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Supplemental Information

The Circadian Clock Protein CRY1

Is a Negative Regulator of HIF-1α

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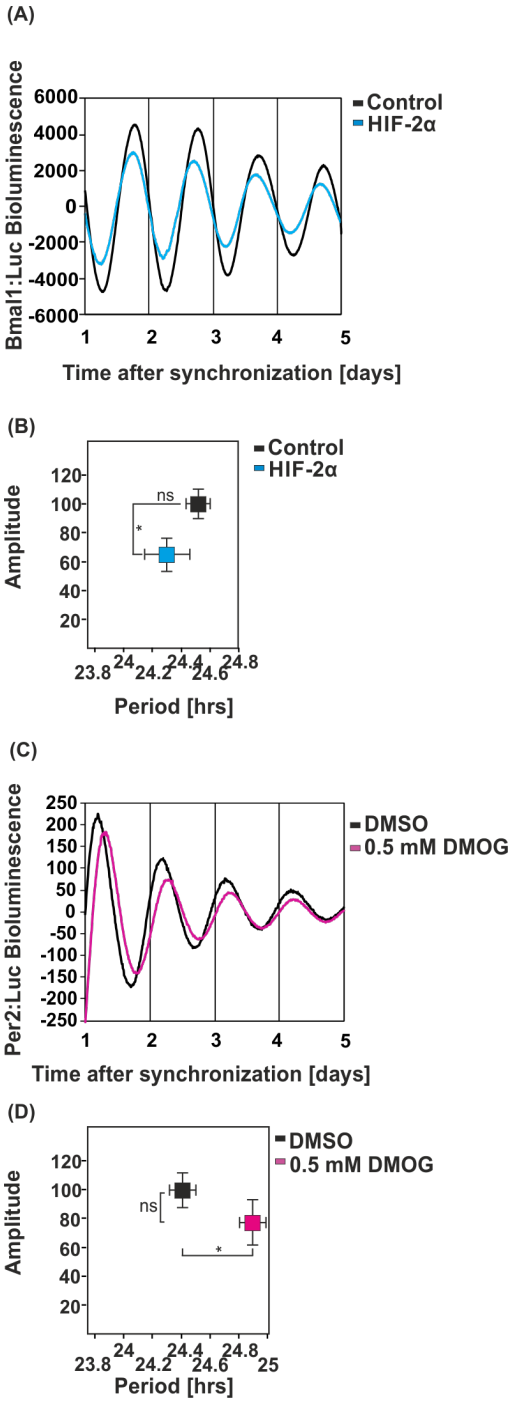

Figure S1 (refers to Fig.2)**. The circadian oscillation in NIH 3T3 cells is affected by HIF-2α and DMOG.** (A) Representative examples of the bioluminescence oscillations in NIH 3T3 cells cotransfected with an mBmal1:luciferase reporter construct and either empty vector (black line) or HIF-2α (blue line). (B) Graphical illustration of the amplitude (y-axis) and period length (x-axis) upon overexpression of HIF-2α. Data are mean ±SEM. * $p < 0.001$ (C) Representative examples of the bioluminescence oscillations in NIH $3T3^{mPer2:Luc}$ cells treated with DMSO as a mock and 0.5 mM DMOG. (D) Graphical illustration of the amplitude (y-axis) and period length (x-axis) upon DMOG treatment. Data are mean ±SEM. *$p < 0.05$.

