Supplemental Materials and Methods

Cell Culture Conditions

U2OS, HEK293T and MEF cells were used in this study. HEK293T and MEF cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and penicillin-streptomycin in 35-mm or 10-cm plates. U2OS cells were cultured in McCoy's 5A medium with 10% FBS and penicillin-streptomycin in 35-mm or 10-cm plates. The cells were maintained in a humidified incubator at 37°C with 5% CO2. All live cell imaging studies were performed at 37°C with CO2. All cell lines generated in this study were derived from U2OS cells, which are originally derived from human bone osteosarcoma epithelial cells of a female patient.

For temperature entrainment, U2OS cells were seeded in 3.5 mm cell culture dishes at 20% confluency, were subjected to a 5-day entrainment with temperature cycles (12 hr at 33°C and 12 hr at 37°C) and then released into constant conditions at 37°C. Medium changes were performed every two days during the transition from 33°C to 37°C. On day 6, cells were harvested and fixed with PFA (paraformaldehyde; Sigma-Aldrich, # 158127) at indicated time points.

Vector construction, transfection, lentivirus production and stable cell generation

The PER2-EGFP, PER2 (L730G)-EGFP, PER2(1-500)-EGFP, PER2(501-950)-EGFP, and PER2(951-1255) constructs were generated previously (19) and subcloned into the pLX_317 backbone vector using the NEBuilder HiFi cloning kit to generate the lentiviral vectors. The PER2(527-818 S/T-A)-EGFP mutant vector was partially synthesized by Genscript before subcloned into the PER2-EGFP lentiviral vector.

For transfection, Lipofectamine 3000 (Invitrogen) or polyethyleneimine (PEI) transfection reagents were used according to the manufacturer's instructions. Briefly, cells were seeded in appropriate culture plates and allowed to adhere overnight. The transfection mixture were prepared by diluting the transfection reagents in Opti-MEM medium and adding DNA to the mixture. The mixtures were incubated at room temperature for 20 minutes and then added to the cells. After incubation, the transfection medium was replaced with fresh growth medium, and cells were allowed to recover before further analysis. For the generation of U2OS knock-in and

knockout cells, we utilized the Lonza 2D electroporation system (program: X-001) and the Amaxa® Cell Line Nucleofector® Kit V for transfection.

The lentivirus vector and packaging plasmids were co-transfected into HEK293T cells at approximately 70% confluency in a 10 cm dish, and the medium was changed 24 hours later. After 48 hours of incubation, the cell culture supernatant was transferred to a 15 mL centrifuge tube and centrifuged at 3000 RPM for 10 minutes. The supernatant was then filtered through a 0.45 micron syringe filter and collected into a new sterile tube. The viral solution was further concentrated using the Lenti-X™ Concentrator (Takara: 631231) according to the manufacturer's instructions and stored at -80°C for long-term use. The U2OS cells were seeded in a six-well plate and transduced with the lentiviral filtrates 24 hours later in the presence of 8 μg/ml of polybrene. Selection was performed under the pressure of 1 μg/ml of puromycin until control cells completely died. The resultant stably transduced cells were confirmed by confocal microscopy and harvested for further experiments.

Mice and tissue preparation

C57BL/6J mice were obtained from Jackson Laboratory. Per1-3 triple KO mice and Cry1-2 double KO mice were described previously (69, 70). All animal experiments described in this study were conducted in accordance with the guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) at UT Southwestern Medical Center (APN 2016-101376-G) and were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals used in this study were of same genders and within an age range of 8–16 weeks. All mice were housed in pathogen-free barrier facilities under a 12 h/12 h light/dark cycle and at an ambient temperature of 23°C.

All mice were individually housed. After 10 to 12 days of entrainment in 12L:12D, mice were either maintained in LD conditions (ZT 0 is the onset of light) or released in constant darkness (CT condition). At the indicated time points, mice were anesthetized by Ketamine/Xylazine (20 mg/kg Ketamine; 16 mg/kg Xylazine) or at CT4 and CT12 (CT: circadian time. CT12 - onset of activity), followed by immediate cardiac perfused with 0.01M phosphate buffer solution (PBS) and freshly prepared 4 % paraformaldehyde (PFA; Sigma-Aldrich, # 158127) in 0.01M PBS. Anesthesia in DD for CT4 and CT12 was administered under the safe-red light (with Kodak GBX-2 filter) and perfusion was done under the room light. The

brains were removed and post-fixed overnight in 4 % PFA in 0.01M PBS. Brains were cryoprotected using 30% sucrose (Crystalgen, # 300-777-1000) solution and embedded in O.C.T. compound (Tissue Plus, Fisher HealthCare, #4585), frozen, and stored at -80 °C until sectioned. 50 mm coronal sections containing the SCN were collected in PBS.

Western Blot Analysis

The protein concentration of samples was determined by Bradford assay. 50 μg of total protein extracts were separated by SDS-PAGE, transferred onto a PVDF membrane (Millipore), and detected using pierce ECL western blotting substrate (Thermo scientific:32106). The intensities of the bands were quantified using Fiji Image J software. Protein samples with high PER2 levels were diluted to bring relative PER2 levels of different samples to be similar.

Circadian Bioluminescence Recording

To examine the circadian clock phenotype of the cells of interest, the cells were electroporated with a Per2(E2)-Luc reporter (48) using the Lonza 2D electroporation system (program: X-001) and Amaxa® Cell Line Nucleofector® Kit V. Stable cells were selected with neomycin for 7 days, synchronized with dexamethasone, and bioluminescence was recorded in real-time with the LumiCycle (Actimetrics) at 37°C. The detailed methods for real-time measurement of luminescence were previously described (48, 79).

Fluorescence recovery after photobleaching

FRAP experiments were conducted with a Zeiss LSM 880 confocal laser scanning microscope equipped with a Plan-Apochromat 63×/1.4 objective lens. Circular regions with diameters ranging from 1 to 3 μm were bleached using a 100% 488 nm/100 mW argon ion laser. The fluorescence signal from the control and bleached area was captured over 70 frames, with acquired images being 512 × 512 pixels, 1.54μs pixel dwell, and 943ms scan time. Fluorescence recovery data were analyzed using FIJI ImageJ software, and the results were averaged using GraphPad Prism.

Generation of Knock-in cell lines

The knock-in cell lines of PER2-EGFP, BMAL1-mScarlet-I, and CRY1-mScarlet-I were generated using a protocol modified from Gabriel et al (30). To increase knock-in efficiency and eliminate cells with random insertions, we made two main changes. First, we substituted the randomly inserted marker CD4 with mCherry or EYFP. This change allowed us to screen out negative clones with random insertions by using direct fluorescent sorting with FACS. Secondly, to achieve efficient nuclear electroporation with minimal cell death, we used the Lonza™ Nucleofector™ Transfection 2b Device with nuclear transfection reagents. The nuclear transfection device significantly increased the efficiency of positive clone selection.

