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Supplemental Information

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Mitochondrial Dynamics and Bioenergetics

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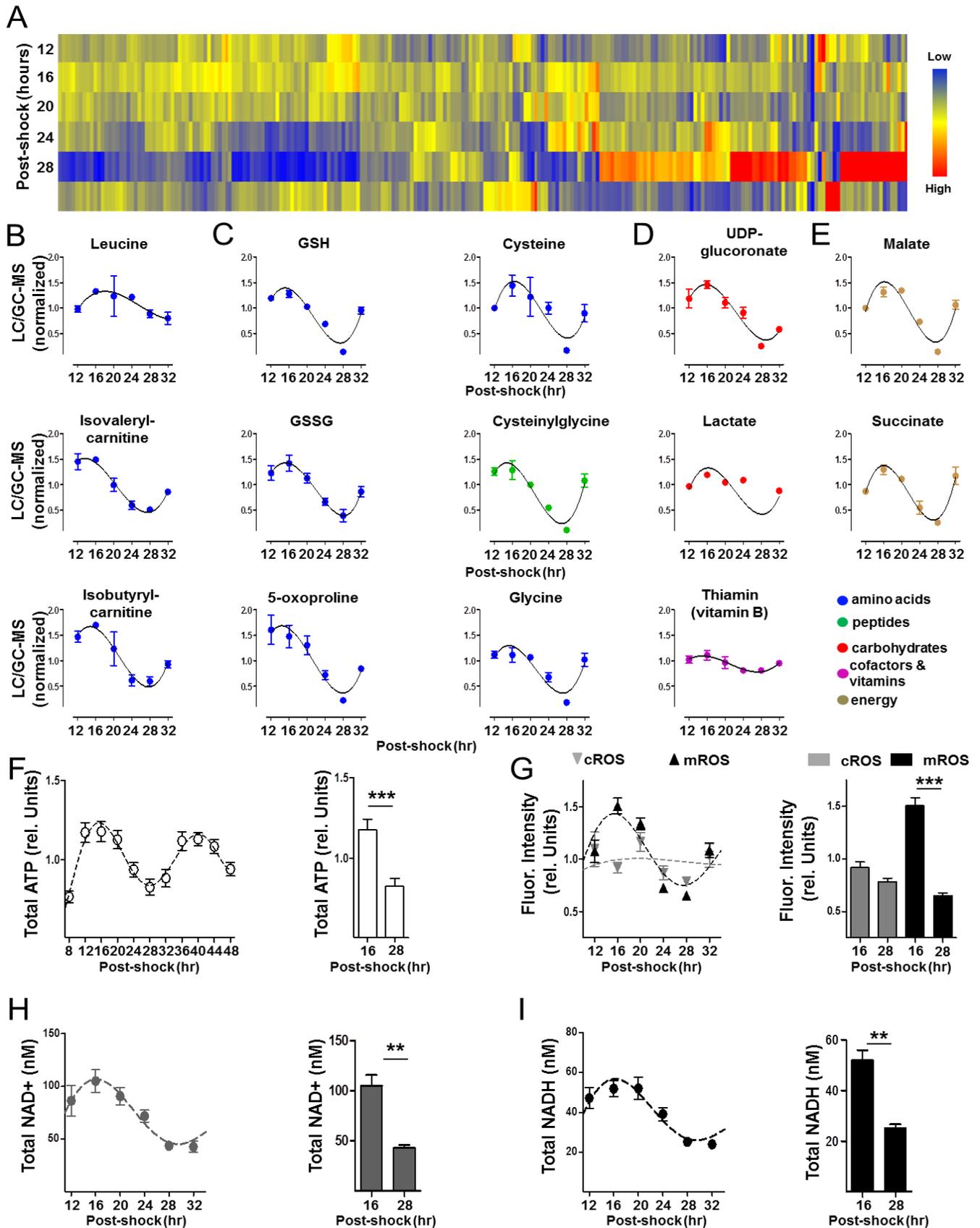
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Circadian control of DRP1 activity regulates mitochondrial dynamics and bioenergetics.

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Supplementary figure titles and legends

Figure S1, related to Figure 1



(A) 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 \pm 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, $P=1.78*10^{-15}$). Right panel displays relative total ATP level at 16 hours post-shock (peak of ATP content) and at 28 hours (trough of ATP content).

