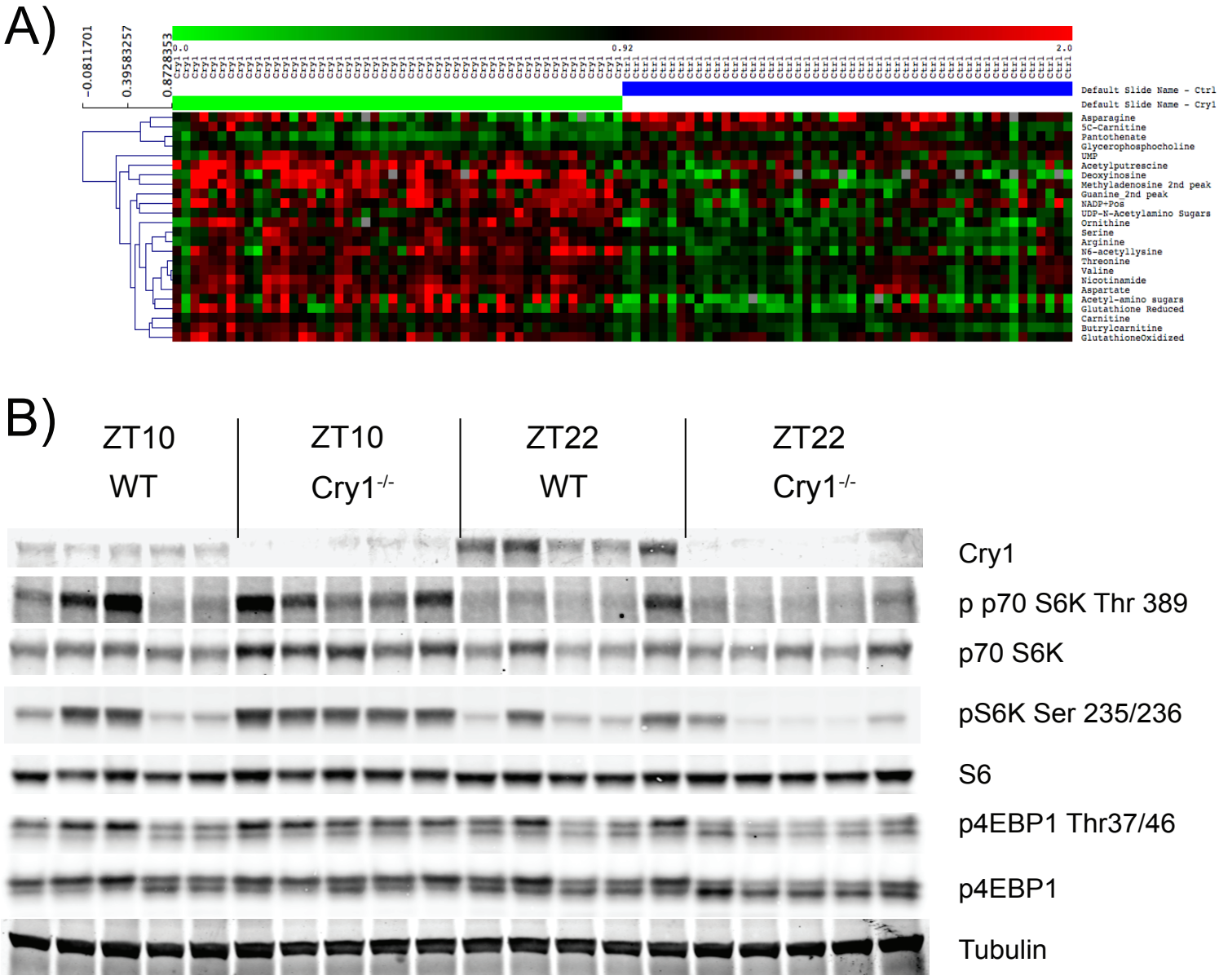

A)
-0.0811701
0.39583257
0.8728353
0.0
0.92
2.0
Default Slide Name - Ctrl
Default Slide Name - Cry1
Asparagine
5C-Carnitine
Pantothenate
Glycerophosphocholine
UMP
Acetylputrescine
Deoxyinosine
Methyladenosine 2nd peak
Guanine_2nd peak
NADP+Pos
UDP-N-Acetylamino Sugars
Ornithine
Serine
Arginine
N6-acetyllysine
Threonine
Valine
Nicotinamide
Aspartate
Acetyl-amino sugars
Glutathione Reduced
Carnitine
Butrylcarnitine
GlutathioneOxidized
B)
ZT10 WT
ZT10 Cry1-/-
ZT22 WT
ZT22 Cry1-/-
Cry1
p p70 S6K Thr 389
p70 S6K
pS6K Ser 235/236
S6
p4EBP1 Thr37/46
p4EBP1
Tubulin

