Supplemental Information

Circadian Control of DRP1 Activity Regulates Mitochondrial Dynamics and Bioenergetics

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Supplementary figure titles and legends

Figure S1, related to Figure 1

A

B

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>LC/MS-MS (normalized)</th>
<th>Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>Low</td>
<td>12 16 20 24 28 32</td>
</tr>
<tr>
<td>Isovaleryl-carnitine</td>
<td>High</td>
<td>12 16 20 24 28 32</td>
</tr>
<tr>
<td>Isobutyryl-carnitine</td>
<td>Low</td>
<td>12 16 20 24 28 32</td>
</tr>
</tbody>
</table>

C

D

E

F

G

H

I

Legend:

- **a**mno acids
- **peptides**
- **carbohydrates**
- **cofactors & vitamins**
- **energy**
(A) Heat plots for all identified metabolites in synchronized human U2OS cells.

(B-E) Accumulation profiles of oscillating metabolites involved in branched-chain amino acid metabolism (B), GSH/GSSG metabolism (C), glycolysis (D), and TCA cycle (E), presented as mean ± SEM. Raw peak values for all metabolites were normalized to have a median of 1.

(F) Relative total ATP levels from serum-shocked human skin fibroblasts measured at the indicated time points (JTK_Cycle, $P=1.78 \times 10^{-15}$). Right panel displays relative total ATP level at 16 hours post-shock (peak of ATP content) and at 28 hours (trough of ATP content).

(G) Cytosolic (cROS) and mitochondrial (mROS) reactive oxygen species levels were evaluated in serum-shocked human skin fibroblasts (JTK_Cycle, $P_{mROS}=5.40 \times 10^{-21}$, $P_{cROS}=0.489$). Right panel displays cROS and mROS levels at 16 hours post-shock (peak) and at 28 hours (trough).

(H) Total NAD$^+$ content assessed from brain of non-fasted wild-type mice kept in constant darkness every 4 hours for 24 hours (JTK_Cycle, $P=1.23 \times 10^{-6}$). Right panel displays relative total NAD$^+$ level at CT4 (peak) and at CT16 (trough).

(I) Total NADH content from brain of non-fasted wild-type mice kept in constant darkness, assessed every 4 hours for 24 hours (JTK_Cycle, $P=0.000168$). Right panel displays relative total NAD$^+$ level at CT4 (peak) and at CT16 (trough).

All data are represented as mean ± SEM of at least three independent samples (n = 4 or 6 per time point) (F-I). **$P<0.01$, ***$P < 0.001$ for Student’s two-tailed t-test comparing time points (e.g. 16h versus 28h).
Figure S2, related to Figure 1 & 2

A

Total ATP (rel. Units)

CTRL AraC

12 16 20 24 28 32

Post-shock (hr)

B

BrdU-positive cells (% on CTRL)

16 28 16 28

Post-shock (hr)

CTRL AraC

C

Period length (hr)

CTRL 2DG AraC

D

Total ATP (rel. Units)

0 4 8 12 16 20 24

CT (hr)

E

NAD+ (nmol/g protein)

0 4 8 12 16 20 24

CT (hr)

F

NADH (nmol/g protein)

0 4 8 12 16 20 24

CT (hr)

G

OCR (pmoles/min)

Post-Shock: 16 h 28 h

H

ECAR (mP/min)

I

TP12

0 μm 25

J

TP16

0 μm 25

K

TP20

0 μm 25

L

TP24

0 μm 25

M

TP28

0 μm 25

N

TP32

0 μm 25

O

TP36

0 μm 25

P

TP40

0 μm 25
(A) Left panel, relative total ATP contents from serum-shocked human skin fibroblasts treated with cytosine β-D-arabinofuranoside (AraC, 100 μM) compared to non-treated cells (CTRL) measured at the indicated time points in cells (n=6 per time point, JTK_Cycle, $P_{\text{CTRL}}=4.69\times10^{-12}$, $P_{\text{AraC}}=3.0\times10^{-10}$). Right panel displays relative total ATP level at 16 hours post-shock (peak of ATP content) and at 28 hours (trough of ATP content) in control and treated conditions.

(B) Percentage of BrdU-positive cells in absence and presence of AraC at 24 hours post-shock. All data are represented as mean ± SEM of at least three independent samples (n = 4 or 6 per time point) (A, B). **P<0.01, ***P < 0.001 for Student’s two-tailed t-test comparing single time points between CTRL and treated cells.

(C) Circadian period length determined in dexamethasone - synchronized human skin fibroblasts transfected with Bmal1::luciferase reporter in presence of 2 deoxy-glucose (4.5 g/L) and AraC (100 μM) compared to control (CRTL).

(D) Left panel shows relative total ATP levels measured in brain of wild-type mice kept in constant darkness (WT Brain) every 4 hours for 24 hours (7 time points, n=4 for each, JTK_Cycle, $P=0.0077$). Right panel displays relative total ATP level at circadian time 4 (CT4; peak of ATP content) and 16 (CT16; trough of ATP content).

(E) Total NAD$^+$ measured in serum-shocked human skin fibroblasts at the indicated time points (6 time points, n=6 for each, JTK_Cycle, $P=2.18\times10^{-8}$). Right panel displays relative total NAD$^+$ level at 16 hours post-shock (peak) and at 28 hours (trough).

(F) Total NADH measured in serum-shocked human skin fibroblasts at the indicated time points (6 time points, n=6 for each, JTK_Cycle, $P=6.68\times10^{-5}$). Right panel displays relative total NAD$^+$ level at 16 hours post-shock (peak) and at 28 hours (trough).