Figure S1

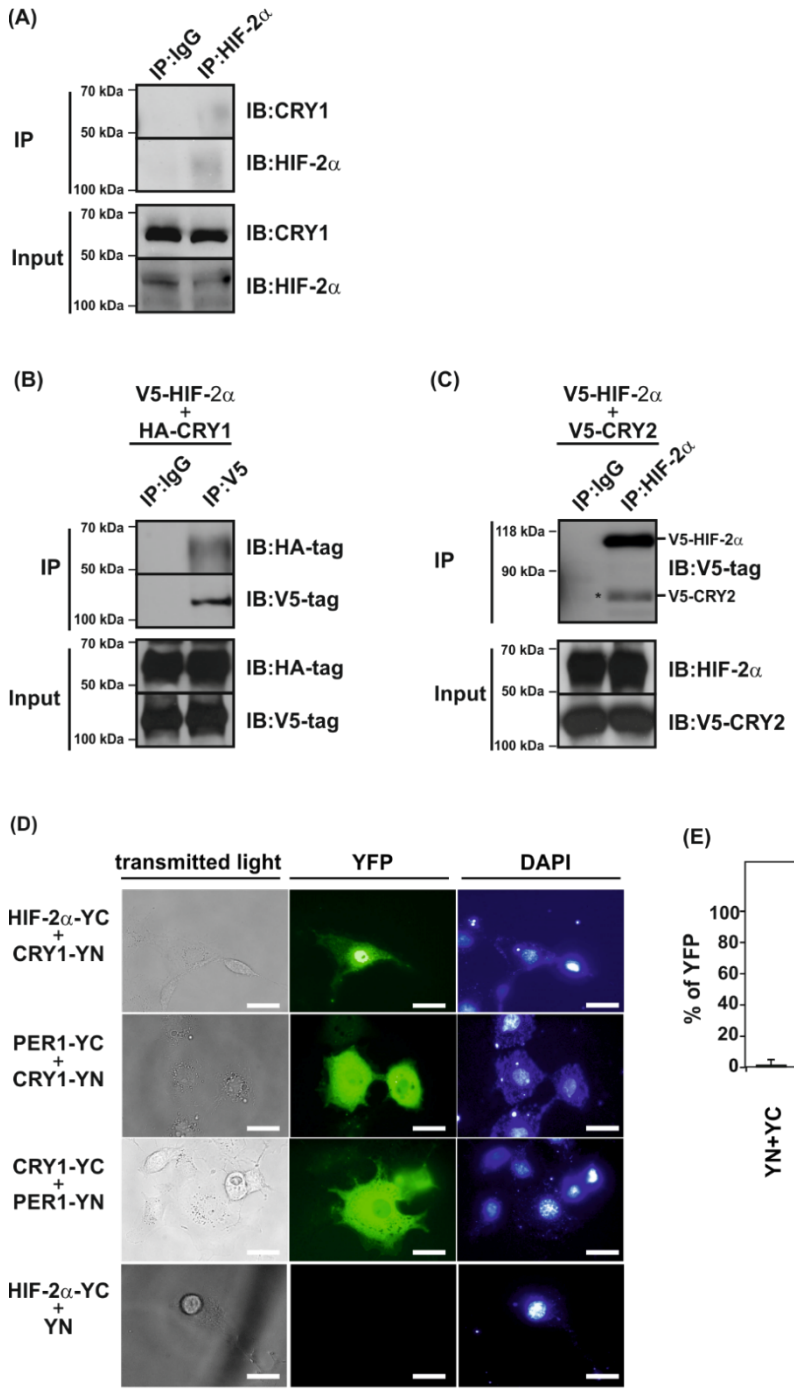

Figure S2

Figure S2 (refers to Fig.4)**. CRY1 interacts with HIF-2α.** (A) Coimmunoprecipitation assays (IPs) from wild-type (WT) MEFs cultured under hypoxia in the presence of MG-132. Blots from anti-HIF-2α IPs were probed with the CRY1 and HIF-2α antibody. The blots shown are representative of two independent experiments. (B, C) Co-immunoprecipitation assays with HEK-293 cells, expressing HIF-2α with either HA-tagged CRY1 or V5-tagged CRY2. (B) Blots from anti-V5 tag IPs were probed with HA-tag and V5-tag antibody. (C) Blots from anti-HIF-2α IPs were probed with V5-tag antibody (C*) Please note that both plasmids are V5-tagged. Therefore, the IPs were done with HIF-2α Abs and probed with anti V5-tag Abs thus resulting in recognition of both overexpressed proteins. (D) BiFC analysis. COS-7 cells cotransfected with the expression vectors for HIF-2α-YC, CRY1-YN, CRY-YC, PER-YN or PER-YC and a vector encoding only YN were cultured on glass slides for 24 hrs. The fluorescence detection was performed using specific filter sets for YFP and DAPI.
Scale bar: 10 µm. (E) Quantification of the BiFC signal. Cells were transfected as in (D) and quantified by flow cytometry (cf. Materials and Methods). The CMV-YFP signal was set to 100%. Data are mean ±SEM. * $p < 0.05$.

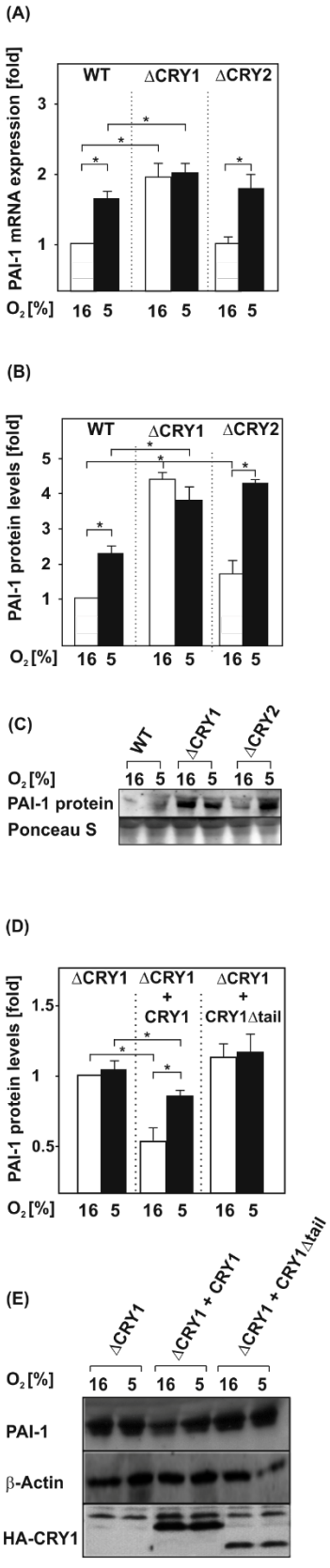

Figure S3 (refers to Fig.7A,B)**. CRY1 but not CRY2 modulates hypoxia-dependent transcription of PAI-1**. Wild-type (WT) MEFs, or MEFs deficient for CRY1 (ΔCRY1) or CRY2 (ΔCRY2) were cultured under normoxia (16% O_2) and hypoxia (5% O_2). The PAI-1 mRNA and protein levels were measured after 4 hrs and 24 hrs by qRT-PCR and Western blot, respectively. (A) Quantitative qRT-PCR data. (B) Quantification of the data from Western blots representatively presented in (C). The values under normoxia were set to 1. Values represent means ± SEM of at least three independent experiments. Statistics, Student's t-test for paired values: *significant difference, $p \leq 0.05$. (D, E) CRY1-deficient cells were transfected with a plasmid encoding CRY1 or CRY1-Δtail. The PAI-1 protein was measured by Western blot with an antibody against PAI-1. The values under normoxia were set to 1. Values represent means ± SEM of at least three independent experiments.