The PER2-EGFP donor vector was synthesized by Genscript and subcloned into a CMV-mCherry vector. The three sgRNA plasmids, the PER2-EGFP donor vector, and the i53 plasmid (Addgene:74939) were transfected into U2OS cells by using Lonza 2D system (program: X-001) with Amaxa® Cell Line Nucleofector® Kit V. After 7 days of blasticidin selection, cells were sorted using a BD® LSR II Flow Cytometer and cells that are CFP^+ $mCherry^-$ were sorted into a mixed population. After 7 days of culture, a CAG-CRE plasmid (Addgene:13775) was transfected into the cells using the Lonza 2D system (program: X-001) with Amaxa® Cell Line Nucleofector® Kit V. After 7 days of blasticidin selection, cells were sorted using a BD® LSR II Flow Cytometer and cells that were CFP+ and mCherry – were sorted into a mixed population. After 7 days of culture, a CAG-CRE plasmid (Addgene:13775) was transfected into the cells using the Lonza 2D system to remove the loxp sites. Afterwards, the cells were sorted into single clones in four 96-well plates. Positive clones were further confirmed by PCR, Western blot, and confocal microscopy. The knock-in cell clones used in this study are homozygous clones.

The knock-in of BMAL1 mScarlet-I and CRY1 mScarlet-I were designed similarly to the PER2-EGFP knock-in, except that the donor vectors were cloned into a CMV-EYFP backbone vector. We created these knock-in cells in the PER2-EGFP KI cell background. Since the PER2-EGFP fluorescence signal is near background level and cannot be detected by FACS, we selected CFP+ and EYFP- cells as candidate double knock-in cell clones. After confirmation by PCR, Western blot, and confocal microscopy, the double KI cells were examined by LumiCycle (LumiCycle, Actimetrics) to detect the presence of normal circadian rhythms. All knock-in cell clones used in this study are homozygous clones.

Knock-in Point mutation and *Per* gene disruption

The PER2 727-731 in-frame deletion was generated in the PER2-EGFP KI background. Single-stranded DNA (ssDNA) donor with PER2 (VL729/730GG) mutation was synthesized by IDT and co-transfected with sgRNA and Cas9 plasmids using the Lonza 2D system with Amaxa® Cell Line Nucleofector® Kit V. After 7 days of puromycin selection, cells were sorted into single clones in four 96-well plates. The cell clone with in-frame deletion of aa 727-731 were identified using PCR and DNA sequencing: one allele has the in-frame deletion while the other allele has a frameshifting mutation. In addition, we obtained some cell clones with homozygous *Per2* disruption. Disruption of *Per1* gene in the PER2-EGFP KI background was achieved by co-transfecting the two *Per1* sgRNAs and Cas9 plasmids. The resulted clonal cells were screened for clones with both allele of *Per1* gene disrupted by frameshifting mutations.

Airyscan confocal imaging analyses

Airyscan confocal imaging analyses of cells was performed using a ZEISS LSM880 confocal microscope equipped with Airyscan. Cells were seeded in 35 mm glass bottom dishes (Cellvis: D35-20-1-N), synchronized by adding fresh medium containing 1 μM dexamethasone when cells reached 50% confluency. Cells were fixed with paraformaldehyde at the indicated time points. Nuclei were stained with DAPI. Laser power was adjusted to 0.5-2% following the manufacturer's instructions. Cells with fewer than 10 passages were used because they exhibit a robust circadian rhythm. To image cellular fluorescence signals, excitation laser wavelengths of 488 nm were used for detecting EGFP while 561 nm was used to detect BMAL1/CRY1-mScarlet. Airyscan detector was employed to detect the signals in super-resolution mode. Imaging was conducted with a 40x/1.3 Plan-Apochromat oil DIC M27 objective, and the pinhole sizes for EGFP and mScarlet were 80 μm, respectively. The frame scan mode was used with a pixel dwell time of 1.19 μs. Filters used were BP420-480+BP495-550, MBS 488/561, and laser power was set to ~2% for PER2-EGFP knock-in cells. For BMAL1 or CRY1 mScarlet knock-in cells, filters BP570-620+LP645 and MBS 488/561 were used, with laser power set to ~0.5-2%. Images were averaged 2 times and saved in .czi format, then processed using ZEN 2.3 SP1 software before being converted to .tiff or .ims files for further analyses. Live cell imaging studies were performed at 37°C with CO2.

To distinguish the true knock-in signal from autofluorescence, U2OS cells and PER2 knock-in cells were fixed with paraformaldehyde after 10 hours of dexamethasone

synchronization. Lambda scans ((https://www.microscopyu.com/tutorials/lambda-stack-basic-concepts) were performed on a ZEISS LSM880 confocal microscope. The GFP signal was isolated by subtracting its wavelength from all other wavelengths, which were then merged to create a background signal.

Imaris 9.9.1 (Bitplane) software for MAC to track PER2, BMAL1, and CRY1 spots. Time series images of PER2, BMAL1, and CRY1 were captured either by light sheet microscopy (30 ms/frame) or ZEISS LSM880 with Airyscan (0.35-1 s/frame) and exported to Imaris. Spot positions were detected using the Imaris (Bitplane) spot detection function, followed by manual corrections. The tracks were evaluated, and any errors were corrected manually in Imaris.

For 3D imaging, we used a ZEISS LSM880 confocal microscope with airyscan. The obtained images were reconstructed at full resolution. To mark the 3D spots, we utilized IMARIS 9.9.1 (Bitplane) software running on a MAC and created 3D models using the spots rendering method. The grain size for the spots area detail level was set to 0.3 μm, with background subtraction and a filter intensity mean above 11.1. To analyze colocalization, we used Imaris Spot-to-Spot colocalization and colocalization distance analysis, with thresholds set at below 0.1 μm or below 0.04 μm.

For SCN immunofluorescence analyses, SCN slices were washing twice with PBS and permeabilized with 0.2% Tween 20 in PBS for 15 min at room temperature. Next, the slices were blocked with 2% bovine serum albumin (BSA) and 0.1% Tween20 in PBS for 1 h at room temperature. Primary antibodies diluted in the blocking solution and incubated overnight at 4°C (PER2,1:500 PER21-A; CRY1,1:1000# ab54649; CRY1,1:1000# this study). After washing twice with PBS, the slices were incubated with fluorescence-labeled secondary antibodies (1:2000) in the blocking solution for 1 h at room temperature in the dark. The slices were then washed twice with PBS, stained with DAPI (Life Technologies) for 10 min at room temperature in the dark, and washed twice with PBS. Finally, the slices were analyzed using a Zeiss LSM 880 Airyscan confocal microscope.

Light-Sheet Microscopy of PER2-EGFP KI cells

Cells were imaged with a high-resolution oblique plane light-sheet microscope as described previously (62, 63). Briefly, cells were prepared the same day on 35 mm Mattek dishes with #1.5 coverslips and imaged in in an environment chamber that provided humidity,

temperature, and CO2 control. Illumination was provided with 488 and 561 nm lasers, and 525/50 and 593/LP emission filters were used to isolate the fluorescence emission arising from green- and red-emitting fluorophores, respectively, prior to detection with a Hamamatsu Flash 4.0 scientific CMOS camera. Imaging was performed in a 2D format whereby an oblique cross-section of the cell is visualized with a 30-millisecond exposure time for ~500 timepoints, thereby allowing the observation of rapid PER body dynamics.

