Supplemental Figures

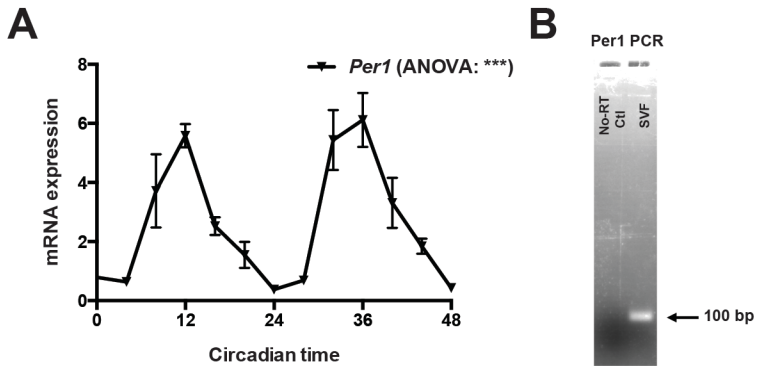

Figure S1. APCs contain a circadian clock that oscillates *in vivo*.
Related to Figure 1.

(A) Quantification by RT-qPCR of the expression levels of *Per1* clock genes in liver tissue immediately lysed from WT mice housed in constant darkness across an *in vivo* time-course (N = 3 mice per time-point). Data analyzed by one-way ANOVA and *post hoc* Bonferroni.

(B) Gel depicting specific PCR products for no-RT control and SVF samples using TaqMan primers for *Per1*.

****P* <0.001. Error bars represent ± SEM.

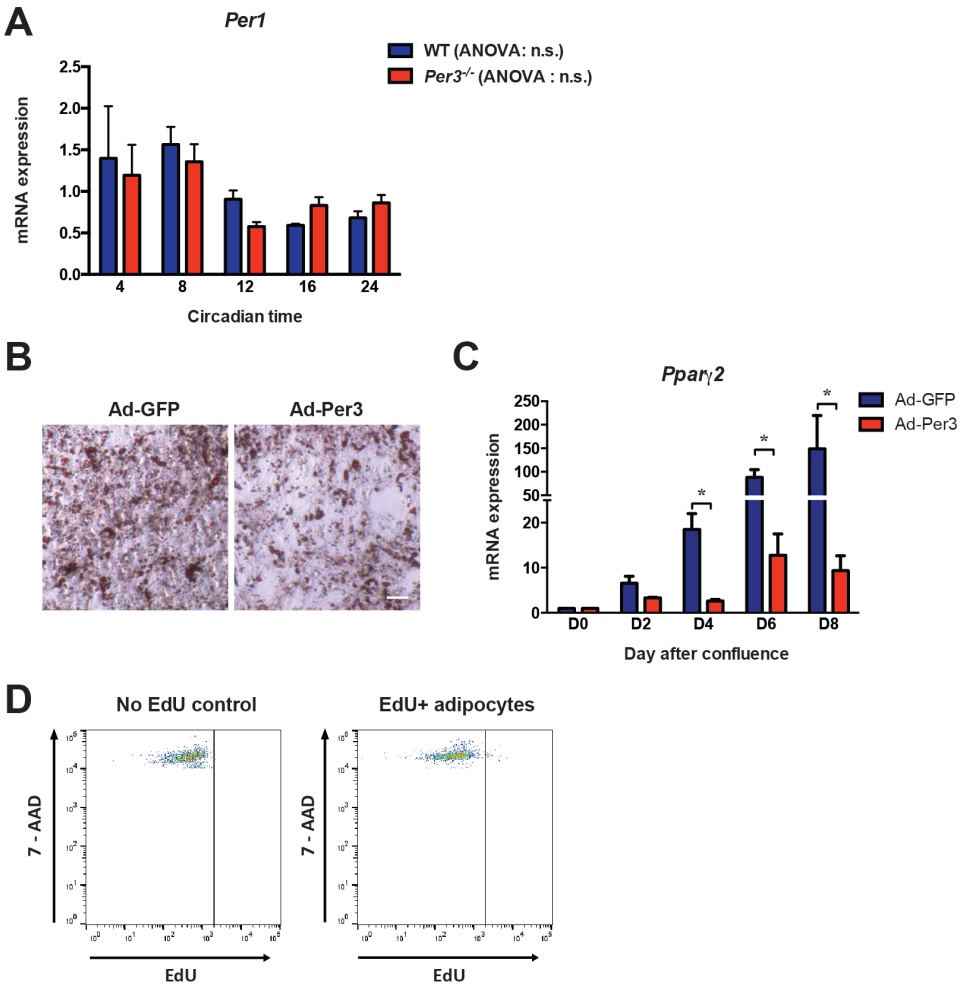

Figure S2. *Per3* regulates adipogenesis in APCs *in vivo*. Related to Figure 2.