Figure S4

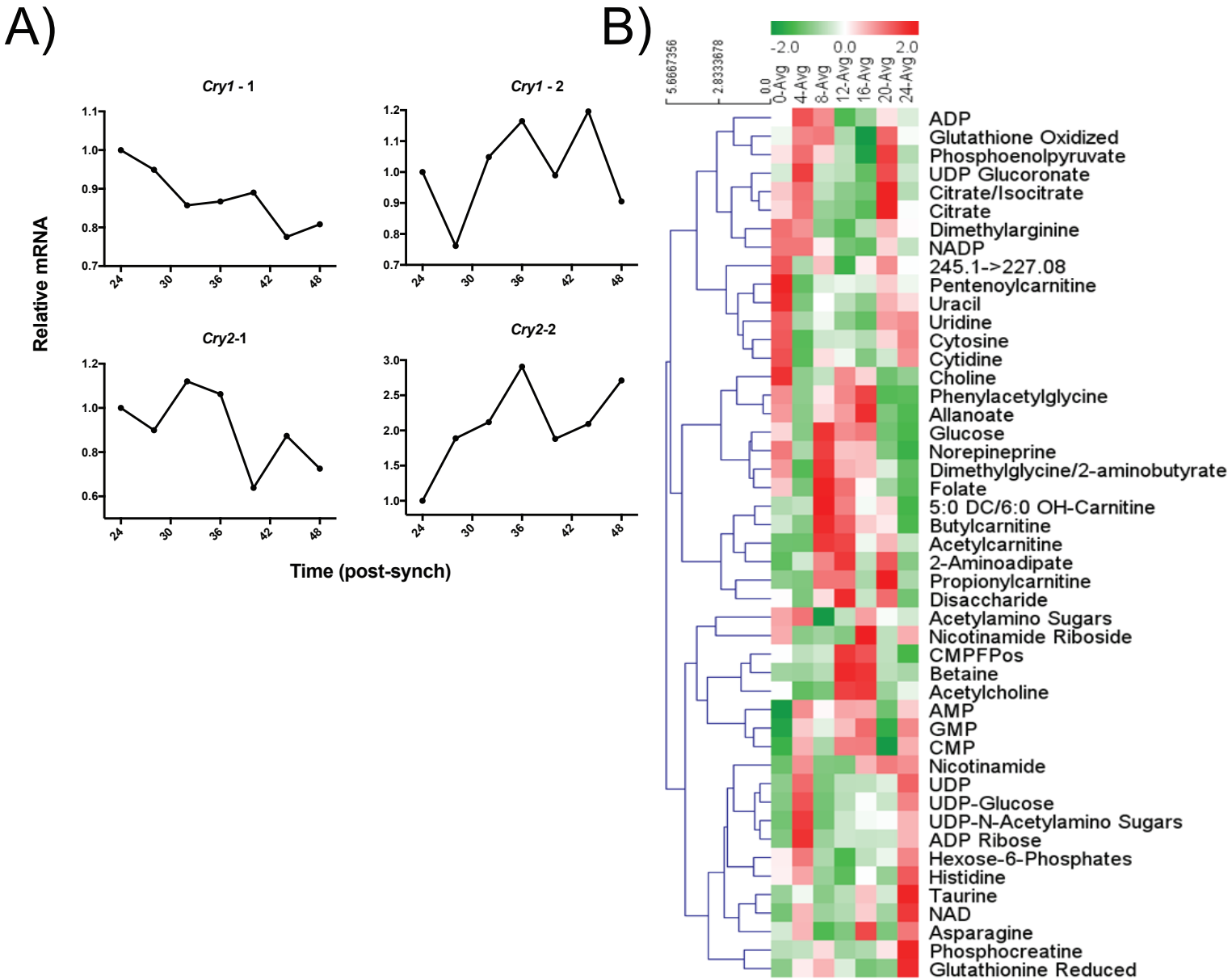

A)
Cry1 - 1
Cry1 - 2
Cry2-1
Cry2-2
Relative mRNA
Time (post-synch)
B)
-2.0
0.0
2.0
5.6667356
2.8333678
0.0
0-Avg
4-Avg
8-Avg
12-Avg
16-Avg
20-Avg
24-Avg
ADP
Glutathione Oxidized
Phosphoenolpyruvate
UDP Glucoronate
Citrate/Isocitrate
Citrate
Dimethylarginine
NADP
245.1->227.08
Pentenoylcarnitine
Uracil
Uridine
Cytosine
Cytidine
Choline
Phenylacetylglycine
Allanoate
Glucose
Norepineprine
Dimethylglycine/2-aminobutyrate
Folate
5:0 DC/6:0 OH-Carnitine
Butylcarnitine
Acetylcarnitine
2-Aminoadipate
Propionylcarnitine
Disaccharide
Acetylamino Sugars
Nicotinamide Riboside
CMPFPos
Betaine
Acetylcholine
AMP
GMP
CMP
Nicotinamide
UDP
UDP-Glucose
UDP-N-Acetylamino Sugars
ADP Ribose
Hexose-6-Phosphates
Histidine
Taurine
NAD
Asparagine
Phosphocreatine
Glutathionine Reduced