STED microscopy

PER2-EGFP KI cells was synchronized by adding fresh medium containing 1 μM dexamethasone when cells reached 50% confluency. 10 hr after synchronization, the cells were twashed three times with warm PBS and fixed with 4% paraformaldehyde (PFA) for 20 min at 20°C. After washing twice with PBS, cells were permeabilized with 0.2% Tween 20 in PBS for 10 min at room temperature. Next, the cells were blocked with 2% bovine serum albumin (BSA) and 10% (v/v) glycerol in PBS for 1 h at room temperature. Primary antibodies diluted 500-1000 times in the blocking solution were added and incubated overnight at 4°C. After washing twice with PBS, the cells were incubated with fluorescence-labeled secondary antibodies (goat anti-mouse STAR red or goat anti-rabbit STAR orange (1:2000)) in the blocking solution for 1 h at room temperature in the dark. The cells were then washed twice with PBS, stained with DAPI (Life Technologies) for 10 min at room temperature in the dark. For GFP nanobody staining, the primary antibody nano GFP 635P (1:1000 dlution) in the blocking solution were added and incubated overnight at 4°C. The cells were then washed twice with PBS, stained with DAPI (Life Technologies) for 10 min at room temperature in the dark.

The STED imaging was performed with the Facility Line of Abberior Instrument with Olympus 60X oil objective. Single z plain 2D STED image was taken using the Lightbox software. For dual color STED Image, line scan mode was used. STAR RED (Abberior) was imaged with 640 nm excitation laser and 775 nm depletion laser. STAR ORANGE was imaged with 561 excitation laser and 775 nm depletion laser. Line scan mode was used to image STAR RED and STAR ORANGE with parameters, Excitation 640 nm 15% with 30% STED, 775 nm 10% with 20% STED. Dwell Time 5 μs and pixel size 10 nm were used. Images were averaged 5 times. For DAPI, 405 mm excitation with confocal mode was used. All images were taken with 1.0 AU pinhole based on 640 nm.

Immunofluorescence assays

Cells were washed three times with warm PBS and fixed with 4% PFA for 20 min at 20°C. After washing twice with PBS, cells were permeabilized with 0.2% Tween 20 in PBS for 10 min at room temperature. Next, the cells were blocked with 2% bovine serum albumin (BSA) and 10% (v/v) glycerol in PBS for 1 h at room temperature. Primary antibodies diluted 500-1000 times in the blocking solution were added and incubated overnight at 4°C. After washing twice with PBS, the cells were incubated with fluorescence-labeled secondary antibodies (1:2000) in the blocking solution for 1 h at room temperature in the dark. The cells were then washed twice with PBS, stained with DAPI (Life Technologies) for 10 min at room temperature in the dark, and washed twice with PBS. Finally, the cells were analyzed using a Zeiss LSM 880 confocal microscope.

CRY1 antibody generation

CRY1 polyclonal antibody was raised against mouse CRY1(496-606) recombinant protein in guinea pigs. CRY1-specific antibodies were purified from the antiserum via negative selection followed by affinity selection using NHS-activated Sepharose 4 Fast Flow resin (Cytiva Cat# 17090601) conjugated to Cry1-/- mouse liver and kidney total protein lysates and mCRY1(496-606) recombinant protein, respectively.

Immunodepletion assay

PER2-EGFP KI; BMAL1-mScarlet-I KI and PER2-EGFP KI; CRY1-mScarlet-I KI U2OS cells were harvested after a 10-hour DXMS synchronization and used to prepare cell extracts. To prepare mice liver extracts, C57BL/6J mice were maintained under a 12:12 hours light-dark (LD) cycle for 14 days and liver tissue were collected at ZT18. The harvested U2OS cells and liver tissue were lysed using RIPA buffer (containing 50mM Tris•HCl at pH 7.6, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 1x protease inhibitor). The protein concentration was determined using the Bio-Rad Protein Assay Dye Reagent. Subsequently, 30 μg of protein extract was incubated with 0.04 μg of specific antibodies or control IgG bound to 10 μL of magnetic beads (#ThermoFisher 26162) for 3 hours. The unbound fractions were then analyzed by western blotting for protein of interest.

Supplemental Figures and Supplemental Data files

Figure S1. (A) Design of stable PER2-EGFP expression plasmid by viral transduction. (B) Western blot result showing the PER2-GFP levels in U2OS cells that stably overexpresses PER2-EGFP and in cells transiently transfected with the PER2-EGFP expression plasmid. The protein sample from the transiently transfected cells were analyzed at 20x-150x dilutions. (C) Representative confocal images (left) and statistics (right) of nuclear and cytoplasmic PER2-EGFP percentage at 24, 48, 72, and 98 h after transfection of the PER2-EGFP expression plasmid into U2OS cells. Scale bar: 5 μm. (D) Representative images of U2OS cells that transiently transfected with a plasmid that expresses PER2- EGFP(A206K). The image was taken 48 hrs after transfection. Scale bar: 5 μm. (E) Western blot result showing the PER2-EGFP level in the cells that stably overexpress PER2-EGFP or PER2(L730G)-EGFP. (F) Percentages of cells that stably overexpress PER2-EGFP, PER2(L730G)-EGFP, or PER2(527 818 S/T-A)-EGFP that contain nuclear condensates. Data are presented as mean ±SD, unpaired two-tailed Student's t test, $***p < 0.0001$.

Figure S2. (A) Western blot result showing the PER2-EGFP level in the PER2-EGFP KI and PER2-EGFP KI cells treated by Per2-specific siRNA (si-PER2) and in PER2-EGFP KI cells in which Per2 gene was disrupted by CRSPR/Cas9. Bottom: sequencing result showing the homozygous two-nucleotide deletion in the Per2 gene in the PER2 KO cells. (B) Time course 2D Airyscan confocal imaging results of nuclear GFP signals in PER2-EGFP KI cells after dexamethasone synchronization. (C) Time course 2D Airyscan confocal imaging results of nuclear GFP signals in PER2-EGFP KI cells after synchronization by temperature cycles. (D) Densitometric analyses of Western blot results of PER2-EGFP levels in the nuclear and cytoplasmic fractions of the PER2-EGFP KI cells at hr 4 and hr 10 after dexamethasone synchronization. (E) Western blot results comparing the levels of PER2-GFP in the KI cells to that of defined amount of recombinant GFP protein (2x10-10g). Quantification of western blot analysis and estimation of PER2 molecules per nuclear body. Data are presented as mean ±SEM, n=3.

Figure S3. (A) Sum of nuclear fluorescence intensity per cell (left) and mean of fluorescence intensity of nuclear PER2 bodies (right) in the PER2-EGFP KI cells with/without hexanediol (1.5%) treatment by hexanediol. (B) Mean of fluorescence intensity of PER2 bodies in the indicated cells. (C) DNA sequencing results showing the homozygous disruption of the Per1 gene in the PER2-EGFP KI and PER2(727-731D)-EGFP KI cells.

Figure S4. (A) (left panels) Airyscan fluorescence imaging results showing the specific BMAL1-mScarlet-I and CRY1-mScarlet-I signals in the indicated double KI cells at hr 10 after dexamethasone synchronization. Scale bar: 5 μm. (Right) Western blot analysis using an RFP antibody to detect the indicated BAML1-mScarlet-1 or CRY1-mScarlet-1 protein in the indicated KI cells. (B-C) Enlarged 2D imaging results in Figure 5B-C showing the colocalization of PER2 with BMAL1/CRY1-mScarlet-I in the double KI cells. Scale bar: 0.5 μm. (D) Airyscan fluorescence imaging results of the indicated double KI cells at hr 32 after dexamethasone synchronization.