Statistics, Student's t-test for paired values: *significant difference, $p \leq 0.05$.

Figure S3

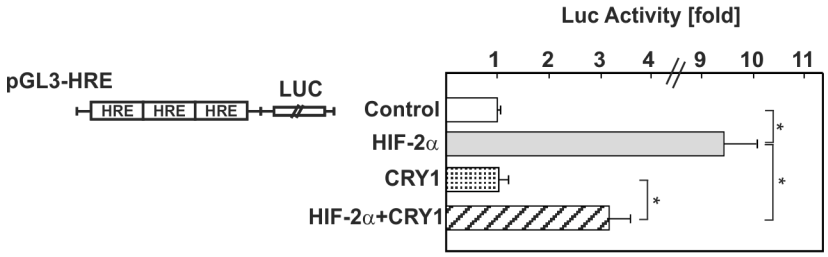

Figure S4

Figure S4 (refers to Fig.7C)**. HIF-2α-dependent transactivation of the HRE-driven promoter Luc construct is downregulated by CRY1.** Luciferase gene constructs containing 3 HIF-binding HREs (pGL3-HRE) in front of the SV40 promoter were cotransfected with expression vectors encoding CRY1, HIF-2α or both in combination. Cells were cultured under normoxia (16% O_2) and hypoxia (5% O_2) for 24 hrs before luciferase assay; In each experiment the Luc activity of pGL3-HRE transfected cells at 16% O_2 was set to 1. Values are means ± SD of four independent culture experiments, each performed in duplicate. Statistics, Student's t-test for paired values: *significant difference, $p \leq 0.01$.

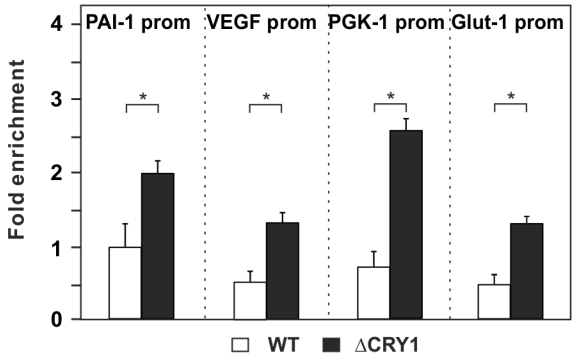

Figure S5

Figure S5 (refers to Fig.7D)**. Deficiency of CRY1 favors binding of HIF-2α to HRE-containing promoters.** ChIP-qPCR analyses were performed in wild-type or CRY1-deficient MEFs, cultured under hypoxia for 16 hrs; DNA fragments were co-precipitated with antibodies against mouse HIF-2α and amplified by qPCR using primers specific for the mouse PAI-1, VEGF-A, PGK-1 and GLUT-1 promoters. Chromatin immunoprecipitation without antibody or using IgG instead of antibody served as an additional specificity control. Differences in HIF-2α DNA binding efficiency in wild-type and ΔCRY1 MEFs were calculated by the fold enrichment method relative to the IgG control. Values are means ± SEM of three independent experiments. *significant difference, $p \leq 0.05$.

