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Supplemental Information

Intense Light-Mediated Circadian Cardioprotection via Transcriptional Reprogramming of the Endothelium

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Figure S1

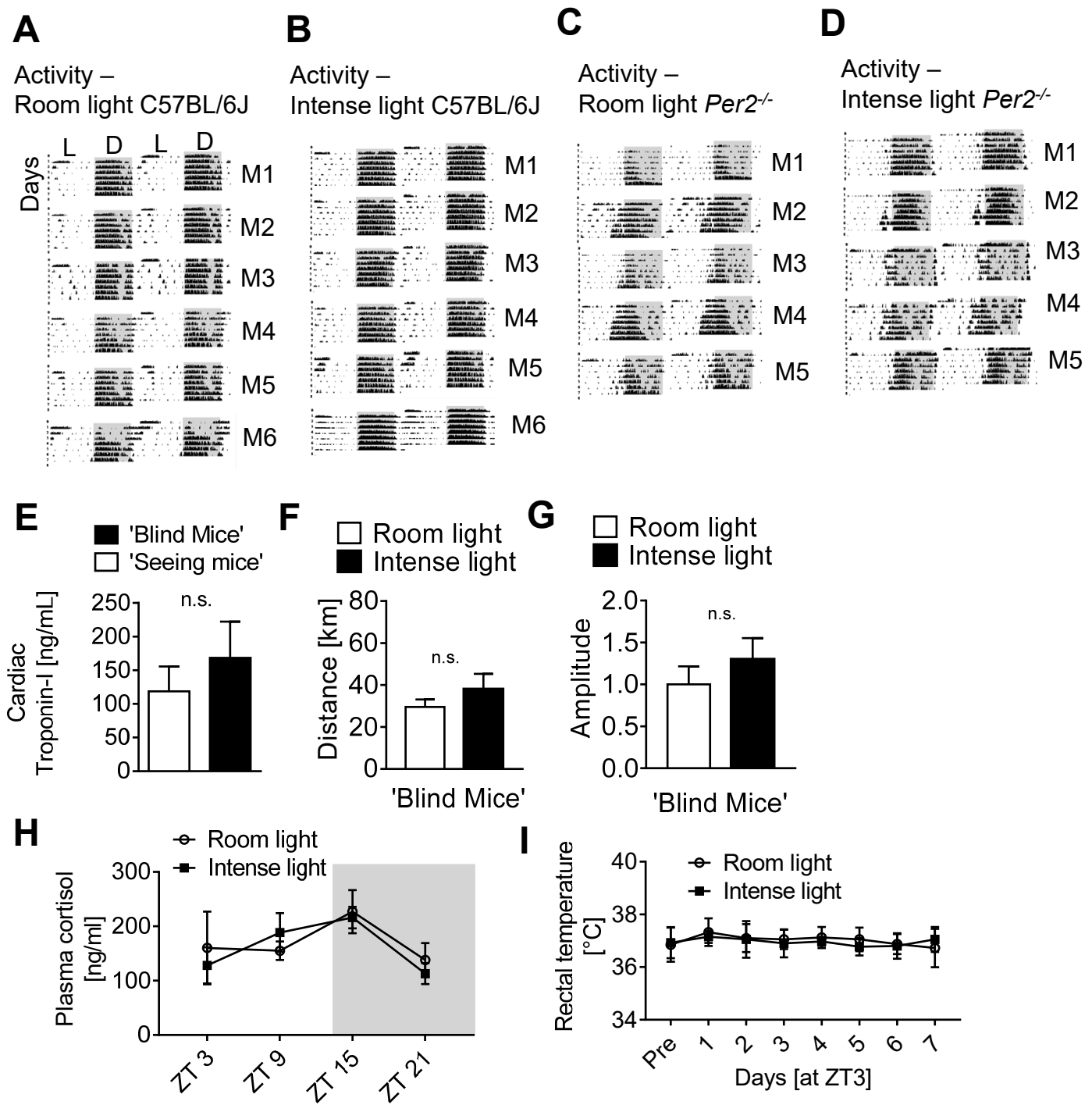
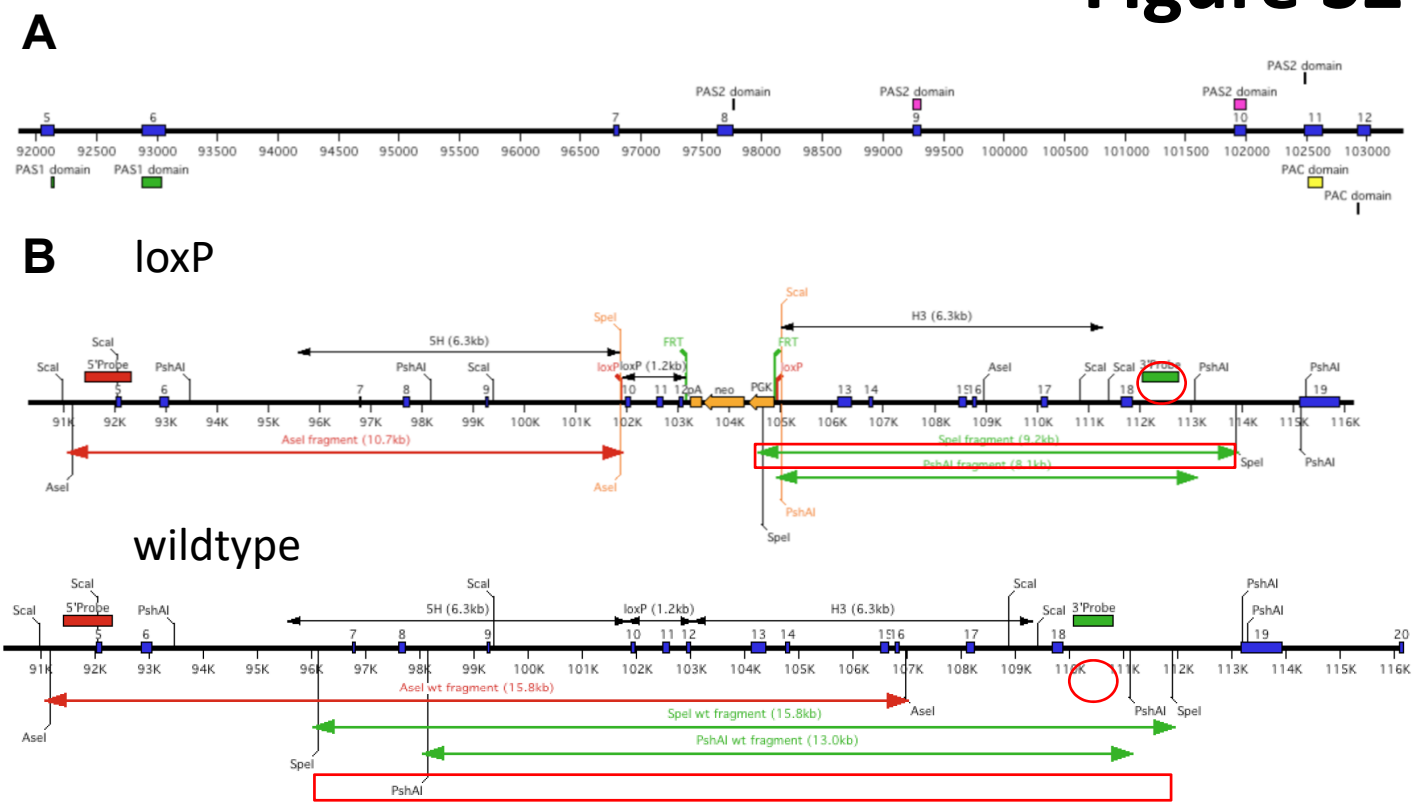
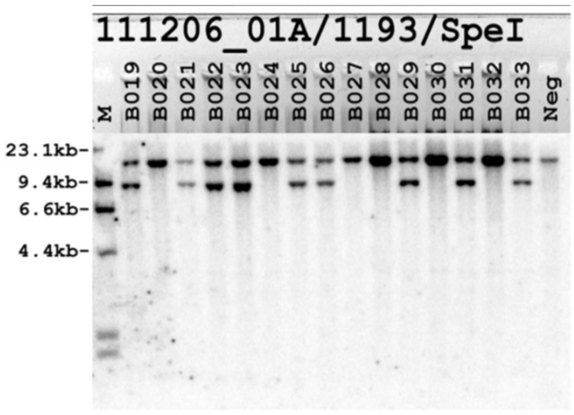
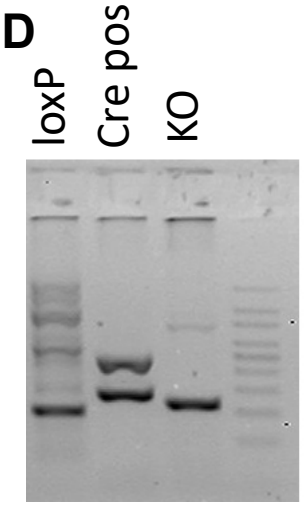