Figure S5. (A) 3D mask result of PER2-EGFP and BMAL1-mScarlet-I bodies in the PER2-

EGFP and BMAL1-mScarlet-I double KI cells using Imaris software. Colocalization spots are identified based on the colocalization distance <0.1 μm. (B) 3D mask result of PER2-EGFP and CRY1-mScarlet-I bodies in the PER2-EGFP and BMAL1-mScarlet-I double KI cells using Imaris software. Colocalization spots are identified based on the colocalization distance <0.1 μm.

Figure S6. (A) Number of PER2-BMAL1 colocalization foci (left) and PER2-CRY1 colocalization foci (right) at different time points after dexamethasone synchronization. Colocalization distance<0.04 μm. Data are presented as mean ±SEM, n=8 cells. (B) Percentage of BMAL1 and PER2 foci that are colocalized with each other in the PER2-EGFP and BMAL1-mScarlet-I double KI cells at different time points after dexamethasone synchronization. Colocalization distance<0.04 μm. Data are presented as mean ±SEM, n=8 cells. (C) Percentage of CRY1 and PER2 foci that are colocalized with each other in the PER2-EGFP and CRY1-mScarlet-I double KI cells at different time points after dexamethasone synchronization. Colocalization distance<0.04 μm. Data are presented as mean Å ± SEM, n=8 cells.

Figure S7. (A) Immunostaining (left) and western blot (right) results of the PER2-EGFP KI cells and KI cells treated by Per2-specific siRNA (si-PER2) cell lines showing the specificity of the GFP antibody used. Scale bar: 5 μm. (B) Immunostaining (left) and western blot (right) results of U2OS cells with/without treatment by Cry1-specific siRNA (si-CRY1) showing the specificity of the CRY1 antibody used. Scale bar: 5 μm. (C) Immunostaining (left) and western blot (right) results of BMAL1 in the control MEF and BMAL1 KO MEF cell lines showing the specificity of the BMAL1 antibody used (Ab3350). Scale bar: 5 μm. (D) STED microscopy using BMAL1/CRY1-speific antibody to estimate the sizes of BMAL1 and CRY1 nuclear bodies in the PER2-EGFP KI cells. (E) Percentage of PER2 and BMAL1 bodies that are colocalized with each other at 10 hrs after dexamethasone synchronization. Colocalization distance<0.04μm. Data are presented as mean ± SEM, n=8 cells. (F) Percentage of PER2 and CRY1 bodies that are colocalized with each other at 10 hrs after dexamethasone synchronization. Colocalization distance<0.04μm. Data are presented as mean ± SEM, n=3 cells. (G) Immunofluorecence imaging results showing the nuclear distribution of PER2 and mCRY1 proteins and their co-localization in SCN prepared at the indicated time points. Cells from the core region of SCN were selected here. (H) Immunofluorecence imaging results indicate the lack of mPER2 or mCRY1 nuclear signals in cells of mice SCN tissues (ZT12) of the PER triple KO or CRY double KO mice, respectively. A rabbit polyclonal PER2 antibody and two CRY1 antibody made from mouse and guinea pig were used. Cells from the core region of SCN were selected here.

Supplemental data files

Supplemental data file 1: A movie related to the result in Figure 1D showing the fusion of two small condensates into one.

Supplemental data file 2: A movie showing the 3D images related to Figure 3D showing the nuclear PER2 bodies in the PER2-EGFP KI cells.

Supplemental data file 3: A movie showing the live cell fluorescence imaging of the PER2-EGFP KI cells using an Airyscan super-resolution microscope in 0.35 s sampling intervals.

Supplemental data file 4: A movie related to the result in Figure 4A showing the nuclear movements of PER2 bodies captured by light sheet microscopy in 30ms interval.

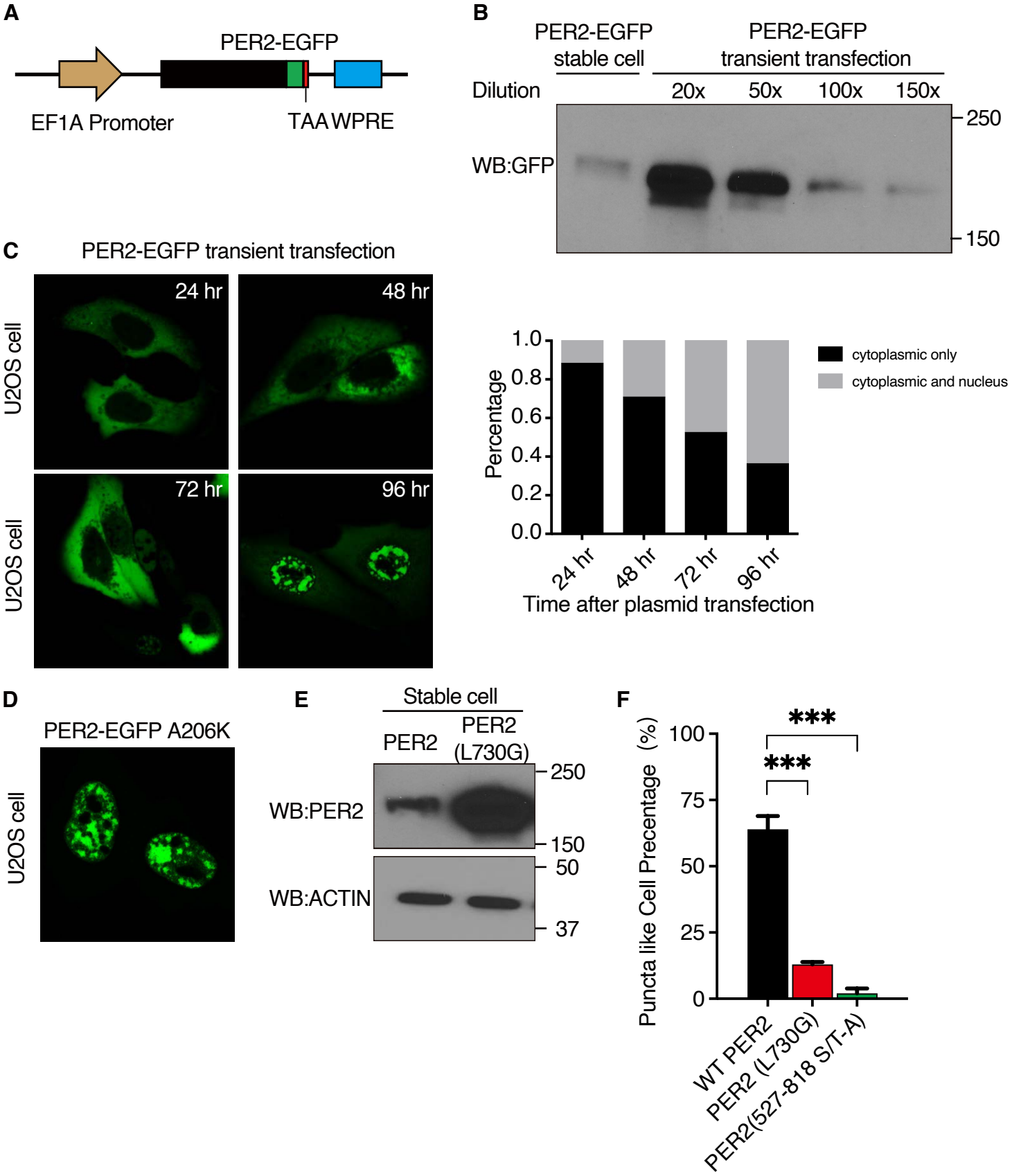
Supplemental data file 5: A movie showing the 3D fluorescence images related to Figure 6A which PER2 (green) and BMAL1 (red) bodies in the PER2-EGFP and BMAL1-mScarlet-I double KI cells at different time points after synchronization.