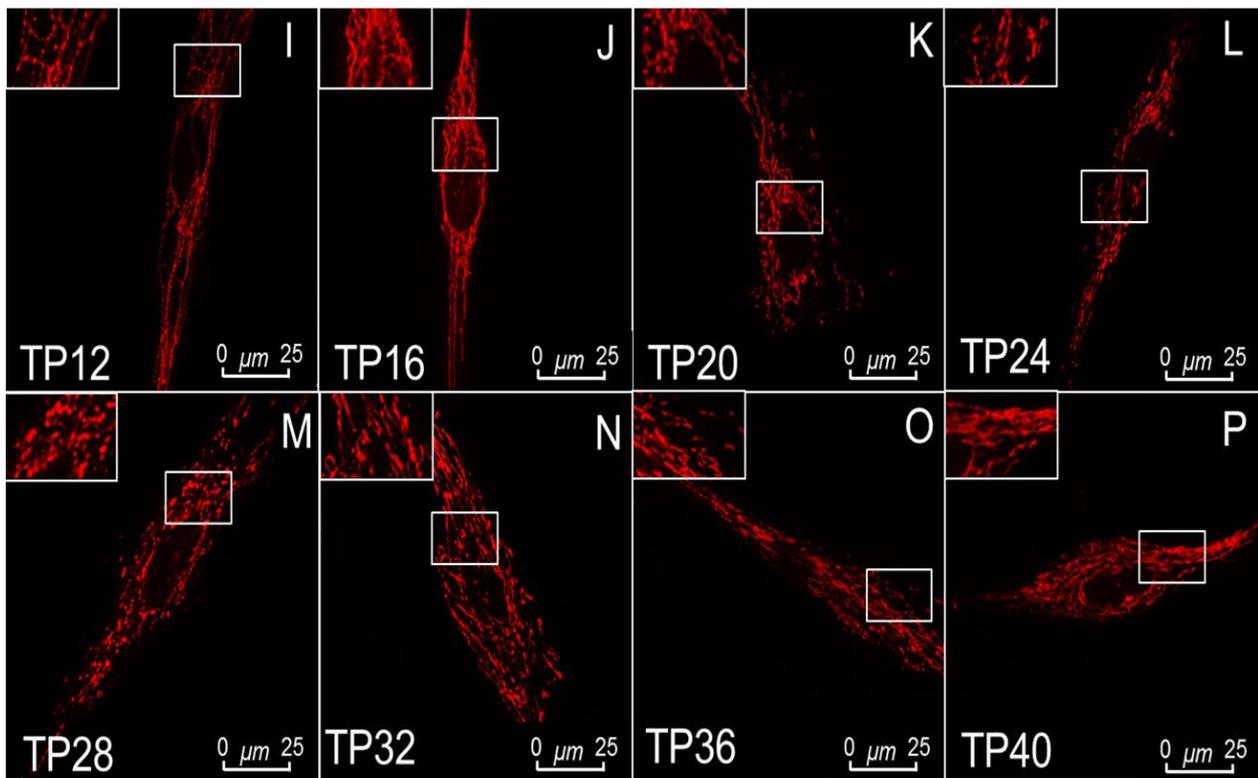
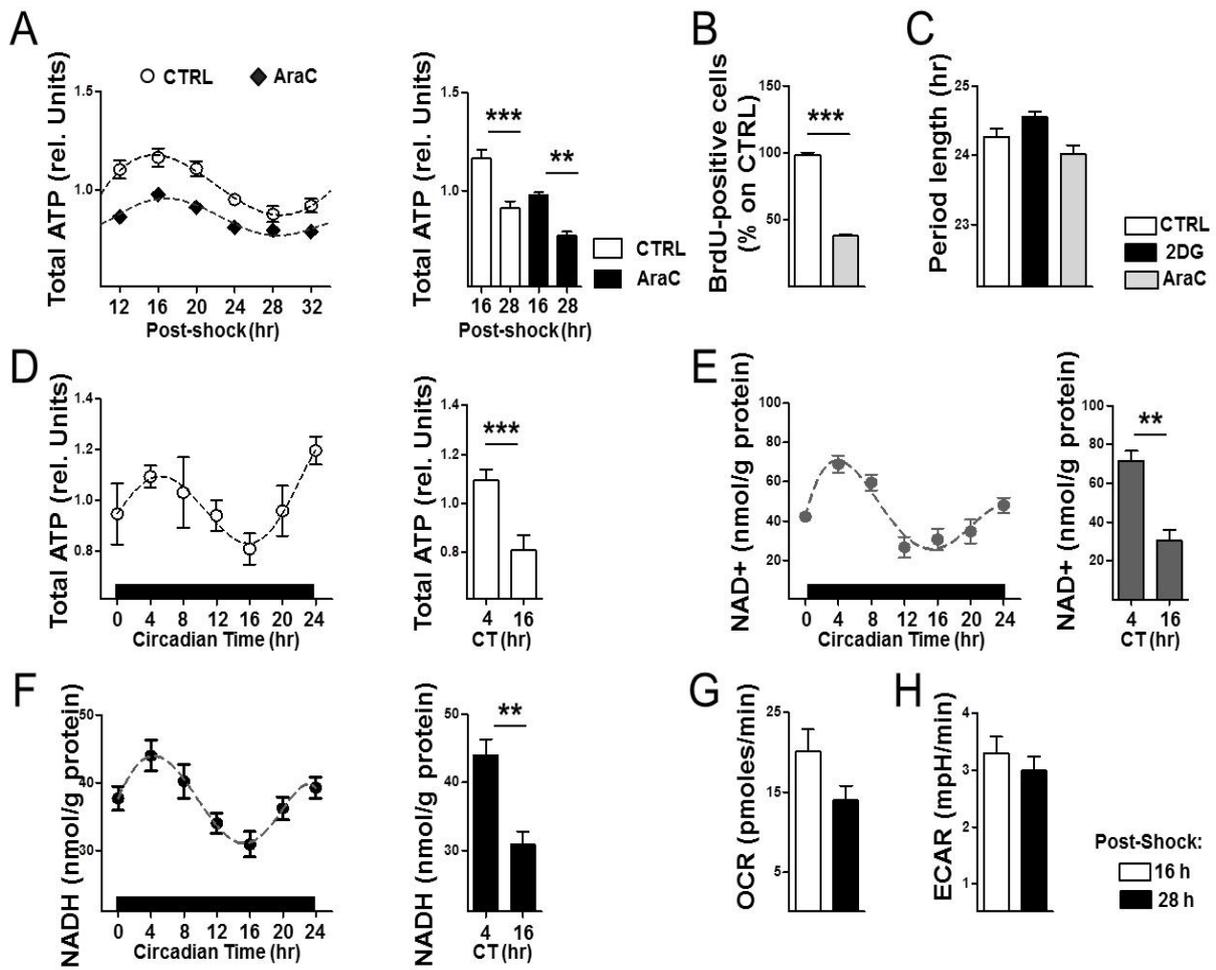
(G) Cytosolic (cROS) and mitochondrial (mROS) reactive oxygen species levels were evaluated in serum-shocked human skin fibroblasts (JTK_Cycle, $P_{mROS}=5.40*10^{-21}$, $P_{cROS}=0.489$). Right panel displays cROS and mROS levels at 16 hours post-shock (peak) and at 28 hours (trough)