Figure S1. Related to Figure 1. (A-D) 7 day wheel running activity graphs (double-plotted) from wildtype or *Per2*$^{-/-}$ mice exposed to 7 days room light versus 7 days intense light (mean±SD, n=5-6, M=mouse, L=light phase, D=dark phase, double-plotted actograms, ***Note***: numbers on the left indicate days. **(E)** Cumulative cardiac troponin measurements (ZT3+ZT15) from 'seeing' compared to enucleated 'blind' wildtype mice subjected to 60 min ischemia and 2 h reperfusion (mean±SD, n=7); (**F-G**) Wheel running measurements during 7 days of room light or intense light housing conditions in 'blind' C57BL/6J mice (mean±SD; n=4). **(H**) Plasma cortisol levels after 7 days of room or intense light exposure in C57BL/6J mice (mean±SD, n=5, (**I**) Rectal temperatures during 7 days of room light or intense light at ZT3 in C57BL/6J mice (mean±SD; n=4).

Figure S2







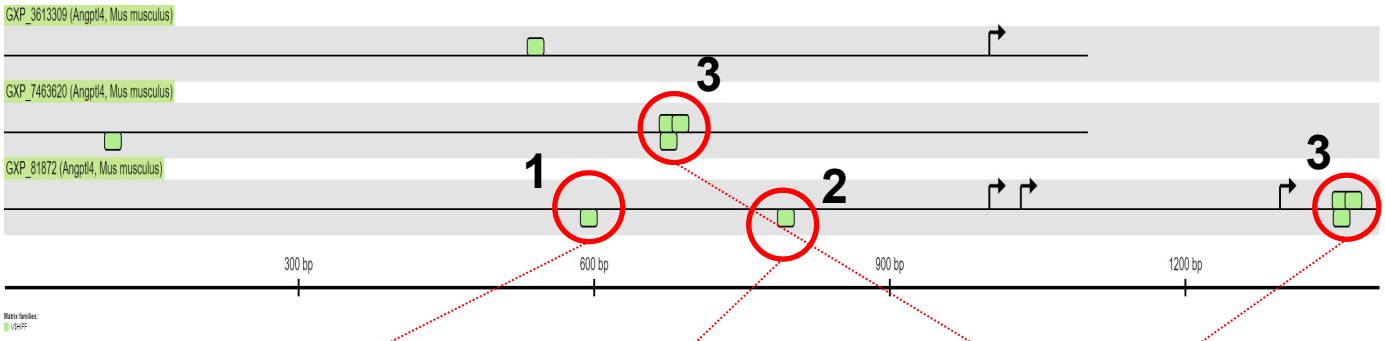
Aorta from Per2$^{loxp/loxp}$
VE-Cadherin-Cre

Figure S2. Related to Figure 3. (A) *Per2*$^{loxp/loxp}$ -strategy: deletion of exons 10, 11 and 12 in the *Per2* gene removes half of the PAS2 domain and all of the PAC domain. This deletion also results in a frameshift mutation introducing an early stop codon. **(B)** Screening strategy. (**C**) Screening: DNA was digested with SpeI and probed with the P3 probe. These mice were the result of a wt (wildtype) /loxP x C57BL/6J mating. The expected sizes were: wildtype 15.8kb and loxP 9.2kb. Correct integration was confirmed by full sequencing. (**D**) PCR-Genotyping of aortic tissue from a *Per2*$^{loxP/loxP}$ -VE-Cadherin-Cre mouse.

Figure S3

A

Murine *Angptl4* promoter regions



B

Matrix: V$ARNTL.01
Family: V$HIFF
Matrix Similarity: 0.945
Position: 586 - 602
Genomic Position: chr 17: 33781974 - 33781990
Sequence: cagagttaCGTGgcaga

C

Matrix: V$HRE.03
Family: V$HIFF
Matrix Similarity: 0.943
Position: 786 - 802
Genomic Position: chr 17: 33781774 - 33781790
Sequence: ataagcgACGTgactgt

D

Matrix: V$ARNT.01
Family: V$HIFF
Matrix Similarity: 0.95
Position: 1349 - 1365
Genomic Position: chr 17: 33781211 - 33781227
Sequence: cgcgaacaCGTGgagcg

Matrix: V$CLOCK_BMAL1.01
Family: V$HIFF
Matrix Similarity: 0.94
Position: 1350 - 1366
Genomic Position: chr 17: 33781210 - 33781226
Sequence: gcgctcCACGtgttcgc

Matrix: V$HIF1.02
Family: V$HIFF
Matrix Similarity: 0.931
Position: 1362 - 1378
Genomic Position: chr 17: 33781198 - 33781214
Sequence: agcgcaccCGTGggcag

Figure S3. Related to Figure 3. (A) Mouse *Anptl4* promotor regions (Genomatix). HIFF family binding sites are depicted by green boxes. (**B-D**) Primers for the ChIP assay covered the regions marked with a red circle (*Angptl4*-HRE1-3, **see Figure 3**).