Supplemental data file 6: A movie showing the 3D fluorescence images related to Figure 6B which PER2 (green) and CRY1 (red) bodies in the PER2-EGFP and CRY1-mScarlet-I double KI cells at different time points after synchronization.

Supplemental data file 7: A movie showing live cell imaging of the BMAL1-mScarlet-I KI cells using an Airyscan super-resolution microscope in ~1s sampling intervals showing the nuclear movements of BMAL1 bodies.

Supplemental data file 8: A movie showing live cell imaging of the CRY1-mScarlet-I KI cells using an Airyscan super-resolution microscope in ~1s sampling intervals showing the nuclear movements of CRY1 bodies.

Figure S1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WB:GFP
250
150
C
PER2-EGFP transient transfection
U2OS cell
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48 hr
72 hr
96 hr
Percentage
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0.6
0.4
0.2
0.0
cytoplasmic only
cytoplasmic and nucleus
Time after plasmid transfection
D
PER2-EGFP A206K
U2OS cell
E
Stable cell
PER2
PER2 (L730G)
WB:PER2
WB:ACTIN
250
150
50
37
F
Puncta like Cell Precentage (%)
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50
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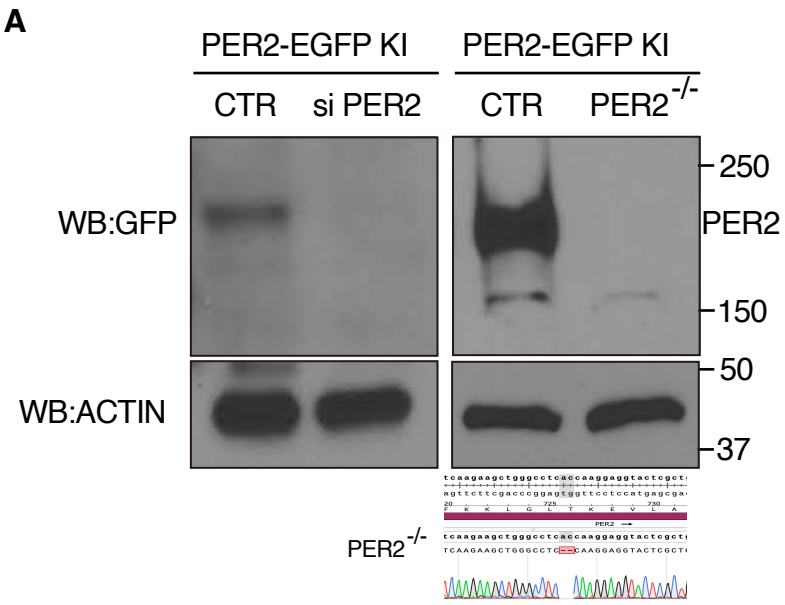
WT PER2
PER2 (L730G)
PER2(527-818 S/T-A)

Figure S2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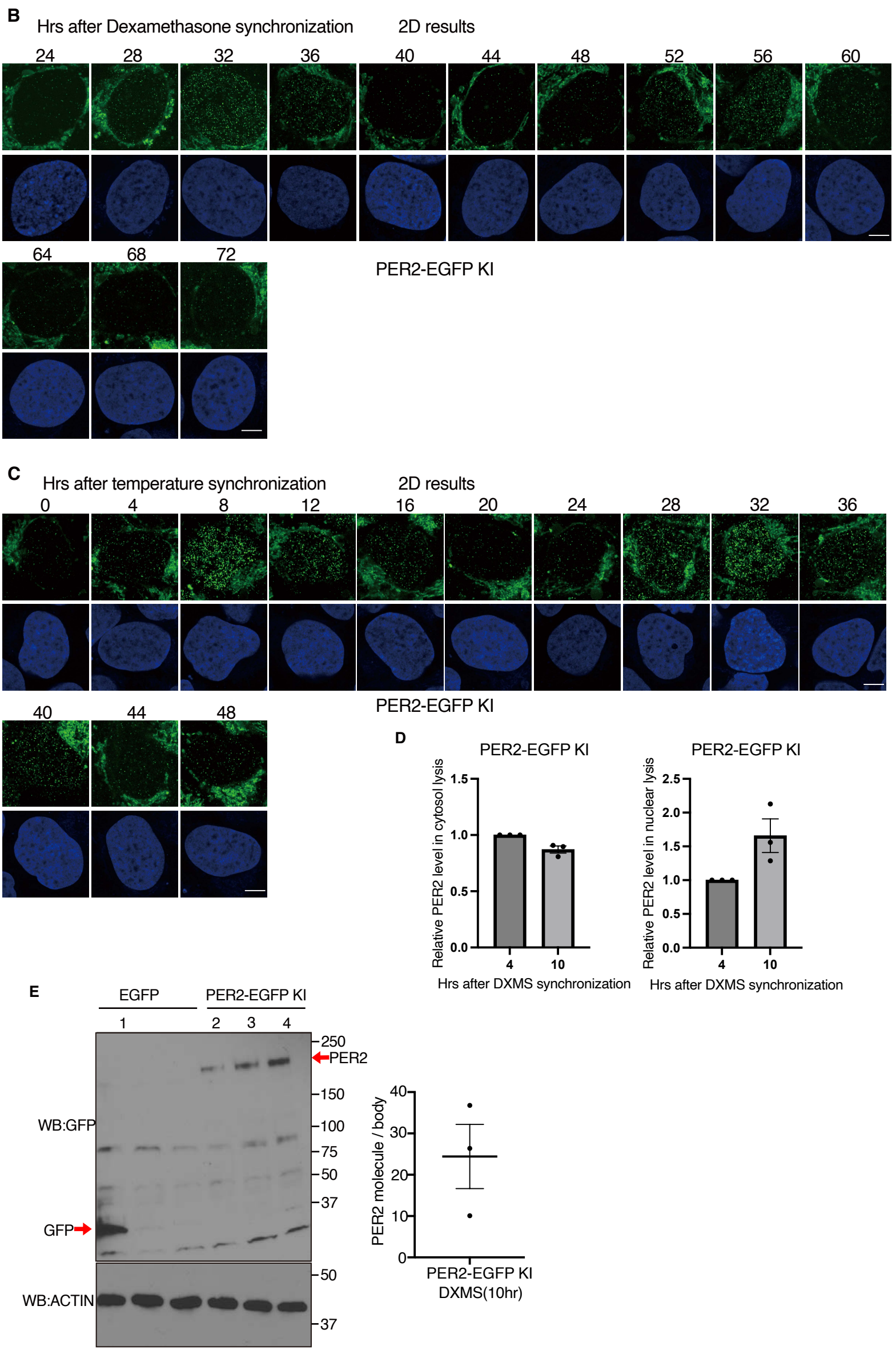
PER2-/-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2D results
24
28
32
36
40
44
48
52
56
60
64
68
72
PER2-EGFP KI
C
Hrs after temperature synchronization
2D results
0
4
8
12
16
20
24
28
32
36
40
44
48
PER2-EGFP KI
D
PER2-EGFP KI
Relative PER2 level in cytosol lysis
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1.0
0.5
0.0
4
10
Hrs after DXMS synchronization
PER2-EGFP KI
Relative PER2 level in nuclear lysis
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2.0
1.5
1.0
0.5
0.0
4
10
Hrs after DXMS synchronization
E
EGFP
PER2-EGFP KI
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2
3
4
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PER2
150
100
75
50
37
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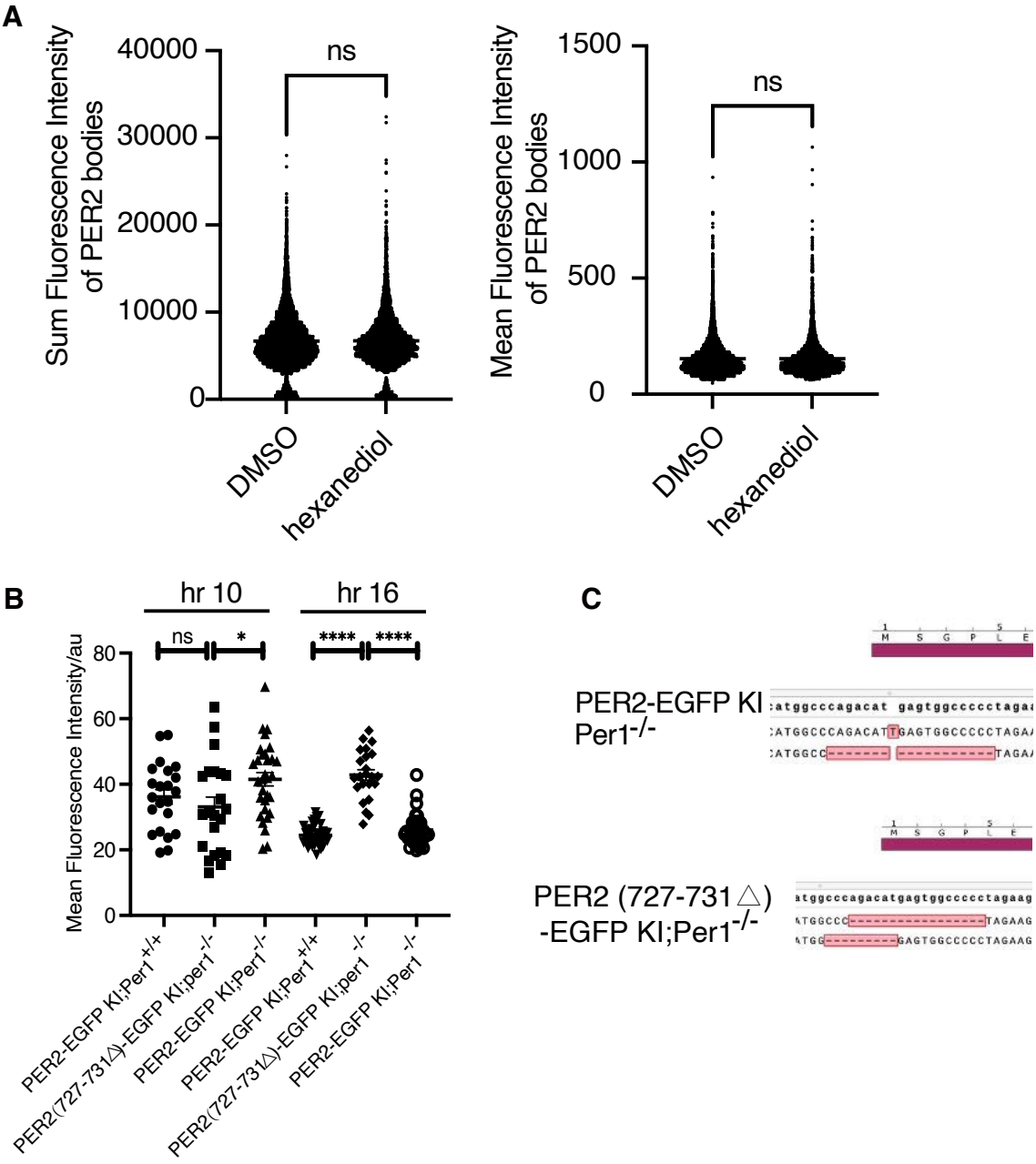
DXMS(10hr)