All data are represented as mean ± SEM (D-F). **P<0.01, ***P < 0.001 for Student’s two-tailed t-test comparing single time points (16 versus 28 hours).

(G) OCR related to the proton leak (independent to ATP production) at 16 hours post-shock and 28 hours post-shock in human skin fibroblast.

(H) Extracellular Acidification Rate (ECAR) corresponding to glycolytic rate at 16 hours post-shock and 28 hours post-shock in human skin fibroblast.

All data are represented as mean ± SEM of three independent samples (n= 11 per time point).

(I-P) Mitochondrial network morphology assessed at 4 hours intervals for 8 time points in synchronized fibroblasts (I, K, L, N, O) Intermediate network; (J, P) Tubular network; (M) Fragmented network. For each representative image, a zoom-in image is provided (400%). Scale bar = 25 μm.
Figure S3, related to Figure 2
(A-H) Mitochondrial network morphology assessed at 4 hours intervals for 8 time points in synchronized A172 glioma cells transfected with a GFP plasmid containing a mitochondrial targeting sequence. (A, C, G) Intermediate network; (B, H) Tubular network; (D, E, F) Fragmented network. For each representative image, a zoom-in image is provided (400%). Scale bar = 25 μm.

(I) Mitochondrial network morphology assessed in liver sections from non-fasted wild-type mice kept in darkness condition at CT0 (i) and CT12 (ii). Scale bar = 50 μm.

(J) Quantification of mitochondrial interconnectivity corresponding to the conditions A to H. On average 10’000-20’000 mitochondrial organelles were analyzed per time point (n = 25-30 images per time point; JTK_Cycle, P = 0.000607).

(K) Quantification of mitochondrial interconnectivity at CT0 and CT12 (n=6 sections per condition,.). On average 2’500-8’500 mitochondrial units were analyzed per time point.

Data are represented as average ± SEM (J, K). ***P<0.001 for Student’s two-tailed t test comparing single time points.
Figure S4, related to figure 4

A: mRNA abundance of Complex I, IV, and V over time.

B: mRNA levels of MFN1, MFN2, OPA1, DRP1, and hFIS1 over time.

C: Total ATP levels in CTRL and Mdivi-1 conditions over time.

D: mRNA abundance of Bmit1 across different time points.

E: Bioluminescence over time in the presence of Compound C.

F: Bioluminescence in CTRL and P110 conditions over time.
(A) Relative mRNA expression of complex I, IV and V subunits at 16 hours post-shock (corresponding to the peak in gene expression) and 28 hours post-shock (corresponding to the trough in gene expression) (JTK_Cycle, P_{NDUFA2}=3.96*10^{-5}, P_{NDUFBS}=8.57*10^{-6}, P_{NDUFC1}=4.88*10^{-15}, and P_{NDUFV2}=7.61*10^{-5}).

(B) Profile of relative mRNA expression of nuclearly-encoded genes related to mitochondrial fusion (MFN1, MFN2 and OPA1) and mitochondrial fission (DRP1 and hFIS1) in serum-shocked human skin fibroblasts (JTK_Cycle, P_{MFN1}=0.809, P_{MFN2}=0.426, P_{OPA1}=0.175, P_{DRP1}=0.215, P_{hFIS1}=0.827).

(C) Left panel, relative total ATP levels from serum-shocked human skin fibroblasts treated with Mdivi-1 (50 μM) compared to non-treated cells (CTRL) measured at the indicated time points (n=6 per time point, JTK_Cycle, P_{CTRL}=5.36*10^{-20}, P_{Mdivi-1}=0.8581). Right panel, relative total ATP level at 16 hours post-shock (peak of ATP content) and at 28 hours (trough of ATP content) in control and treated conditions.

All data are represented as mean ± SEM of at least three independent samples (n=6 per time point.) (A- C). *P<0.05, **P<0.01, ***P<0.001 for Student’s two-tailed t test comparing single time points (A, B) or comparing single time points between CTRL and treated cells (C).

(D) Relative mRNA expression of Bmal1 evaluated from in Drp1^{−/−} MEFs compared to Drp1^{lox/lox} MEFs at 12, 18, 24, 30 and 36 hours post-shock (n=6 per time point, JTK_Cycle, P_{Drp1^{lox/lox}}=0.000951, P_{Drp1^{−/−}}=0.864).

(E, F) Representative bioluminescence records determined in dexamethasone - synchronized human skin fibroblasts transfected with Bmal1::luciferase reporter in presence of (E) an AMPK inhibitor (compound C, 1 μM) and (F) a DRP1 inhibitor, P110 (1 μM), compared to control (CTRL) (n = 3).
Table S2: Primer sequences, related to STAR Methods section: “Quantitative real-time PCR”.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Probe ID (Applied Biosystems)</th>
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<td><strong>Fusion</strong></td>
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<td>ATP5L (ATP synthase)</td>
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<tr>
<th>Primer</th>
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<td><strong>BMAL1</strong></td>
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<td><strong>PER1</strong></td>
<td>forward, 5'-CGCCTAACCCCGTATGTGA-3'  &lt;br&gt;reverse, 5'-CGCCTACCTCCTCCTCCTGTC-3'  &lt;br&gt;probe, 5'-Yakima Yellow-CGCATCCATTCCGGGTACGAAAGCTC-BHQ1-3'</td>
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<tr>
<td><strong>PER2</strong></td>
<td>forward, 5'-GGGCAGCCTTTCGACTATTCT-3'  &lt;br&gt;reverse, 5'-GCTGGTGTCCTTACCGGATCTGACT-3'  &lt;br&gt;5'-Yakima Yellow-CATTGGGTTTCCGTCCGCGG-CBH1-3'</td>
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