(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, $P=1.23*10^{-6}$). 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 \pm 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) Left panel, relative total ATP contents from serum-shocked human skin fibroblasts treated with cytosine β -D-arabinofuranoside (AraC, 100 μ M) compared to non-treated cells (CTRL) measured at the indicated time points in cells (n=6 per time point, JTK_Cycle, $P_{CTRL}=4.69*10^{-12}$, $P_{AraC}=3.0*10^{-10}$). 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 \pm 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 μ M) compared to control (CTRL).

(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*10⁻⁸). 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*10⁻⁵). Right panel displays relative total NAD⁺ level at 16 hours post-shock (peak) and at 28 hours (trough)).

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

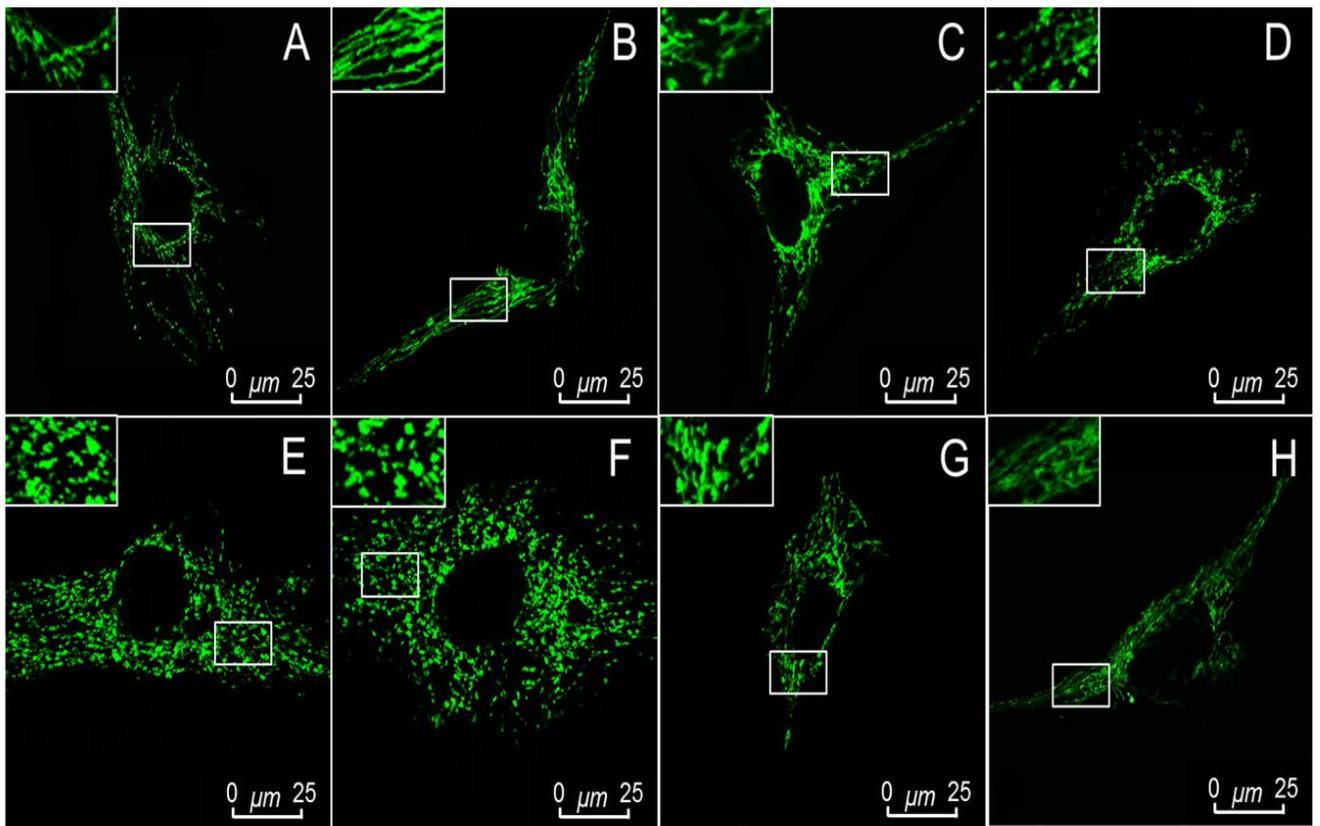
(G) OCR related to the proton leak (independent to ATP production) at 16 hours post-shock and 28 hours post-shock in human skin fibroblast.

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

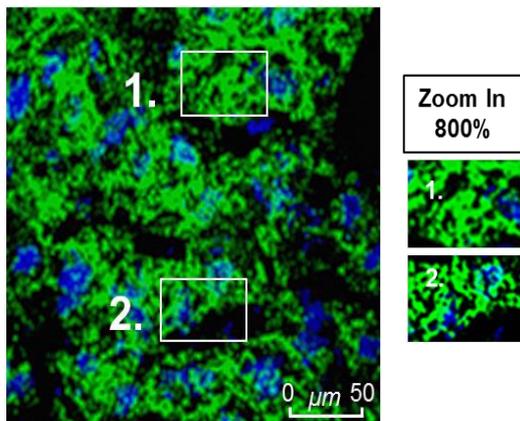
All data are represented as mean \pm SEM of three independent samples (n= 11 per time point).

(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 μ m.