Figure S4

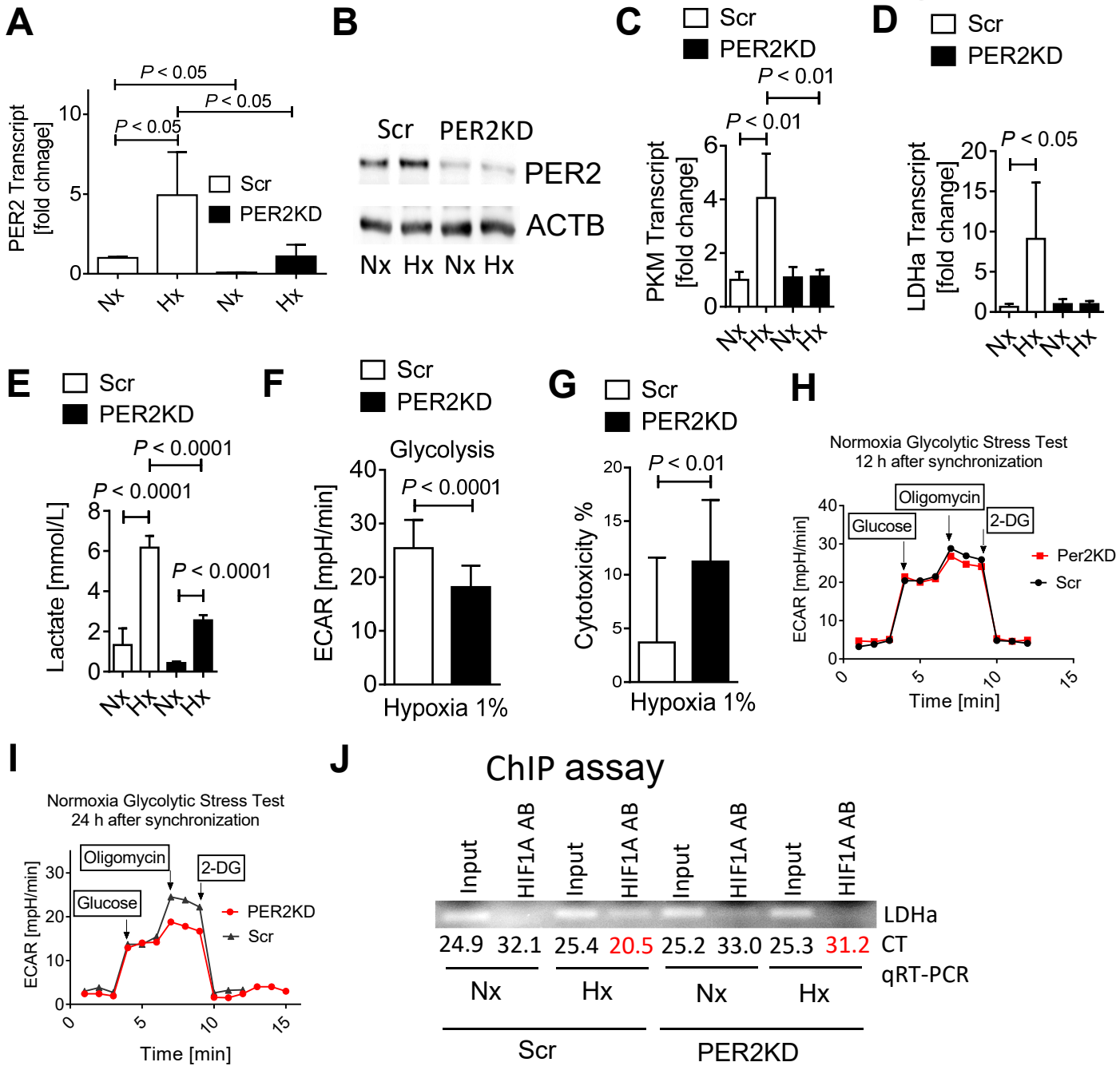


Figure S4. Related to Figure 4. HMEC-1 controls (Scr; treated with lentiviral scrambled shRNA) or HMEC-1 PER2 knockdown (KD; treated with lentiviral PER2 shRNA) were synchronized via serum starvation and exposed to 24 h of normoxia (Nx) or 1% hypoxia (Hx) in all experiments unless specified otherwise. (**A, B**) PER2 transcript or protein levels (mean±SD, n=3). (**C, D**) Transcript expression of pyruvate kinase (PKM) or lactate dehydrogenase (LDHa) (mean±SD, n=3). (**E**) Lactate levels in cell supernatants (mean±SD, n=3). (**F**) Glycolytic stress test (mean±SD, n=10). (**G**) LDH-Cytotoxicity (mean±SD, n=10). (**H,I**) Glycolytic stress tests 12 or 24h after cell synchronization (mean±SD, n=10) under normoxic conditions. (**J**) Chromatin immunoprecipitation (ChIP) analysis to detect HIF1A protein binding to the human LDHa promoter. qRT-PCR for the human LDHa promoter was performed for quantification. PCR products analyzed on a 2% agarose gel (top, **not** quantitative) or quantitative CT values form the qRT-PCR are shown (bottom, n=3).