Figure S5

Table S1

	Organism	Circadian / Diurnal	Sample studied	Total Length	Resolution	Replicates? (Number)	Total samples	Metabolites measured
Current study	Mouse Human	Dark / Dark N/A	Liver U2 OS Primary Hepatocytes	48 h 48 h 24 h	1 h 2 h 4 h	Y (2) N Y (2-3)	96 24 16	179 137 147
(Thaiss et al., 2016)	Mouse	Light / Dark	Feces Serum	48 h	6 h	Y (18)	162	ND
(Tran et al., 2016)	Mouse	Light / Dark	Liver Serum	24 h	6 h		4	110 80
(Masri et al., 2016)	Mouse	Light / Dark	Liver	24 h	4 h	Y (5)	30	ND
(Abbondante et al., 2015)	Mouse	Light / Dark	Liver Serum	24 h	4 h	Y (5)	30	306 362
(Gogna et al., 2015)	Fly	Light / Dark & Temperature	Whole body	24 h	2 h	Y (5)	12	14 metabolites / NMR
(Li et al., 2015)	Zebrafish	Light / Dark	Whole larvae	24 h	6 h	Y (3)	12	73 assigned signals by NMR
(Zwighaft et al., 2015)	Mouse	Light /Dark	Liver	24 h	4 h	Y (4)	24	Polyamines / HPLC
(Giskeødegård et al., 2015)	Human	Dim light, constant routine, 24 hour wake/sleep, 24 hour wakefulness	Urine	24 / 24	2-8 h	Y (15)	105	NMR / binning
(Castro et al., 2015; Paschos et al., 2012)	Mouse	Light / Dark	White and Brown Adipose Tissues	24 h	6 h	Y (3)	12	- Fatty acids - Intact lipids - NMR polar
(Davies et al., 2014)	Human	Dim light, constant routine, 24 hour wake/sleep, 24 hour wakefulness	plasma	24 / 24	2 h	Y (12)	144 / 144	171
(Masri et al., 2014)	Mouse	Light / Dark	Liver	24 h	4 h	ND	ND	ND
(Dyar et al., 2014)	Mouse	Light / Dark	Muscle	24 h	4 h	Y (5)	30	277 (only sugars reported)
(Chaix et al., 2014)	Mouse	Light / Dark	Serum	24 h	4 h	N	6	278
(Shostak et al., 2013)	Mouse	Light / Dark and Dark / Dark	Blood / adipose	24 h	6 h	Y (3-5)	12-20	Selected fatty acids / lipid compounds
(Eckel-Mahan et al., 2013)	Mouse	Light / Dark	Liver	24 h	4 h	Y (5)	30	306 total
(Chua et al., 2013)	Human	Dim light, Constant lab conditions, wakefulness	plasma	28 h	4 h	Y (20)	140	LCMS / 263 lipids
(Hatori et al., 2012)	Mouse	Light / Dark	Liver	24 h	3 h	Y (3-4)	24-32	324
(Eckel-Mahan et al., 2012)	Mouse	Light / Dark	Liver	24 h	6 h	Y (5)	20	309 named (Metabolon)
(Dallmann et al., 2012)	Human (pooled)	Dim light / constant protocol / sleep deprivation	Blood plasma	40 h	4 h	Y (10)	100	258 total, 219 across two individuals (Metabolon)
(Fustin et al., 2012)	Mouse	Light / Dark and Dark / Dark	Liver	24 h	4 h	Y (3)	18	CE-TOF-MS; only nucleic acid metabolites reported

Table S2

REAGENT or RESOURCE	SOURCE	IDENTIFIER
36B4 (*Rplp0*)	IDT	TTA TAA CCC TGA AGT GCT CGA C, CGC TTG TAC CCA TTG ATG ATG
REV-ERBα (*Nr1d1*)	Taqman Gene Expression Assay	Mm00520708_m1
REV-ERBβ (*Nr1d2*)	IDT	Mm.PT.51.12747673
BMAL1 (*Arntl*)	Taqman Gene Expression Assay	Mm00500226_m1
Cry1	Taqman Gene Expression Assay	Mm00514392_m1
Cry2	Taqman Gene Expression Assay	Mm01331543_g1
GAPDH	Taqman Gene Expression Assay	Endogenous Human control
ARNTL	Taqman Gene Expression Assay	Hs00154147_m1
CRY1	Taqman Gene Expression Assay	Hs00172734_m1
CRY2	Taqman Gene Expression Assay	Hs00391360_m1
siRNA targeting human CRY1	Qiagen	GS1407
siRNA targeting human *CRY2*	Qiagen	GS1408
siRNA targeting human *ARNTL*	Qiagen	GS406
AllStars Negative Control siRNA	Qiagen	SI03650318