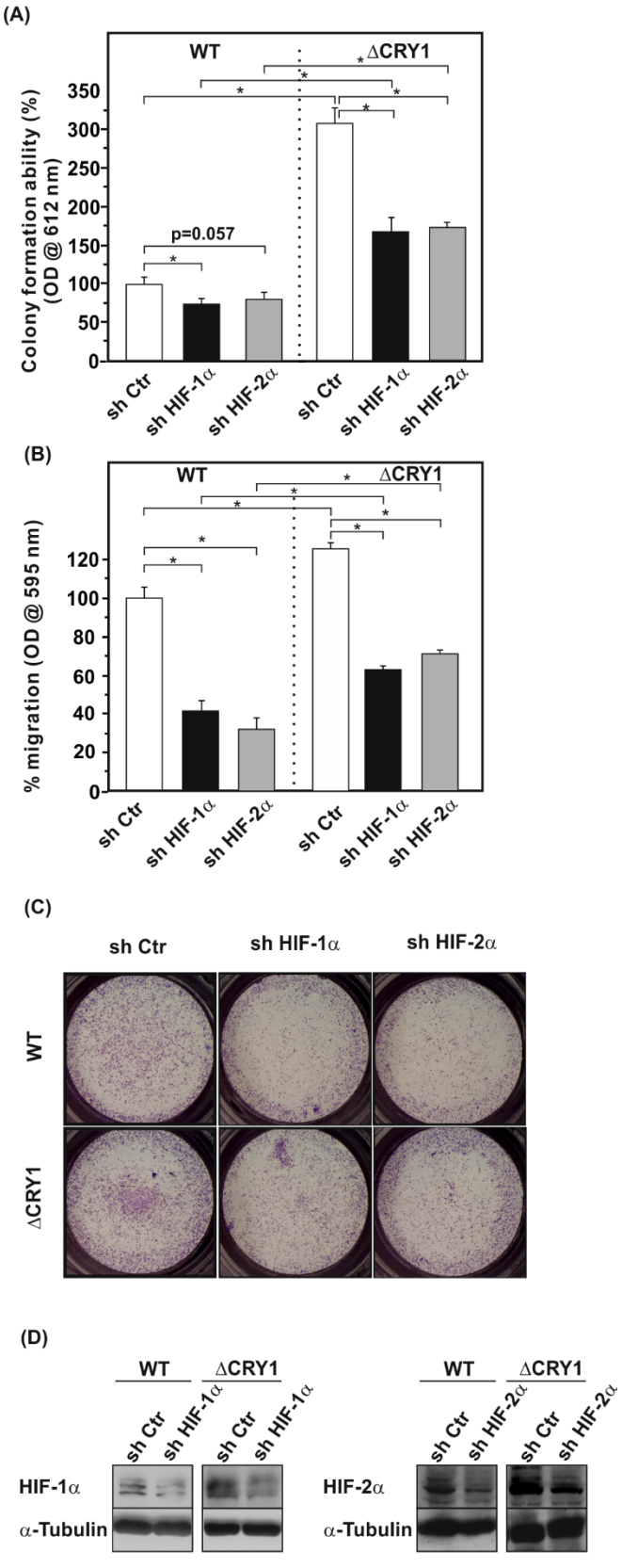

Figure S6 (refers to Fig.10)**. CRY1 and HIFs have opposite roles on cell proliferation and migration.** (A-D) Wild-type (WT) and CRY1-deficient (ΔCRY1) MEFs were transduced with retroviral particles expressing a scrambled non-coding shRNA (shCtr), a shRNA against HIF-1α or shRNA against HIF-2α. (A) Soft agar colony formation. Cells were plated onto soft agar and allowed to grow under hypoxia for 2 weeks. After staining with Resazurin the absorbtion was measured at an excitation wavelength of 584 nm and emission at 612 nm. The OD of the WT cells were set to 100%. Values represent means ± SEM of at least three independent experiments. (B) Transwell migration assay. Cells were seeded into Transwell chambers and incubated under hypoxia overnight. After staining with crystal violet the absorbtion of migrated cells at 595 nm was quantified. The OD of the WT cells was set to 100%. Values represent means ± SEM of at least two independent experiments. Statistics, Student's t-test for paired values: *significant difference, $p \leq 0.05$. (C) Representative photographs of a transwell migration assay. (D) Representative HIF-1α and HIF-2α Western blots in WT and ΔCRY1 MEFs transduced with respective HIF shRNA expressing retroviral particles.

Figure S6

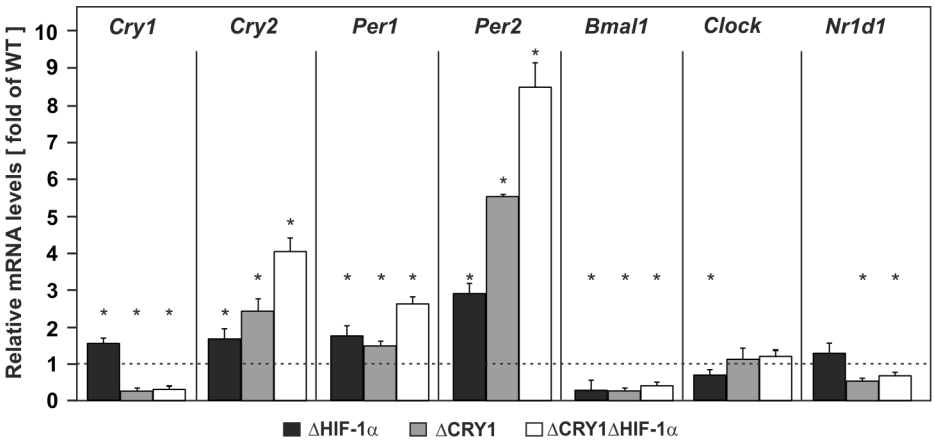

Figure S7

Figure S7 (refers to Fig.10)**. Lack of HIF-1α and CRY-1 regulates expression of clock genes.** Quantification of Cry1, Cry2, Per1, Per1, Bmal1, Clock, and Ndr1d1 mRNA levels in MEFs lacking either Cry1 (ΔCRY1), HIF-1α (ΔHIF-1α) or both (ΔCRY1ΔHIF-1α). The respective mRNA levels were assessed by qRT-PCR and the mRNA levels in wild type (WT) MEFs were set to 1; the values indicate fold-change WT vs the respective knockout. Data are mean ±SD (n=3), * significant difference WT vs. knockout.

Transparent Methods

All biochemical substances and enzymes used were of analytical grade obtained from commercial suppliers.

