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Supplemental Information

Circadian Control of DRP1 Activity Regulates Mitochondrial Dynamics and Bioenergetics

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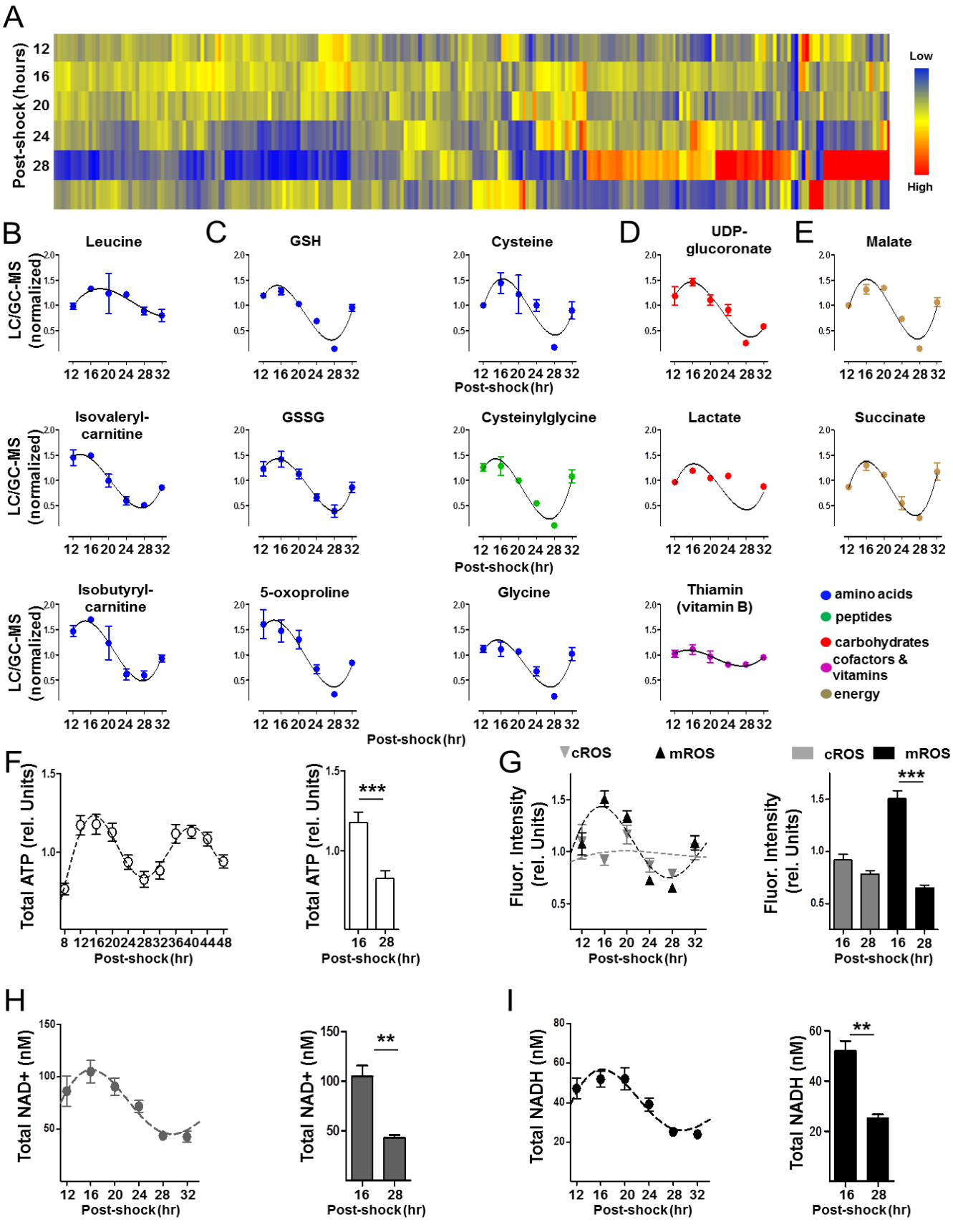
Supplementary figure titles and legends