Figure S3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hexanediol
B
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Mean Fluorescence Intensity/au
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PER2-EGFP KI;Per1+/+
PER2(727-731△)-EGFP KI;per1-/-
PER2-EGFP KI;Per1-/-
PER2-EGFP KI;Per1+/+
PER2(727-731△)-EGFP KI;per1-/-
PER2-EGFP KI;Per1-/-
C
PER2-EGFP KI Per1-/-
PER2 (727-731△) -EGFP KI;Per1-/-

Figure S4

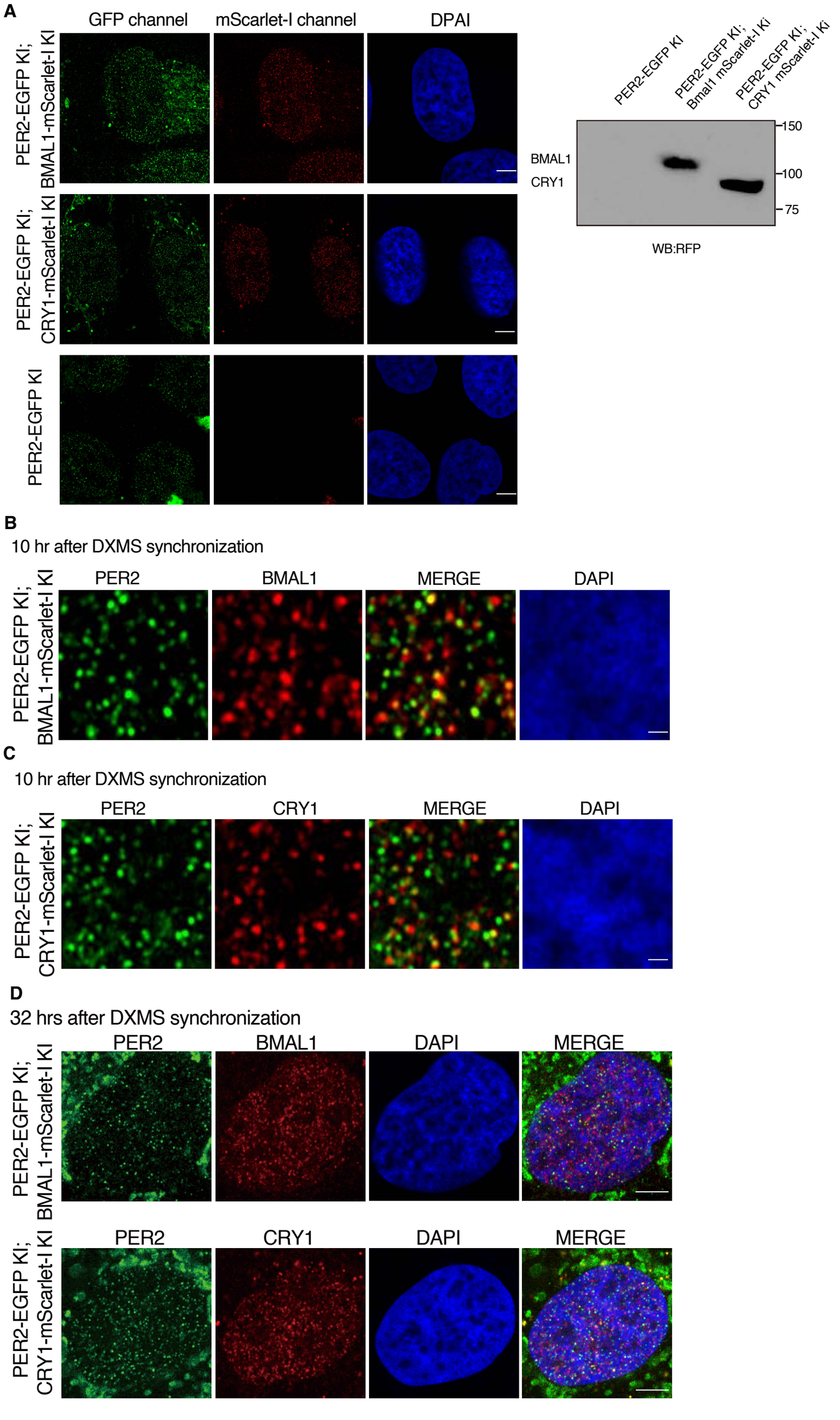

A
GFP channel
mScarlet-I channel
DPAI
PER2-EGFP KI; BMAL1-mScarlet-I KI
PER2-EGFP KI; CRY1-mScarlet-I KI
PER2-EGFP KI
PER2-EGFP KI
PER2-EGFP KI; Bmal1 mScarlet-I Ki
PER2-EGFP KI; CRY1 mScarlet-I Ki
BMAL1
CRY1
150
100
75
WB:RFP
B
10 hr after DXMS synchronization
PER2
BMAL1
MERGE
DAPI
PER2-EGFP KI; BMAL1-mScarlet-I KI
C
10 hr after DXMS synchronization
PER2
CRY1
MERGE
DAPI
PER2-EGFP KI; CRY1-mScarlet-I KI
D
32 hrs after DXMS synchronization
PER2
BMAL1
DAPI
MERGE
PER2-EGFP KI; BMAL1-mScarlet-I KI
PER2
CRY1
DAPI
MERGE
PER2-EGFP KI; CRY1-mScarlet-I KI

Figure S5

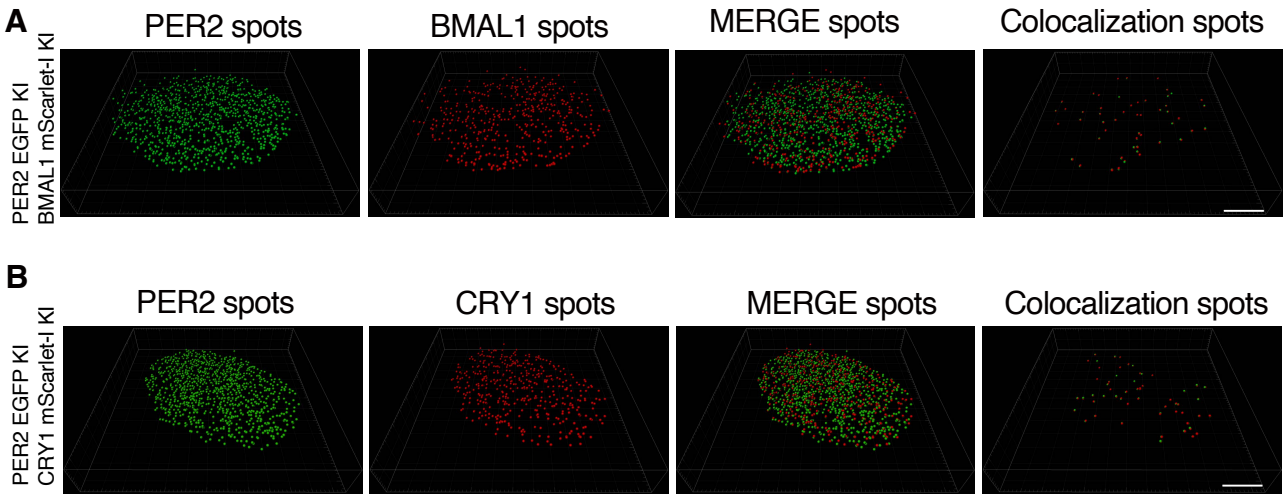

A
PER2 spots
BMAL1 spots
MERGE spots
Colocalization spots
PER2 EGFP KI
BMAL1 mScarlet-I KI
B
PER2 spots
CRY1 spots
MERGE spots
Colocalization spots
PER2 EGFP KI
CRY1 mScarlet-I KI