Animal experiments and monitoring of circadian behavior

All animal experiments were performed according to protocols approved by the National Animal Experiment Board of Finland following the regulations of the EU Directive 86/609/EEC, the European Convention ETS123 and the national legislation of Finland. Inbred C57BL/6N male mice were housed under standard conditions and fed *ad libitum*. For monitoring total physical activity (XT) of 2-month-old C57BL/6N mice under normoxia and hypoxia an automated infrared analyzing system for small animals (LabMaster, TSE Systems GmbH, Bad Homburg, Germany) was used. Before the real experiment the mice were housed individually for 7 days in training cages similar to those used in the actual measurements. Animals were maintained in a cycle of 12 hrs light and 12 hrs darkness (LD) or in continuous darkness (DD) in constant ambient temperature with water and food available *ad libitum*. The activity data were recorded continuously (every 10 min) for 2 weeks under normoxic or hypoxic conditions (17% O_2). All data are presented as a mean ± SEM, $p \leq 0.05$. Activity records were plotted as actograms (http://www.circadian.org/softwar.html) and the period of locomotor activity was determined by the cosinor and chisquare method. One-way Anova with post-hoc Tukey HSD Test, Mann Whitney test as well as Student's t-test were used to make statistical comparisons between the different conditions.

Cell Culture

HeLa, HepG2, COS-7 and HEK-293 cells were cultured in MEM (Sigma-Aldrich); mouse embryonic fibroblasts (MEFs) were cultured in DMEM (Sigma-Aldrich); NIH 3T3 and NIH 3T3 cell stably expressing a Per2:Luc construct (kindly provided by Hiroki R. Ueda [Isojima et al., 2009]) (were maintained in DMEM-Ham-F10 (1:1, Lonza); All cell culture media were supplemented with 10% fetal bovine serum (Biochrom), 1% nonessential amino acids (PAA Laboratories) and 0,5% antibiotics. The cells were cultured in a normoxic atmosphere of 16% O_2, 79% N_2, and 5% CO_2 [by volume] or a hypoxic atmosphere of 5% O_2, 90% N_2, 5% CO_2 [by volume], or in an InVivo2 400 hypoxia work station (Ruskinn Technologies).

Plasmids

For luminescence measurements the pGl4.11-Bmal1:luciferase (Bmal1:Luc) construct (kindly provided by Dr. U. Schibler, Geneva) (Brown et al., 2005), the reporter plasmids pGL3-hPAI-806/+19, containing the human PAI-1 promoter 5'-flanking region from -806 to +19 and the mutant pGL3-hPAI-806HREm (Dimova et al., 2005) as well as the pGL3-HRE and pGL3-HREm luciferase reporter plasmids containing 3 repeats of a wild-type HRE or mutant HRE, respectively, (Liu et al., 2004) were used as a reporters.

The expression plasmids encoding full-length HIF-1α with mutations in proline 402, proline 564, and asparagine 803 as well as full-length HIF-2α were already described (Flügel, Görlach & Kietzmann, 2012). The expression plasmids encoding HA-tagged wild-type CRY1 and HA-tagged CRY1 mutants HA-CRY1mutNLSc (with mutations in a bi-partite nuclear localization signal domain), HA-CRY1Dtail (deletion of the C-terminal tail), HA-CRY1ΔCC (deletion of the coiled-coil (CC) domain), HA-CRY1ΔCCmutNLSc (deletion of the CC domain and mutation in NLS), HA-CRY1ΔCCtail (deletion of the CC domain and tail), HA-CRY1ins 487 were already described (Chaves et al., 2006). The expression vectors for ARNT in p3XFLAG-Myc-CMV™-24 (Sigma-Aldrich) as well as the constructs for BiFC assays were cloned during the course of this study.

The wild-type human HIF-1α cDNA cloned into pCMV-Myc (Clontech) (Flugel et al., 2007) was used as a template to generate expression constructs for various HIF-1α mutants by using the OuickChange mutagenesis kit (Stratagene) followed by digestion and re-ligation (primer sequences are listed in Table S1). The HIF-1α variants are: bHLH (containing HIF-1α amino acids 1-80 encompassing the bHLH domain), bHLH-PAS A (containing HIF1α amino acids 1-200 and lacking PAS B, ODD, ID and CAD), bHLH-PASA-PASB (containing HIF-1α amino acids 1-350 and lacking ODD, ID and CAD), bHLH-PASA-PASB-ODD (containing HIF-1α amino acids 1-603 and lacking ID and CAD), bHLH-PASA-PASB-ODD-ID (HIF-1α amino acids 1-786 lacking the CAD), bHLH-ODD-ID-CAD (lacking PAS A and PAS B domains) and PAS A-PAS B-ODD-ID-CAD (lacking the bHLH domain). The constructs for production of retroviral particles expressing shRNA against Mus musculus HIF-1α (# TG517255), HIF-2α (# TF500609) and scrambled negative shRNA (# TR30013) were purchased from OriGene Technologies Inc. (Rockville, MD). The CRISPR-Cas9 backbone plasmid, pSpCas9(BB)-2A-GFP (PX458) (Addgene #48138) was a generous gift from Dr. Feng Zhang (Ran et al., 2013)