Figure S1, related to Figure 1



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

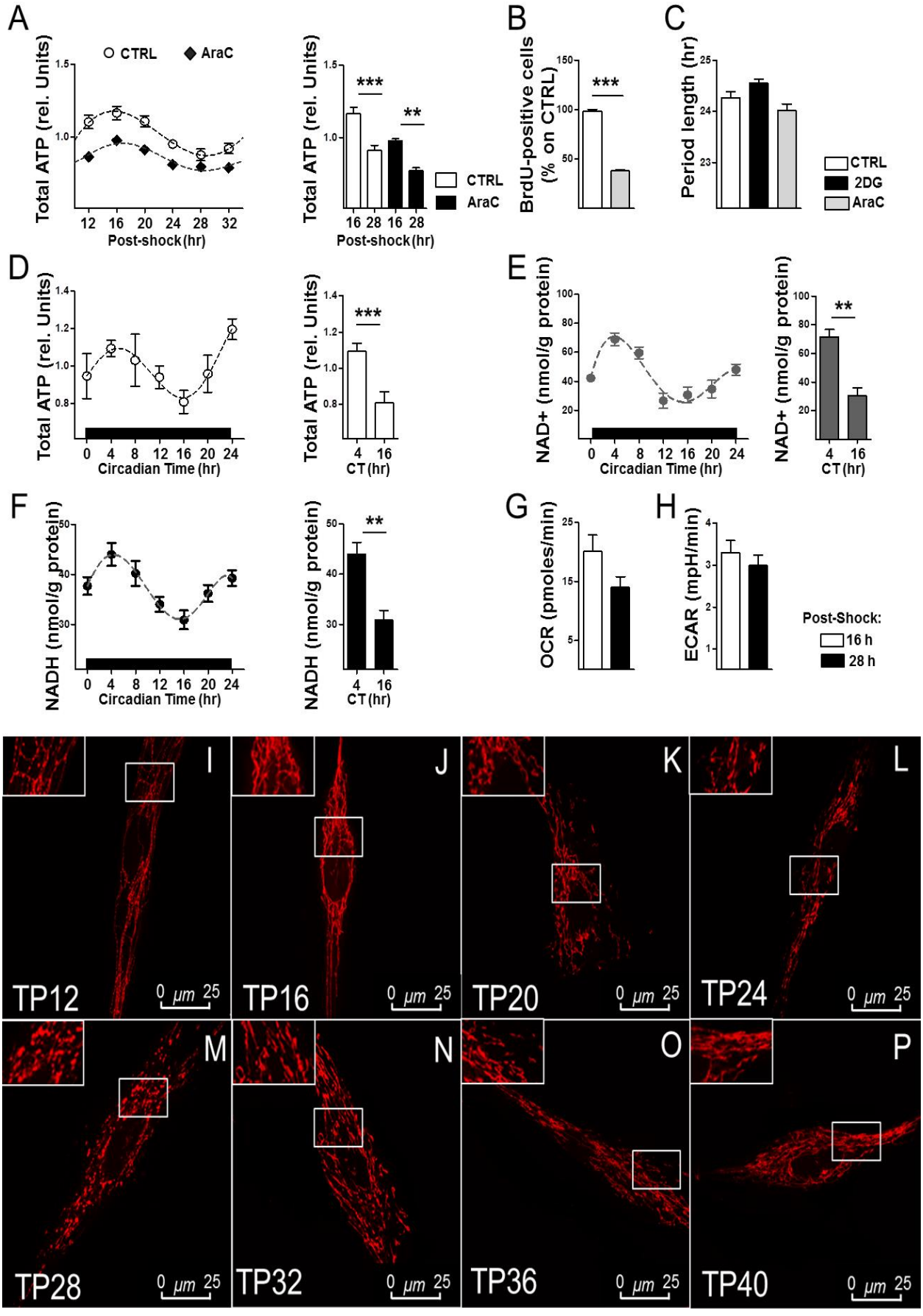
(**G**) Cytosolic (cROS) and mitochondrial (mROS) reactive oxygen species levels were evaluated in serum-shocked human skin fibroblasts (JTK_Cycle, P_{mROS}=5.40*10-21, P_{cROS}=0.489). Right panel displays cROS and mROS levels at 16 hours post-shock (peak) and at 28 hours (trough)

(**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 ± 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 μM) 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 ± 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, $P=2.18*10^{-8}$). 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 ± SEM (**D-F**). **P<0.01, ***P < 0.001 for Student's two-tailed t-test comparing single time points (16 versus 28 hours).