(A) Quantification by RT-qPCR of the expression levels of *Per1* in SVF immediately lysed from WT and *Per3*$^{-/-}$ mice housed in constant darkness across an *in vivo* time-course (N = 3 mice per time-point for each genotype). Data within a genotype was analyzed by one-way ANOVA; the effect of genotype and time was tested by two-way ANOVA followed by *post hoc* Bonferroni.

(B) Levels of adipogenesis in APCs isolated from WT mice and infected with either GFP or *Per3* expressing adenovirus stained 8-days post-infection with Oil Red O. Scale bar represents 100 μm.

(C) Levels of adipogenesis in APCs isolated from WT mice and infected with either GFP or *Per3* expressing adenovirus quantified by measuring the expression levels of *Pparγ2* using RT-qPCR across an *in vitro* time-course. Data analyzed by Student's *t*-test.

(D) Example of a FACS scatter plot of adipocytes isolated from mice without an EdU pulse (No EdU control, left) that were used to establish gates (vertical line) to detect EdU positive cells in mice after in vivo injections with EdU (EdU+ adipocytes, right).

**P* <0.05. Error bars represent ± SEM.

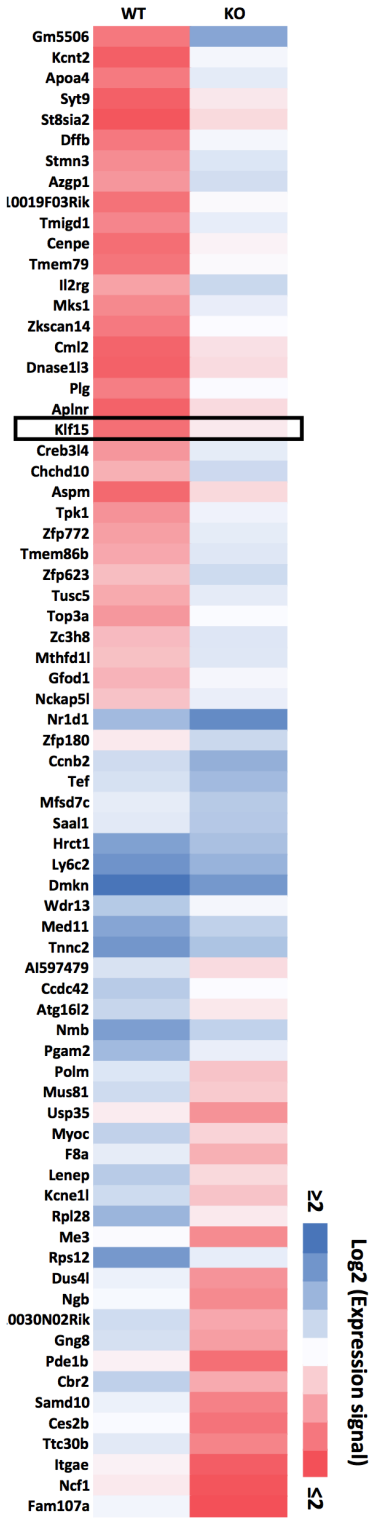

Figure S3. Klf15 expression is increased in APCs isolated from *Per3*$^{-/-}$ mice. Related to Figure 3.

Heat map (log_2) of genes with significant differential expression in APCs isolated from Per3 KO and WT mice. n=2 independent experiments.

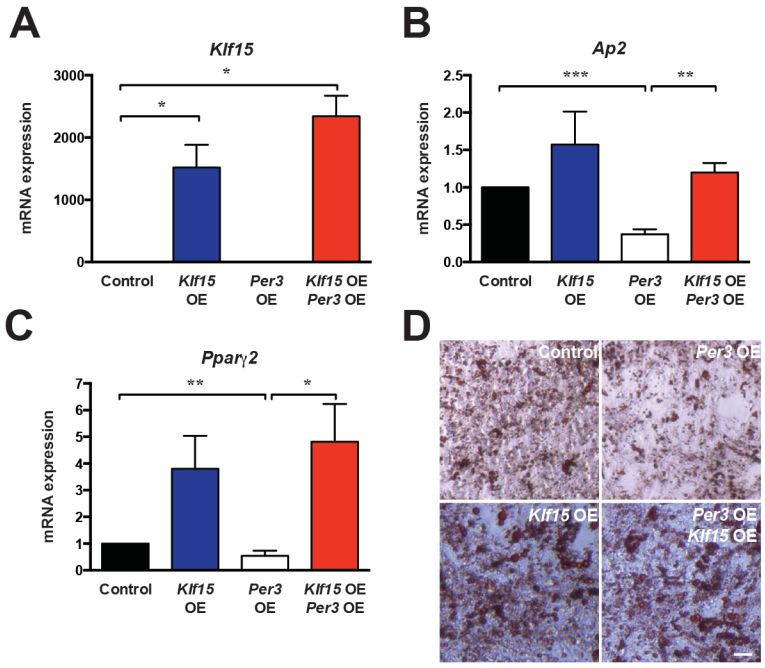

Figure S4. PER3 regulates the expression of *Klf15*.
Related to Figure 3.