Figure S5

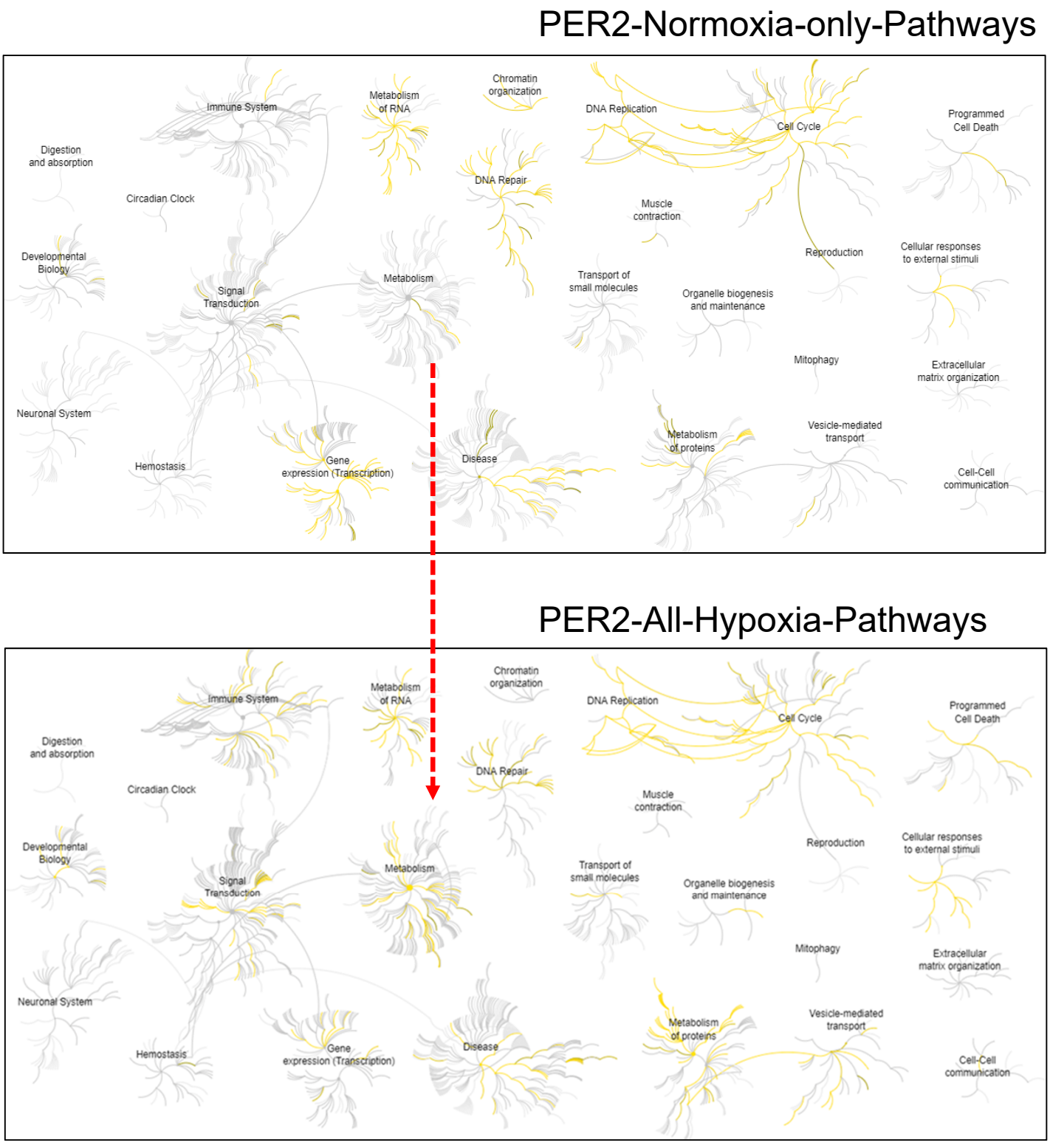


Figure S5. Related to Figure 4. PER2-Normoxia-Hypoxia-Pathways. *Reactome* analysis of an affinity purification–mass spectrometry-based proteomics from hypoxic HMEC-1 cells, indicating as strong involvement of PER2 in metabolic pathways under hypoxia. Yellow depicts PER2 pathways in comparison to all available *Reactome* pathways (grey).

Figure S6

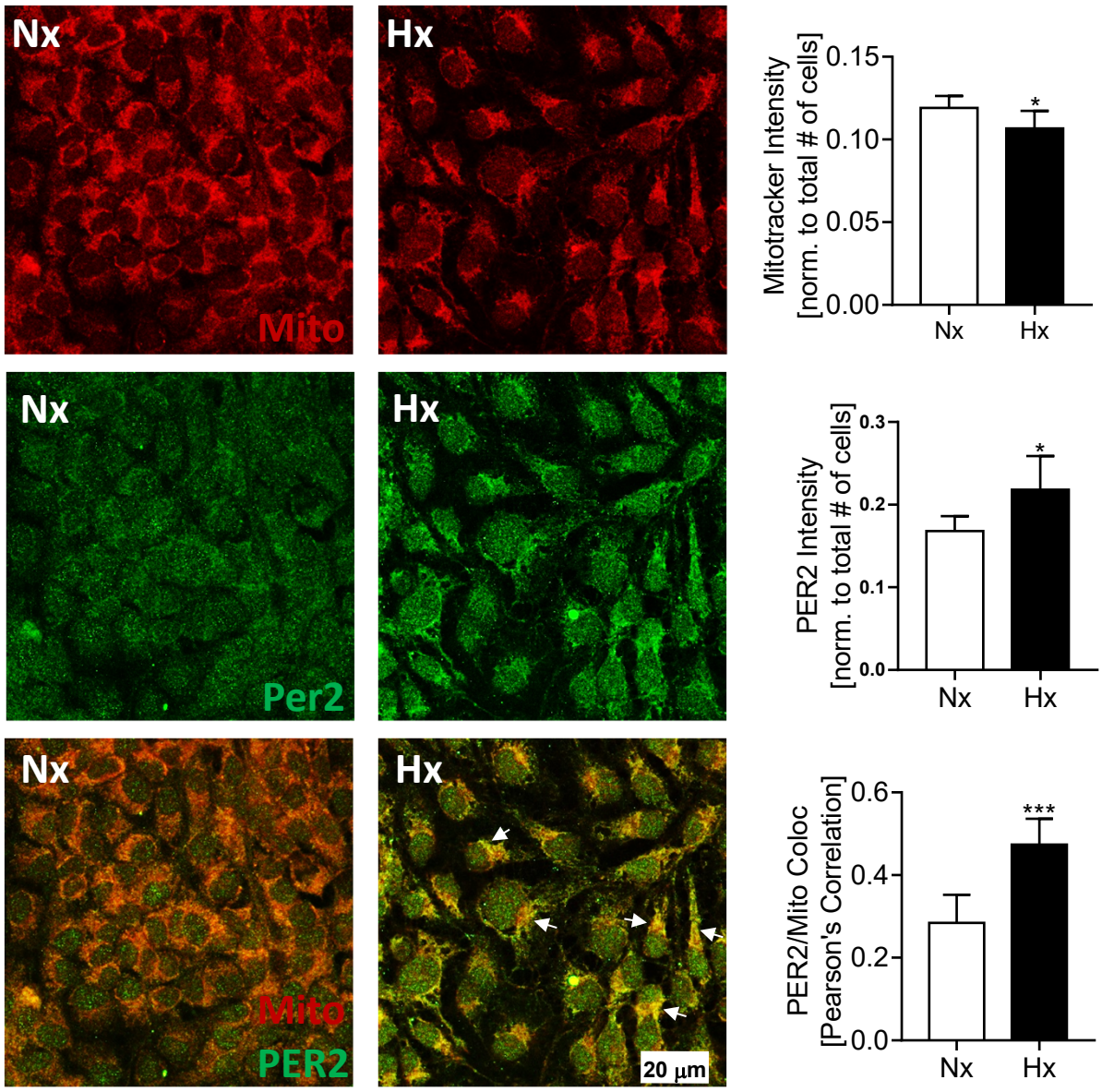


Figure S6. Related to Figure 4. Colocalization of PER2 and mitochondria (upper panel: MitoTracker Red CMXRos staining (red), middle panel: PER2 staining (green), lower panel: overlay MitoTracker Red and PER2 staining). Shown are staining from HMEC-1 cells after 24h of normoxia or 24h of hypoxia 1%. White arrows indicate mitochdrial translocation of PER2 (yellow, mean±SD, n=6, *=P<0.05, ***= P<0.001).