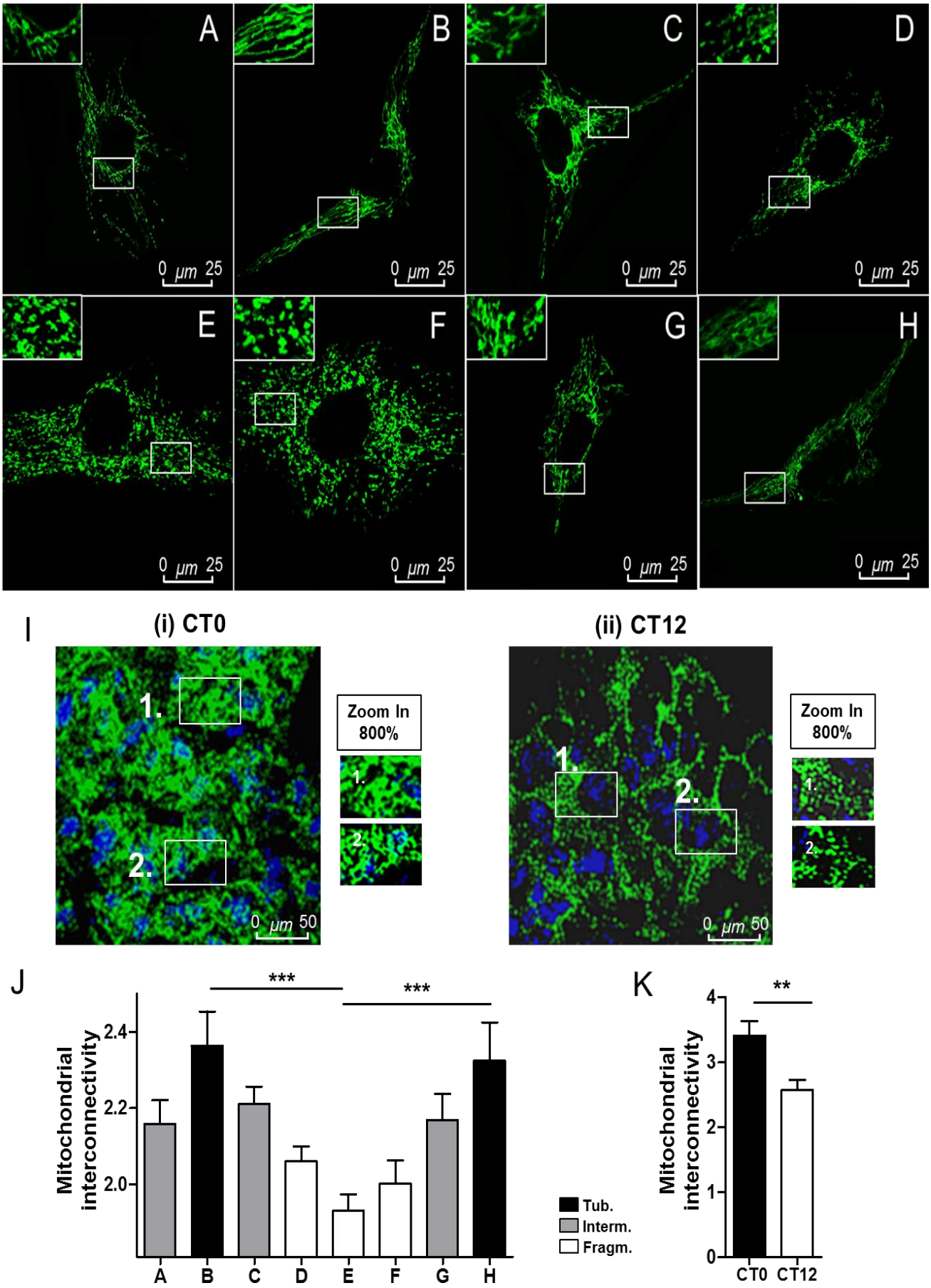
(**G**) OCR related to the proton leak (independent to ATP production) at 16 hours post-shock and 28 hours post-shock in human skin fibroblast.

(**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.