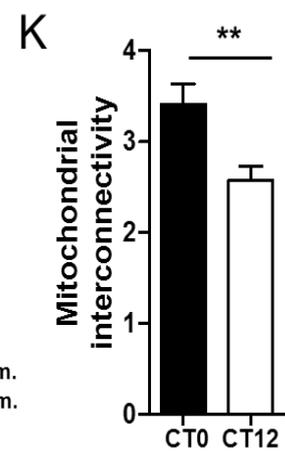
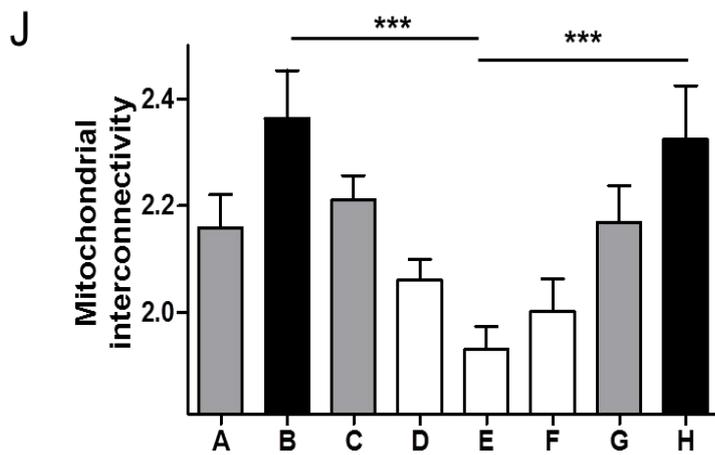
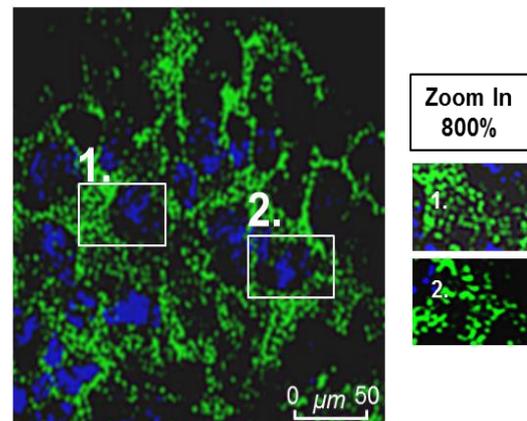
Figure S3, related to Figure 2



I (i) CT0



(ii) CT12



(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 .

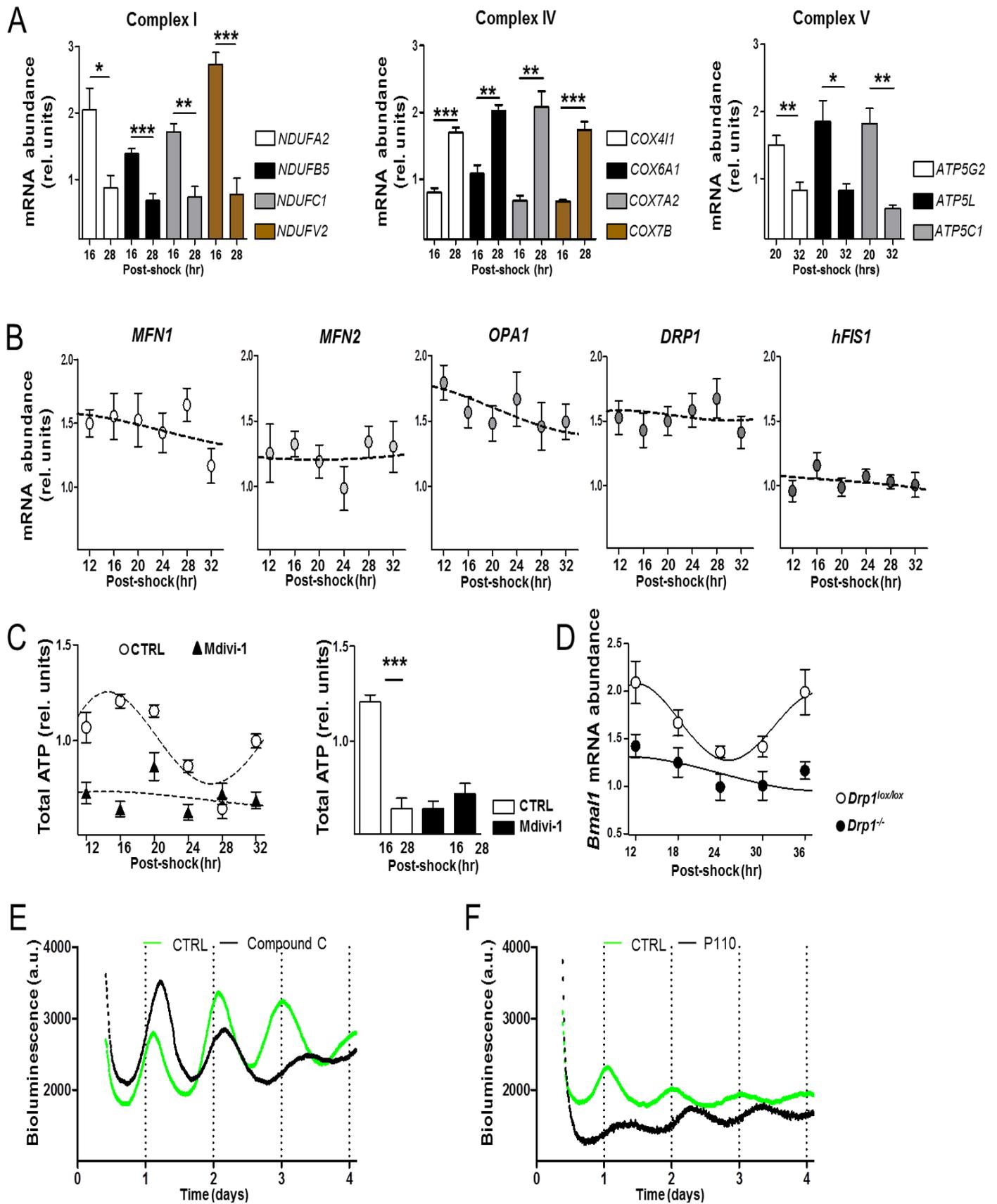
(I) Mitochondrial network morphology assessed in liver sections from non-fasted wild-type mice kept in darkness condition at CT0 (i) and CT12 (ii). Scale bar = 50 μm .