Figure S6

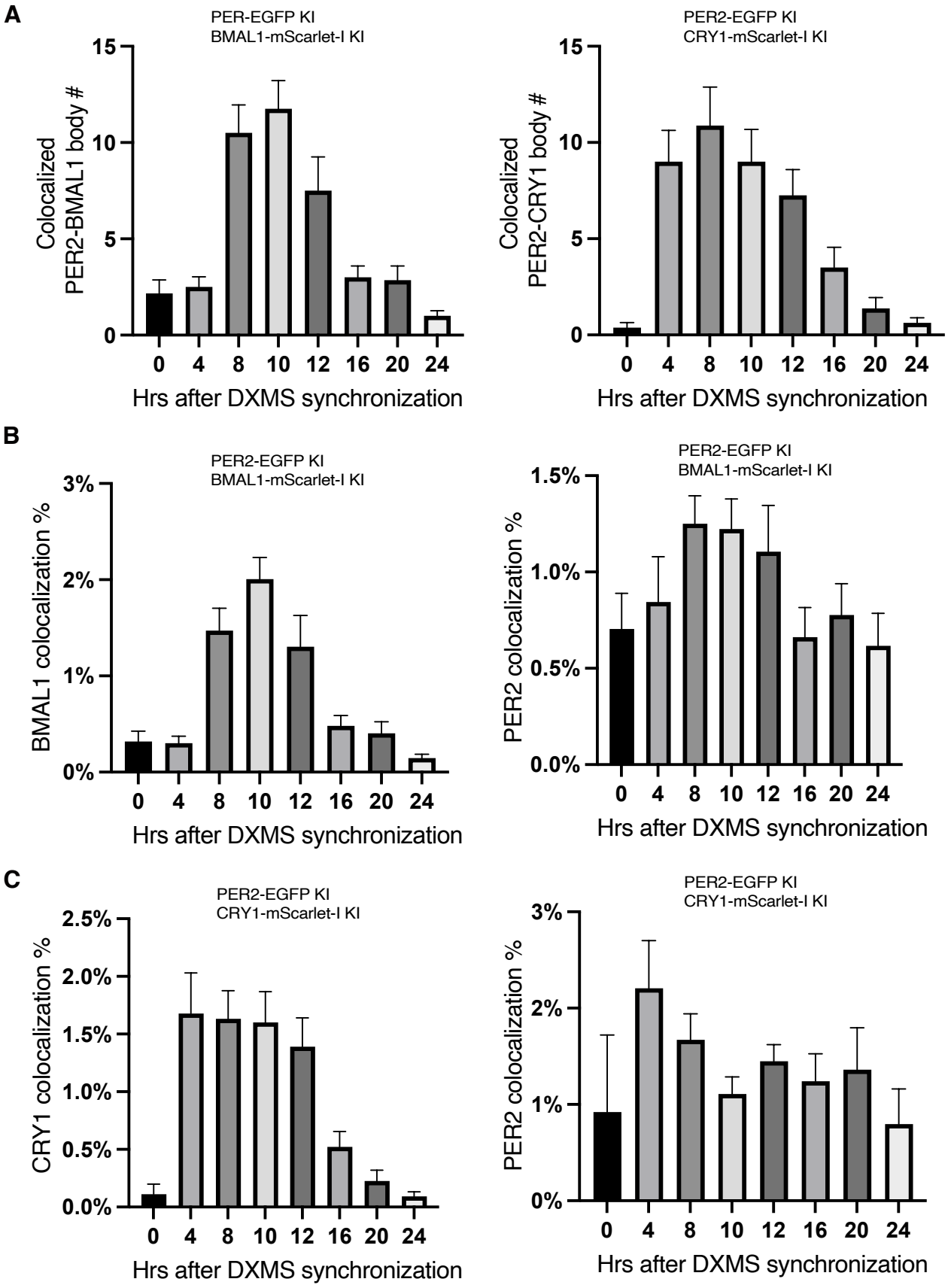
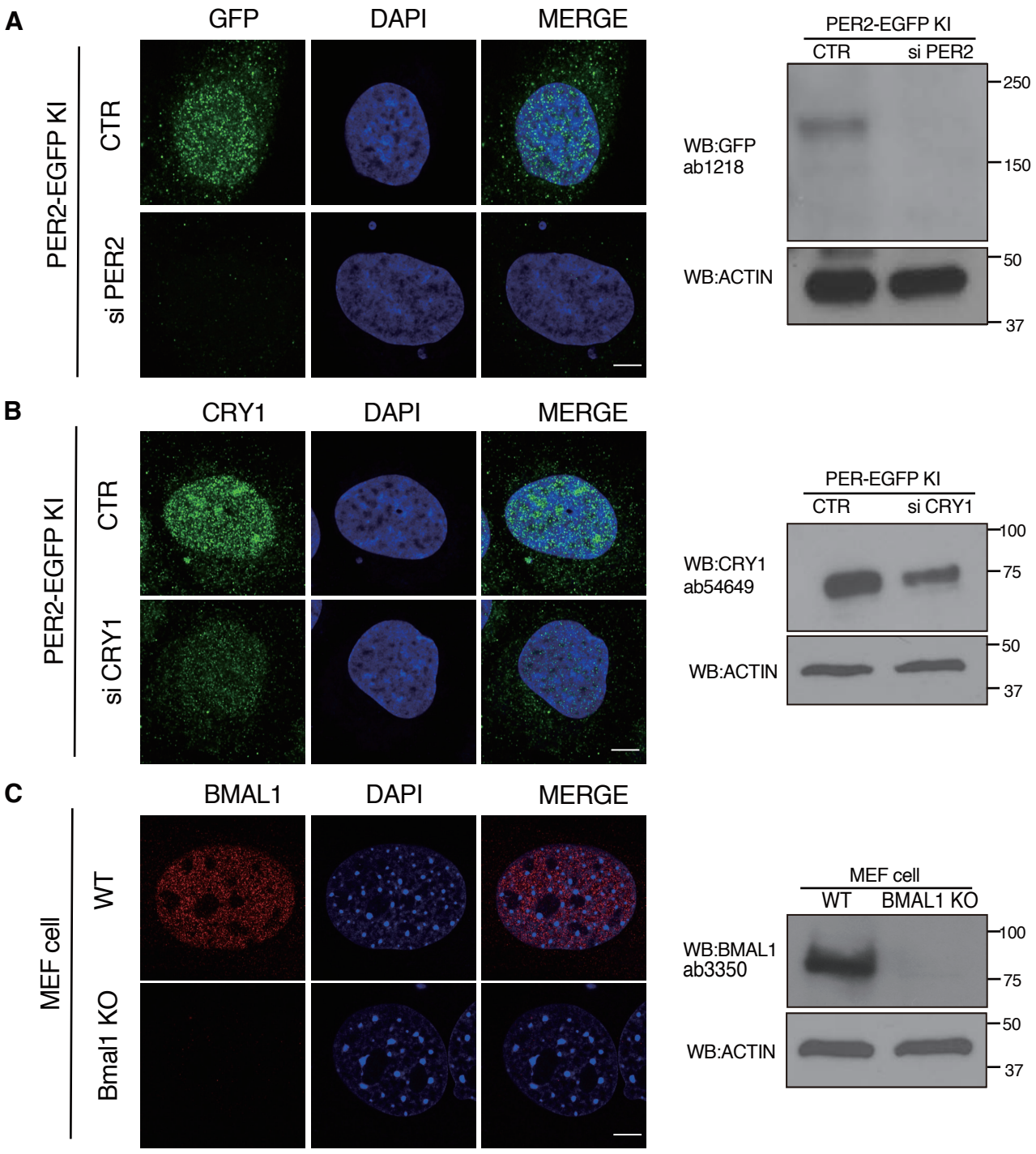

A
PER-EGFP KI
BMAL1-mScarlet-I KI
Colocalized PER2-BMAL1 body #
15
10
5
0
0 4 8 10 12 16 20 24
Hrs after DXMS synchronization
PER2-EGFP KI
CRY1-mScarlet-I KI
Colocalized PER2-CRY1 body #
15
10
5
0
0 4 8 10 12 16 20 24
Hrs after DXMS synchronization
B
PER2-EGFP KI
BMAL1-mScarlet-I KI
BMAL1 colocalization %
3%
2%
1%
0%
0 4 8 10 12 16 20 24
Hrs after DXMS synchronization
PER2-EGFP KI
BMAL1-mScarlet-I KI
PER2 colocalization %
1.5%
1.0%
0.5%
0.0%
0 4 8 10 12 16 20 24
Hrs after DXMS synchronization
C
PER2-EGFP KI
CRY1-mScarlet-I KI
CRY1 colocalization %
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
0 4 8 10 12 16 20 24
Hrs after DXMS synchronization
PER2-EGFP KI
CRY1-mScarlet-I KI
PER2 colocalization %
3%
2%
1%
0%
0 4 8 10 12 16 20 24
Hrs after DXMS synchronization

Figure S7


A
GFP
DAPI
MERGE
PER2-EGFP KI
CTR
si PER2
PER2-EGFP KI
CTR
si PER2
WB:GFP
ab1218
WB:ACTIN
250
150
50
37
B
CRY1
DAPI
MERGE
PER2-EGFP KI
CTR
si CRY1
PER-EGFP KI
CTR
si CRY1
WB:CRY1
ab54649
WB:ACTIN
100
75
50
37
C
BMAL1
DAPI
MERGE
MEF cell
WT
Bmal1 KO
MEF cell
WT
BMAL1 KO
WB:BMAL1
ab3350
WB:ACTIN
100
75
50
37
D
Puncta size (μm)
0.20
0.15
0.10
0.05
0.00
BMAL1
CRY1
E
PER2 and BMAL1 colocalization ratio
15%
10%
5%
0%
PER2
BMAL1
Distance<0.04μm
F
PER2 and CRY1 colocalization %
4%
3%
2%
1%
0%
PER2
CRY1
Distance<0.04μm
G
SCN cell nucleus(Airyscan)
PER2 (Rabbit)
CRY1 (Guinea pig)
DAPI
Merge
CT 4
CT12
H
SCN cell nucleus(Airyscan)
PER2 (Rabbit)
DAPI
Merge
PER triple KO
CRY1 (mouse)
DAPI
Merge
CRY1CRY2 KO
CRY1 (Guinea pig)
DAPI
Merge
CRY1CRY2 KO