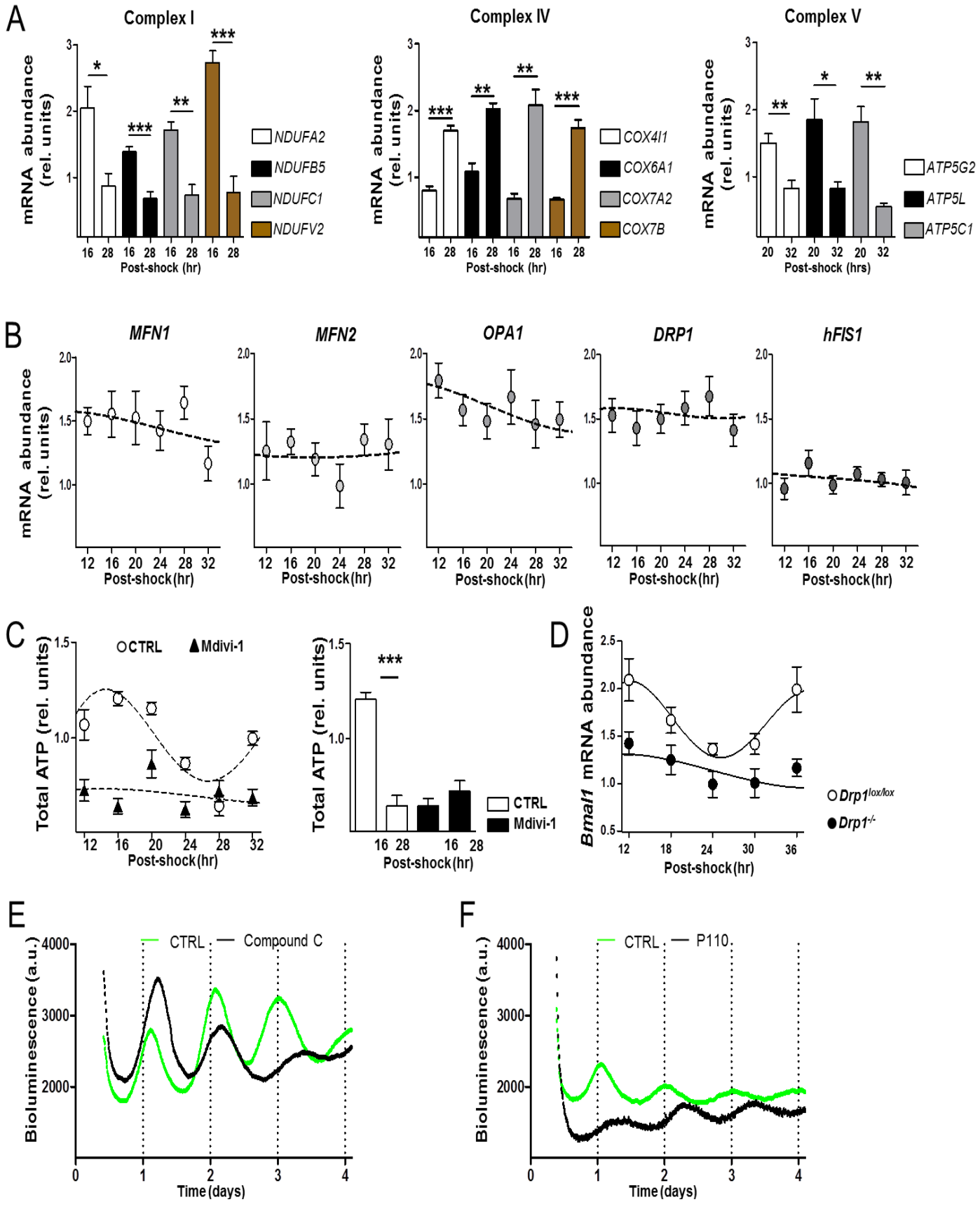
(**I**) Mitochondrial network morphology assessed in liver sections from non-fasted wild-type mice kept in darkness condition at CT0 (i) and CT12 (ii). Scale bar = 50 μm.

(**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**) 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 h*FIS1*) 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".

	Primer	Probe ID (Applied Biosystems)
Fusion	*MFN1*	Hs00250475_m1
	MFN2	Hs00208382_m1
	OPA1	Hs00323399_m1
Fission	*DRP1*	Hs00247147_m1
	FIS1	Hs00211420_m1
OXPHOS	*NDUFA2* (complex I)	Hs00159575_m1
	NDUFB5 (complex I)	Hs00159582_m1
	NDUFC1 (complex I)	Hs00159587_m1
	NDUFV2 (complex I)	Hs00221478_m1
	COX4I1 (complex IV)	Hs00971639_m1
	COX6A1 (complex IV)	Hs01924685_g1
	COX7A2 (complex IV)	Hs01652418_m1
	COX7B (complex IV)	Hs00371307_m1
	ATP5G2 (ATP synthase)	Hs01096582_m1
	ATP5C1 (ATP synthase)	Hs01101219_g1
	ATP5L (ATP synthase)	Hs00758883_s1

Primer	Sequence (Microsynth)
BMAL1	forward, 5'-GAAGACAACGAACCAGACAATGAG-3'
	reverse, 5'-ACATGAGAATGCAGTCGTCCAA-3'
	probe, 5'-Yakima Yellow-TGTAACCTCAGCTGCCTCGTCGCA-BHQ1-3'
PER1	forward, 5'-CGCCTAACCCCGTATGTGA-3'
	reverse, 5'-CGCGTAGTGAAAATCCTCTTGTC-3'
	probe, 5'-Yakima Yellow-CGCATCCATTCGGGTTACGAAGCTC-BHQ1-3'
PER2	forward, 5'-GGGCAGCCTTTCGACTATTCT-3'
	reverse, 5'-GCTGGTGTCCAACGTGATGTACT-3'
	5'-Yakima Yellow-CATTCGGTTTCGCGCCCGGG-BHQ1-3'