Figure S7

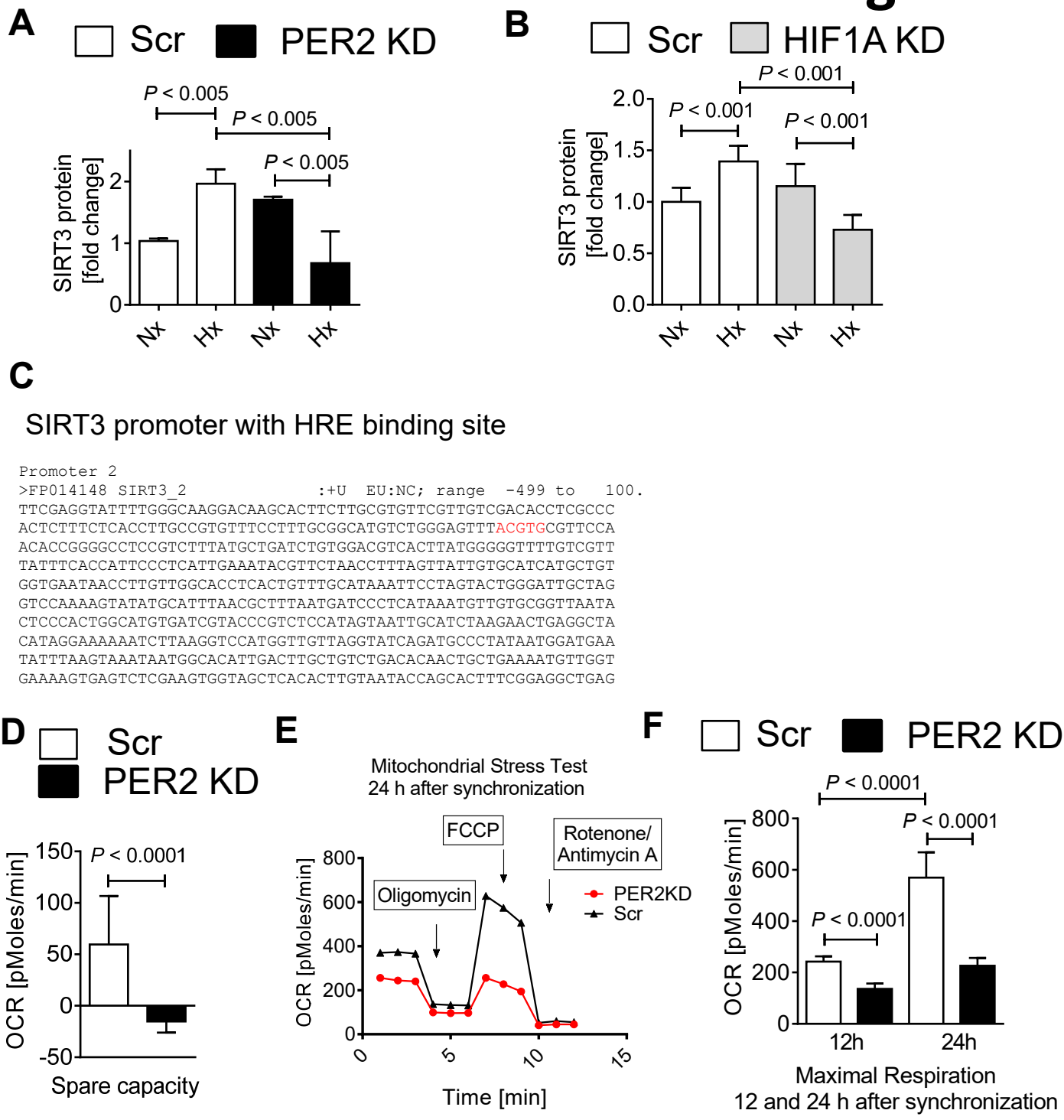
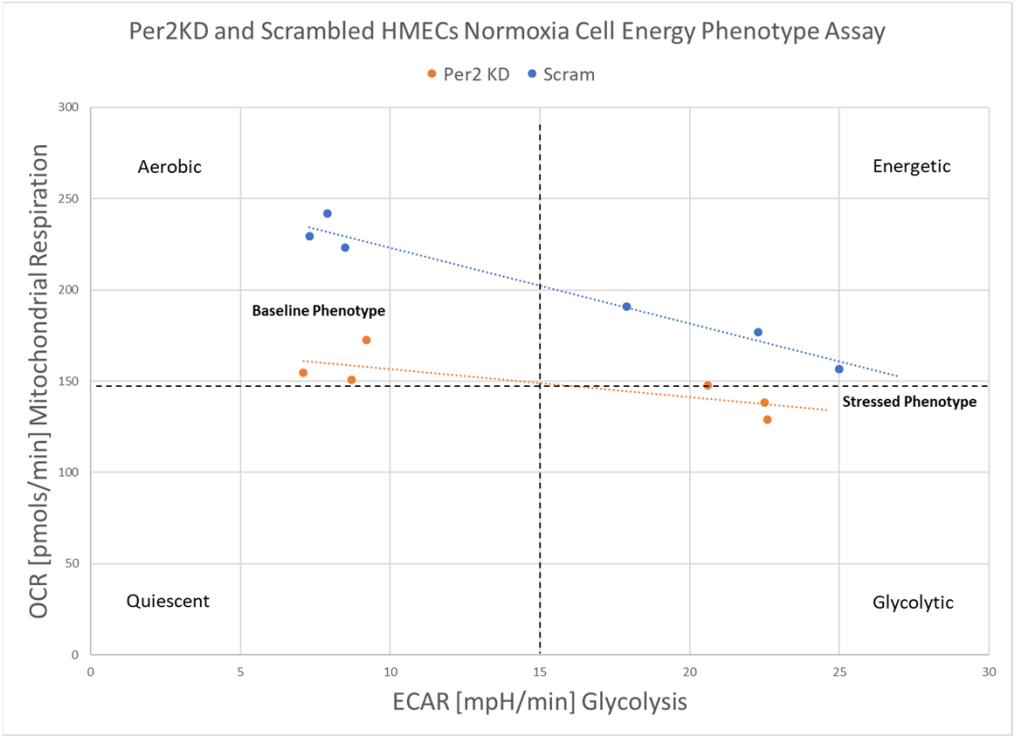


Figure S7. Related to Figure 4 and Figure 5. (A) Quantification of SIRT3 protein from PER2 KD or Scr control HMEC-1 in Hx (hypoxia 1%) or Nx (normoxia; mean±SD, n=5). **(B)** Quantification of SIRT3 protein from HIF1A KD or Scr control HMEC-1s in Hx or Nx (mean±SD, n=5). **(C)** Region of the human SIRT3 promoter containing a hypoxia response element (HRE) binding site (red). **(D)** Mitochondrial stress test in PER2 KD or Scr control HMEC-1 measuring spare capacity 12 h after cell synchronization (mean±SD, n=10). **(E, F)** Mitochondrial stress test administered at 24h after cell synchronization in PER2 KD or Scr control cells (mean±SD, n=5). Differences in maximal respiration between time point 12 and 24h are quantified in (**F**).