RNA preparation and quantitative real-time PCR

Isolation of total RNA was performed using the Qiagen RNeasy® Mini Kit (Qiagen, Switzerland) and with a GenElute mammalian total RNA miniprep kit (Sigma-Aldrich) following the manufacturer's instructions. Reverse transcription was conducted with 1 µg of total RNA and qScript cDNA Synthesis kit (Quanta Bioscience, GE Healthcare). Quantitative real-time PCR was performed in duplicate or triplicate using an iTaq Universal SYBR Green Supermix reaction kit (Biorad, Finland) and Applied Biosystems 7500 Real-Time PCR System (Life Technologies, Finland). All primer sets (Table S1) were validated for their product and amplification efficiency using standard dilution analysis and melting curve analysis. β-Actin, 18S rRNA and Hprt (hypoxanthine-guanine phosphoribosyltransferase) were used as internal controls to normalize the variability in expression levels. The relative quantification of gene expression was determined using the ΔΔCt method (Schmittgen, Livak, 2008, Livak, Schmittgen, 2001).

Transfections and real-time bioluminescence

HepG2 cells, 4 x 10^5 per 60-mm dish were transfected essentially as described (Immenschuh et al., 1998). In brief, 2 µg of the appropriate promoter Firefly luciferase (Luc) constructs were cotransfected in duplicate with 0,5 µg of the respective expression vector for HIFs, ARNT or CRY1, or with empty vector in the controls. After 5 hrs the medium was changed, and the cells were cultured under normoxia and/or hypoxia as indicated.

For real-time monitoring of the circadian oscillations in cell cultures, cell medium was buffered with 25 mM HEPES containing 0.1 mM luciferin (Sigma-Aldrich) for 2 days prior measurement. After synchronization of intracellular clocks by treatment of confluent cultures with 10 µM forskolin, plates were sealed and the bioluminescence was recorded for at least 5 days (75 sec measurements at 10 min intervals) with a LumiCycle 32-channel automated luminometer (Actimetrics). The data were analysed with the Actimetrics software using the running-average method and two sample comparisons were done using a paired Students t-test.

Western blot analysis, co-immunoprecipitations and HIF-1α protein half-life studies

Western blot analysis was carried out as described (Immenschuh et al., 1998). In brief, media or total cellular lysates were collected, and 100 µg of protein was loaded onto a 10% or 7.5% SDS-polyacrylamide gel and, after electrophoresis and blotting, probed with a primary monoclonal antibody directed against human HIF-1α (1:2000, Novus, Littleton, USA), myc-tag (1:1000, Cell Signaling, Frankfurt/M, Germany), or with a primary polyclonal antibody against HA-tag (1:500, Santa Cruz, Heidelberg, Germany), mouse HIF-1α (1:2000, Novus, Littleton, USA), CRY1 (H-84; 1:1000; Santa Cruz Biotechnology), Golgi membrane (1:10000; Biosciences, Goettingen, Germany), β-actin (1:10000, Sigma-Aldrich) and α-tubulin (1:10000, Sigma-Aldrich). The enhanced chemiluminescence system (ECL; Amersham Biosciences) was used for detection.

Co-immunoprecipitation experiments were performed either in hypoxic wild-type MEFs or in HEK-293 cells transiently transfected with full-length HA-tagged CRY1, V5-tagged CRY2, myc-tagged HIF-1α or V5-tagged HIF-2α. Twenty hours post-transfection total cellular protein extracts were isolated and 150 µg protein extracts were cleared with 2 µg of antibody pre-coupled to protein-G-Sepharose (Amersham, Freiburg, Germany). The samples were then subjected to Western blot analyses using anti-HIF-1α, anti-HIF-2α, anti-CRY1, anti-myc-tag, anti-V5tag or anti-HA-tag antibodies. HIF-1α half-life studies were performed under hypoxia in wild-type and ΔCRY1 MEFs treated with cycloheximide (10 µg/mL; Sigma-Aldrich). After harvesting the cells at the indicated time points, the endogenous HIF-1α protein levels were measured by Western blot.

BiFC analyses

For BiFC assays, pCMV-HA (Clontech) was used as a backbone for generation of the pCMV-YN, pCMV-YC, pCMV- HIF-1α-YC, pCMV-CRY1-YN and pCMV-ARNT-YN constructs; their correctness was proven by DNA sequencing. In brief, the sequences encoding HIF, ARNT and CRY were fused to sequences encoding YFP residues 1–154 (YN) or residues 155–238 (YC). The coding regions were connected by linker sequences encoding RSIAT (YN) or RPACKIPNDLKQKVMNH (YC) and were fused to the amino-terminal HA epitope tag in pHA-CMV (Clontech).