(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 \pm SEM **(J, K)**. ***P<0.001 for Student's two-tailed t test comparing single time points.

Figure S4, related to figure 4



(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, $P_{NDUFA2}=3.96*10^{-5}$, $P_{NDUFB5}=8.57*10^{-6}$, $P_{NDUFC1}=4.88*10^{-15}$, 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 μ M) 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 \pm 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 μ M) and (F) a DRP1 inhibitor, P110 (1 μ M), compared to control (CTRL) (n = 3).

Table S2: Primer sequences, related to STAR Methods section: “Quantitative real-time PCR”.

	Primer	Probe ID (Applied Biosystems)
Fusion	<i>MFN1</i>	Hs00250475_m1
	<i>MFN2</i>	Hs00208382_m1
	<i>OPA1</i>	Hs00323399_m1
Fission	<i>DRP1</i>	Hs00247147_m1
	<i>FIS1</i>	Hs00211420_m1
OXPHOS	<i>NDUFA2</i> (complex I)	Hs00159575_m1
	<i>NDUFB5</i> (complex I)	Hs00159582_m1
	<i>NDUFC1</i> (complex I)	Hs00159587_m1
	<i>NDUFV2</i> (complex I)	Hs00221478_m1
	<i>COX4II</i> (complex IV)	Hs00971639_m1
	<i>COX6A1</i> (complex IV)	Hs01924685_g1
	<i>COX7A2</i> (complex IV)	Hs01652418_m1
	<i>COX7B</i> (complex IV)	Hs00371307_m1
	<i>ATP5G2</i> (ATP synthase)	Hs01096582_m1
	<i>ATP5C1</i> (ATP synthase)	Hs01101219_g1
	<i>ATP5L</i> (ATP synthase)	Hs00758883_s1

Primer	Sequence (Microsynth)
<i>BMALI</i>	forward, 5'-GAAGACAACGAACCGACAATGAG-3'
	reverse, 5'-ACATGAGAATGCAGTCGTCCAA-3'
	probe, 5'-Yakima Yellow-TGTAACCTCAGCTGCCTCGTCGCA-BHQ1-3'
<i>PER1</i>	forward, 5'-CGCCTAACCCCGTATGTGA-3'
	reverse, 5'-CGCGTAGTGAAAATCCTCTTGTC-3'
	probe, 5'-Yakima Yellow-CGCATCCATTCGGGTTACGAAGCTC-BHQ1-3'
<i>PER2</i>	forward, 5'-GGGCAGCCTTTCGACTATTCT-3'
	reverse, 5'-GCTGGTGTCCAACGTGATGTACT-3'
	5'-Yakima Yellow-CATTCGGTTTCGCGCCCGGG-BHQ1-3'