Figure S8

A



B

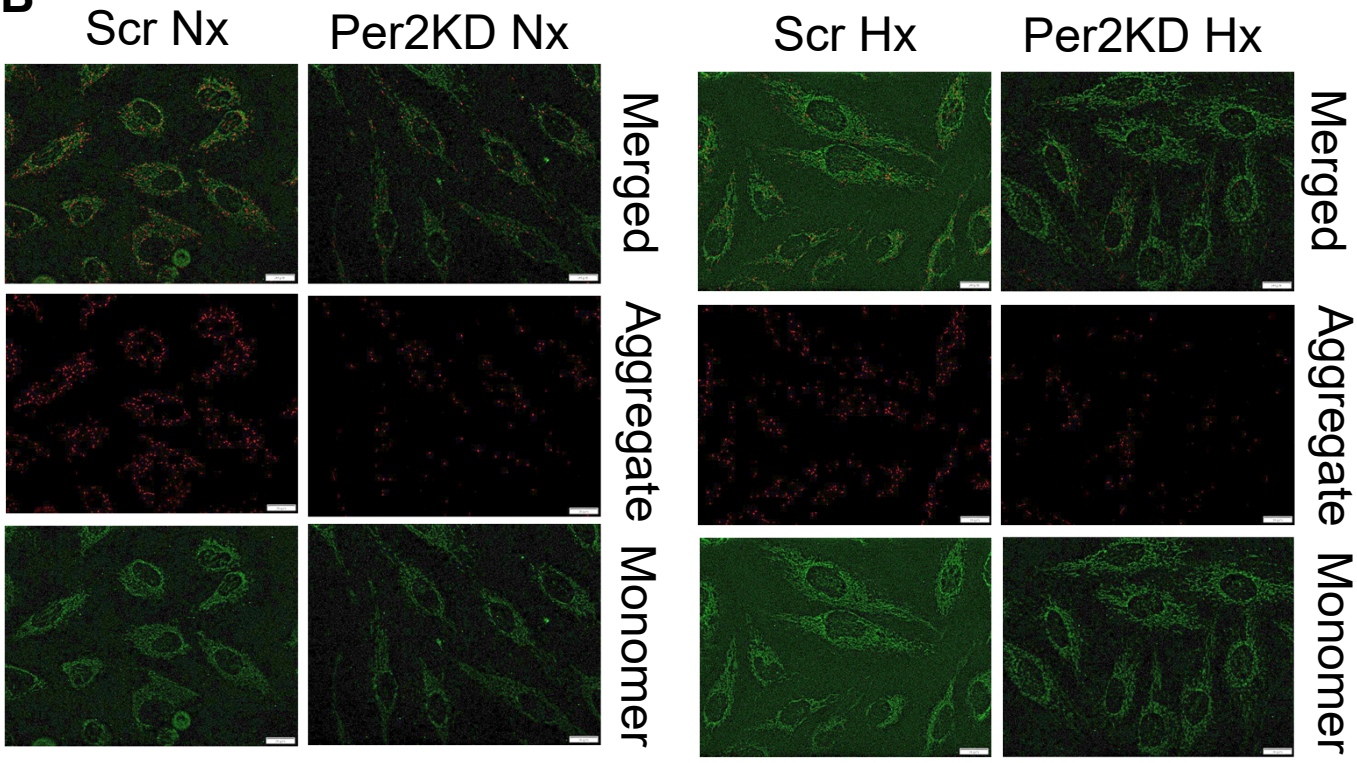
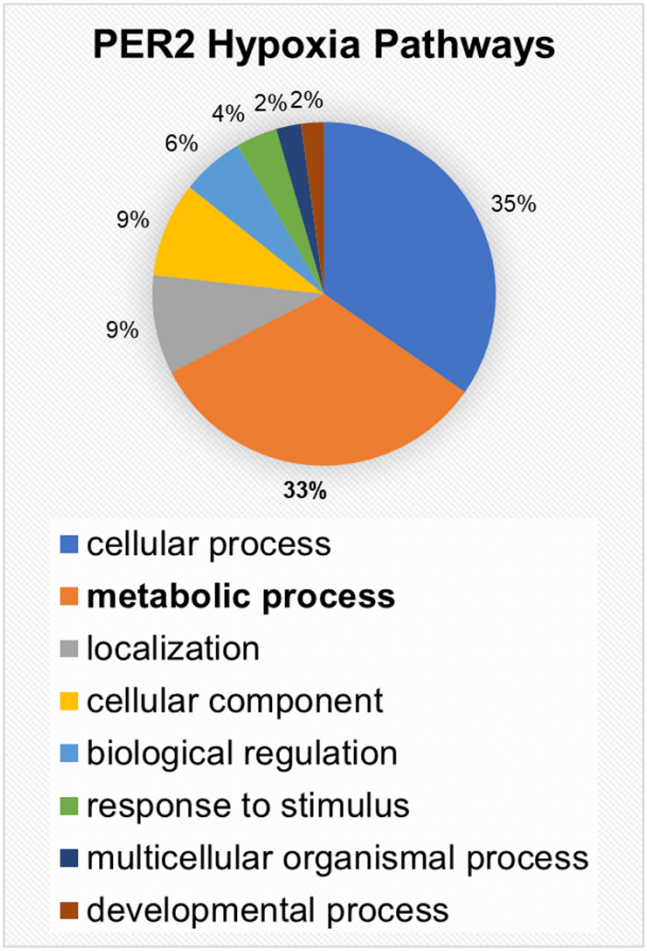
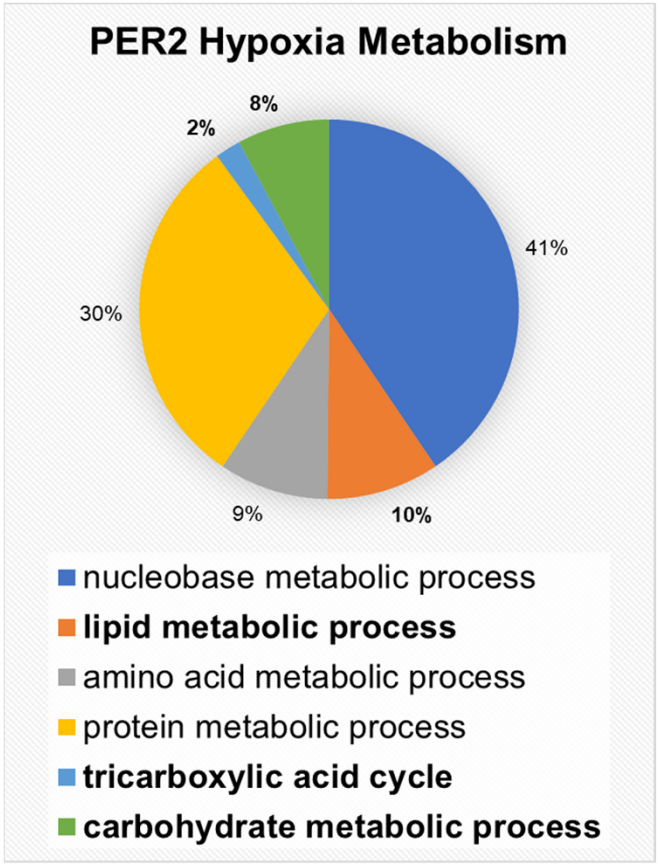
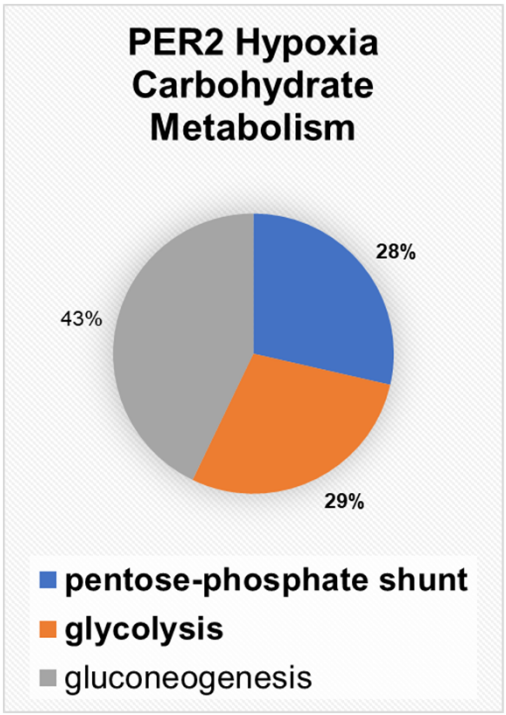