COS-7 cells were cultured in 6-well plates on glass slides to about 50% confluence and cotransfected with expression vectors pCMV-HIF-1α-YC and either pCMV-CRY1-YN, pCMV-ARNT-YN or pCMV-YN (2,5 µg each). The fluorescence was observed 24 h post-transfection using an inverted fluorescence microscope (Carl Zeiss AxioVert 200M). YFP fluorescence was captured using an excitation wavelength of 500 nm and an emission

wavelength of 535 nm. The nuclei of fluorescent cells were stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI).
Quantification of the BiFC Signal was perfomed by flow cytometry. In brief, cells were transfected as above and 48 hrs post transfection cells were washed with PBS and harvested by trypsin-EDTA. Cells were then pelleted by centrifugation at 1000 rpm for 2 min. The cell pellet was resuspended in 1.5 ml of PBS. The BiFC signal was quantified using the CyFlow Space flow cytometer equipped with an appropriate filter set for YFP. In each case, triplicates of 5000 cells were counted and used to calculate the average BiFC signal intensity in each sample using the FloMAX software. The fluorescence for maximal fluorescence in each experiment, against which The BiFC signal intensities were normalized against the signal from full-length YFP which was set to 100%.

Chromatin immunoprecipitation
ChIP analyses were carried out according to the protocol for fast ChIP (Nelson, Denisenko & Bomsztyk, 2006). In brief, confluent wild-type and DCRY1 MEFs cultured under normoxia or hypoxia for 16 hrs were crosslinked with formaldehyde, lysed, and sonicated to obtain DNA fragments in a size from 250 to 500 bp. Then, chromatin was precipitated with a mouse HIF-1α, HIF-2α antibody (Novus, Littleton, USA) or unspecific IgG. DNA from chromatin immunopreciptiations was analyzed by quantitative real-time PCR. Differences in the HIF-1α and HIF-2α DNA binding efficiency in wild-type and ΔCRY1 MEFs were calculated by the fold enrichment method relative to the IgG control using the formula $2^{-(Ct\ [IP] - Ct\ [IgG])}$.

Generation of HIF-1α knockout MEFs by CRISPR-Cas9-mediated genome editing
A 20-bp guide sequence targeting the third exon of mouse HIF-1α (HIF-1α-001, ENSMUST00000021530.7) was designed online using Zhang's laboratory web resource (www.genome-engineering.org); a non-targeting, scrambled sequence (OriGene) was used as a negative control. gRNA-encoding oligonucleotides (Sigma-Aldrich) were cloned into the vector SpCas9(BB)-2A-GFP (PX458, Addgene plasmid ID 48138) using standard procedures as described (Ran et al., 2013). The generation of the HIF-1α control and knockout cells via CRISPR-Cas9-mediated non-homologous end-joining (NHEJ) DNA repair and the screening was performed according to described guidelines (Ran et al., 2013). In brief, the wild-type and ΔCry1 MEFs were transiently transfected with either the genome editing or the scrambled CRISPR-Cas9 construct and 48 hrs post-transfection cells were subjected to single-cell-sorting (BD FACSAria™ III cell sorter). The single-cell clones were expanded and screened for frame-shift mutations; shortly, a region spanning the target site was amplified by PCR from genomic DNA isolated from clonal cell lines. PCR products were subsequently cloned into pUC19 (Invitrogen). 15-20 sequences were analyzed per clone by aligning them to the WT HIF-1α sequences using BLAST and Serial Cloner. All primer sequences are listed in Table S2.

Live Cell Imaging Assays
For real-time quantitative live-cell proliferation analysis, $5x10^3$ cells per well were seeded onto 96-well plates. Live phase contrast recording of cell confluence was performed in the IncuCyte® ZOOM System (Essen BioScience) for 72 h in 3 h intervals. For the scratch wound assays, $4x10^4$ cells per well were seeded onto 96-well Essen ImageLock Plates (Essen Bioscience) in the presence of a proliferation inhibitor (mitomycin, 1µg/ml). The following day the confluent cell monolayer was wounded with the 96 PTFE pin Wound Maker (Essen Bioscience) and the live wound closure was recorded for 45 h in 3 h intervals. In both assays, the confluence analyses were performed using the basic IncuCyte software settings.

Colony formation
Anchorage-independent growth was analyzed in a three-layer soft agar assay as described (Flügel, Görlach & Kietzmann, 2012). In brief, the $1x10^3$ cells per well were seeded in triplicates and incubated at 37°C for 14 days under normoxia and/or hypoxia before the colonies were visualized with resazurin or crystal violet. Cell growth was measured in a Fluroskan Ascent FL type 374 (Thermo Scientific) with an excitation wavelength of 584 nm and emission at 612 nm.

Transwell migration assay
Cell migration assays were conducted *in vitro* in transwell chambers (Becton Dickinson) as described (Flügel, Görlach & Kietzmann, 2012). In brief, serum-starved cells were seeded into the top chamber at a density of $2x10^4$ cells/well in 500 µl of serum-free DMEM; the bottom chamber was filled with 600 µl DMEM containing 10% FBS.

After 18 hrs in a humidified hypoxic incubator, cells were fixed and stained with crystal violet. The absorption of migrated cells at 595 nm was quantified.

Satistics

Statistical analyses were performed with GraphPad Prism version 5 (GraphPad Software). Parameters and experimental details can be found in the figure legends. The Student's t test with a p-value of at least $p \leq 0.05$ was considered statistically significant.

Table S1. (refers to Fig.6) **Primers used for generation of HIF-1α deletion constructs.** The wild-type human HIF-1α cDNA cloned into pCMV-Myc vector (Clontech) was used as a template for generating expression constructs for different HIF-1α mutants. The OuickChange mutagenesis kit (Stratagene) was used to introduce restriction enzyme sites and/or stop codon (underlined) and was followed by digestion and re-ligation. The antisense primers contained the reverse complementary sequence of the sense primer. The correctness of all designed deletion mutants was proven by DNA sequencing.