Figure S8. Related to Figure 5. (A) Cell energy phenotype test using the Seahorse Bioanalyzer in PER2 KD or Scram control HMEC-1 at baseline. Quiescent phenotype = cell not energetic for either metabolic pathway; energetic phenotype = cell uses both metabolic pathways; aerobic phenotype = cell uses predominantly mitochondrial respiration; and glycolytic = cell uses predominantly glycolysis (mean±SD, n=10). (**B**) JC-1 staining results from PER2 KD or Scr control HMEC-1s in Hx (hypoxia, 1%) or Nx (normoxia). Aggregate represents hyperpolarized cells and monomer represents depolarized cells (mean±SD, n=3, white scale bar=20μm).

Figure S9







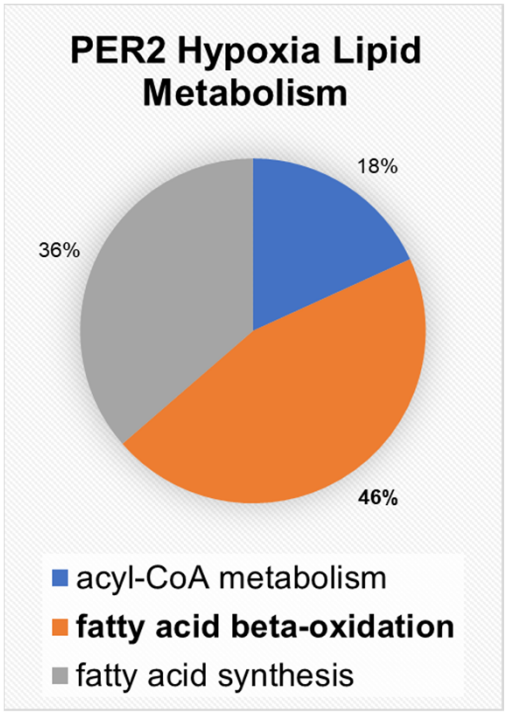


Figure S9. Related to Figure 5. PER2-Hypoxia-only-Pathways. PANTHER (Protein ANalysis THrough Evolutionary Relationships) analysis of an affinity purification–mass spectrometry-based proteomics from hypoxic HMEC-1 cells.

Figure S10

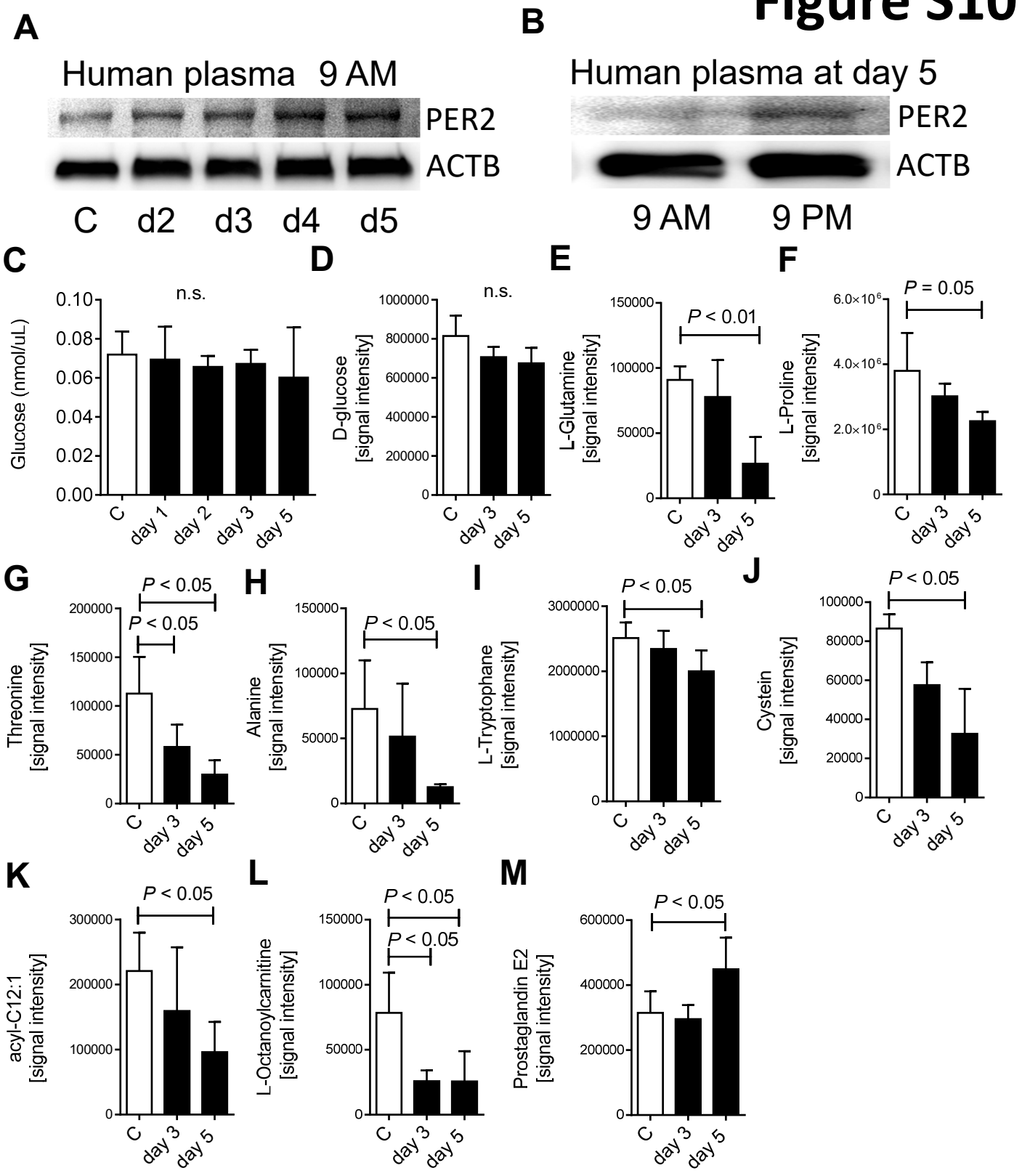


Figure S10. Related to Figure 7. (**A, B**) Immunoblots of plasma PER2 at 9AM and 9PM from one healthy human volunteer exposed to 5 days of intense light for 30 min between 8.30 and 9.00 AM. (**C**) Plasma glucose levels from human healthy volunteers during 5 days of intense light therapy. (**D-M**)Targeted metabolomics in plasma samples from healthy human volunteers exposed to 5 days of 30 minutes intense light from 8.30 to 9.00 AM each morning; (mean±SD; n=3).

Figure S11

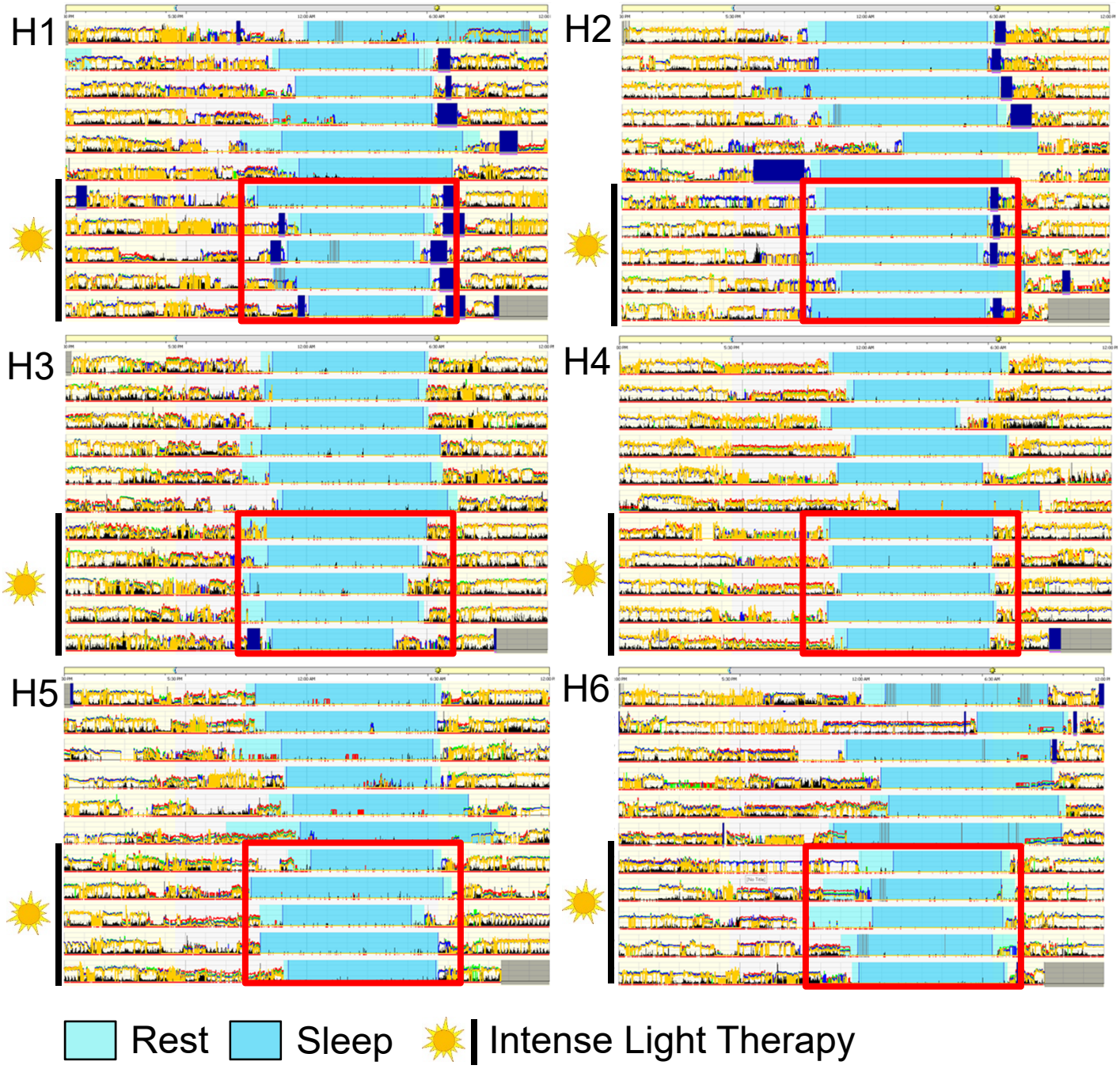


Figure S11. Related to Figure 7. Actigraphy data using a validated accelerometer (Actiwatch 2) from human healthy volunteers during 5 days without and 5 days with intense light therapy (30 min intense light from 8.30 – 9.00 AM; n=6, H=healthy volunteer; **Note**: synchronized sleep phases during intense light exposure [red square] vs no intense light therapy.