Construct	(bp)	Sequence (5'- 3') of the sense mutagenesis primers	Deleted domain	digest
bHLH	**243**	ctggatgctggtgattagggtaccgaagatgacatgaaagcacag	PAS A, PAS B, ODD, ID, CAD	*Kpn I*
bHLH-PAS A	**528**	gcgaagctttttctctgaatgtagggtaccctaactagccg	PAS B, ODD, ID, CAD	*Kpn I*
bHLH-PAS APAS B	**1215**	cctgatgctttaacttagctggtaccagccgctggagacac	ODD, ID, CAD	*Kpn I*
bHLH-PAS A-PAS B- ODD	**1830**	cagtattccagtagactcaggtaccagaacctactgctaatgccacc	ID, CAD	*Kpn I*
bHLH-PAS A-PAS B- ODD-ID	**2379**	ctgctggggcaatcataggatgaaagtggggtaccacagctgacc	CAD	*Kpn I*
bHLH-ODD-IDCAD	**1800**	gctatttgcgtgtgaggaagcttctggatgctggtg ggacaagtcaccacaggaaagcttaggatgcttgccaaaagagg	PAS A, PAS B,	*Hind III*
PAS A-PAS B-ODD-ID-CAD	**2238**	ggtgatttggatattgaagaggccatggaggcccagatgaa	bHLH	*Sfi I*

Table 2S. (refers to Fig.4, 7, 10) **Primer sequences**

Gene/ Construct	Forward (5'-3')	Reverse (5'-3')
qPCR primers		
Pai-1	GTGAATGCCCTCTACTTCAGTG	GCTGCCATCAGACTTGTGGAA
Hif1α	GGGGAGGACGATGAACATCAA	GGGTGGTTTCTTGTACCCACA
Hif2α	TCCTTCGGACACATAAGCTCC	GACAGAAAGATCATGTCACCGT
ActB	GTTGTCGACGACGAGCG	GCACAGAGCCTCGCCTT
18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Hprt	CGAAGTGTTGGATACAGGCC	GGCAACATCAACAGGACTCC

Cited2	CGCCAGGTTTAACAACTCCCA	TGCTGGTTTGTCCCGTTCAT
Glut1	CTCTGTCGGCCTCTTTGTTAAT	CCAGTTTGGAGAAGCCCATAAG
Cry1	CACTGGTTCCGAAAGGGACTC	CTGAAGCAAAAATCGCCACCT
Cry2	CACTGGTTCCGCAAAGGACTA	CCACGGGTCGAGGATGTAGA
Per1	TGAAGCAAGACCGGGAGAG	CACACACGCCGTCACATCA
Per2	GAAAGCTGTCACCACCATAGAA	AACTCGCACTTCCTTTTCAGG
Bmal1	TGACCCTCATGGAAGGTTAGAA	GGACATTGCATTGCATGTTGG
Clock	CTTCCTGGTAACGCGAGAAAG	GTCGAATCTCACTAGCATCTGAC
Nr1d1	ATGTCCCGAGAGCTACATGAC	CCTGCTCCTGAACATCGAACT
BiFC		
pCMV-YC	CGAATTCAAGCTTCTCGAGACGCGTATGGGTAC	CCATACGCGTCTCGAGAAGCTTGAATTCGGGCC
pCMV-YN	AATTCGGGCCCACGCGTGGTACCT	TAGAGGTACCACGCGTGGGCCCG
pCMV-CRY1YC	GAATTCAAGCATGGGGGTGAACGCCGTGC	GGCCCTCTAGAGTTACTGCTCTGCC
pCMV-HIF-1α -YC	CTGAAGCTTGCGGCCGCACGTCCGGCGTGCAAAATC	CATGCAAGCTTCGCGGGTACAATTCCGCAGCTTTTAG
ChIP primers		
Pai-1	GTCTAGACGACCGACCAGCCAAA	GAAATGTCTGGGCTGCCCGC
Pgk-1	GGCATTCTGCACGCTTCAA	GAAGAGGAGAACAGCGCGG
Glut-1	ATTTCTAAGGCCCTGGGTCC	CCTGCCTGATGCGTGTCA
Vegf-A	CAGTTGTCTCTCCTTCAGGGCT	GAAACCCACGTATGCACTGTGTA
ActB	GTGAGTGAGCGACGCGGAGCCAA	CTTACCTGGTGGCGGGTGTGGA
CRISPR-Cas9		
Hif1 (guide)	GCTAACAGATGACGGCGACA	
Hif1a (genotyping, sequencing)	CACCCTTGGTGATTTGCTTGT	CCTCATGGTCACATGGATGAGT
Hif1a	GATATGAATTCACCCTTGGTGATTTGCTTGT	CGATAAGCTTCCTCATGGTCACATGGATGAGT
(pUC19 cloning, sequencing)		
Rom1 (off-target)	GGTGGGAAGGGGAAAGATGG	ACAGTGATTTCTGTCCACAAACTT

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