(A–D) RT-qPCR quantification of the level of adipogenesis in APCs isolated from WT mice infected with adenoviruses expressing GFP (Control), *Per3*, *Klf15* or both *Per3* and *Klf15* measured by quantification of the expression levels of *Klf15* (A), *Ap2* (B), *Pparγ2* (C), and stained with Oil Red O (D). Data analyzed by ANOVA and *post hoc* Bonferroni.

**P* <0.05, ***P* <0.01, ****P* <0.001. Error bars represent ± SEM.

Supplemental Experimental Procedures

Adipocyte Precursor Cells isolation and FACS
APCs were harvested and purified as previously described (Wong et al., 2016). Briefly, animals were euthanized by asphyxiation with CO_2 followed by cervical dislocation and inguinal subcutaneous fat pads were harvested. The adipose depots were minced and incubated in 1 mg/mL collagenase (Worthington) in Dulbecco's modified Eagle's medium (DMEM) for 1h. The cell suspension was filtered through a 100 μm cell strainer, and the SVF was isolated by centrifugation at 300 g/min.

For APC isolation, erythrocyte-free SVF samples were re-suspended in HBSS/2% FBS and antibody labeling was performed as previously described (Wong et al., 2016). Samples were sorted on a BD FACS Aria. Gating was performed using unstained and fluorescence-minus-one-stained controls. Forward-scatter and side-scatter analysis was performed for single cell analysis. Cells were sorted into serum-free DMEM media for gene expression analysis or into complete media for *ex vivo* assays. Data analysis was performed using FlowJo version 10.

Real-time Circadian Monitoring
APCs from m*Per2*^{Luc} mice were isolated as described above. Cells were seeded in 16mm petri dishes and maintained at 37°C in 5% CO_2. Real-time luciferase activity was continuously measured in a Kronos Dio AB2500 (ATTO).

Cell Culture
Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (Atlanta Biologicals), streptomycin (50 μg/mL) and penicillin (50 units/mL) (Life Technologies) at 37°C in 5% CO_2.

Immunocytochemistry
FACS purified APCs were seeded on cover slips in 24 well plates and KLF15 protein expression was assessed by immunofluorescence staining using anti-KLF15 antibody (Abcam, 1:500) as previously described (Aggarwal et al., 2015). Imaging was performed using an Axioplan2 (Zeiss) fluorescence microscope at 20x and 63x. Quantification was performed using images of at least 10 randomly selected 20x fields captured from each genotype (n=3) and the intensity of staining was quantified using Image J software.

RNA Extraction and RT-qPCR
Total RNA was extracted from APCs or SVF using the RNeasy micro kit plus (Qiagen) according to manufacturer's protocol. RNA yield was quantified using a NanoDrop (Thermo Scientific) and equal amounts of RNA were reverse transcribed using random primers (Promega) and the Superscript III kit (Life Technologies). Relative transcript levels were measured by quantitative PCR (qPCR) using the $2^{-\Delta\Delta Ct}$ method on a CFX384 system (Bio-Rad).

For RT-qPCR performed using SYBR chemistry we used the following primer pairs (5' – 3'): mPer3 (Fwd: GTGACAGCAGAGTCCCATGA, Rev: CACTGCCATCTCGAGTTCAA), mAp2 (Fwd: CAGAAGTGGGATGGAAAGTCG, Rev: CGACTGACTATTGTAGTGTTTGA), mPPARγ2 (Fwd: TGCACTGCCTATGAGCACTT, Rev: TGATGTCAAAGGAATGCGAG), mKlf15 (Fwd: TATGCTCGAGGCTCTGAGGGG, Rev: TATTGATATCAGCTGGGCGCT), mTbp (Fwd: ACCCTTCACCAATGACTCCTA, Rev: TGACTGCAGCAAATCGCTTGG), and mRpl19 (Fwd: ATGAGTATGCTCAGGCTACAGA, Rev: GCATTGGCGATTTCATTGGTC).
For RT-qPCR performed using TaqMan chemistry we used the following probe sets (Applied Biosystems): mPer1 (Mm00501813_m1), mPer2 (Mm00478113_m1), mPer3 (Mm00478120_m1), mBmal1 (Mm00500226_m1), mKlf15 (Mm00517792_m1), and mβ-Actin (Mm00607939_s1).

***In vivo* EdU Pulse Chase**
To test for equal uptake 5-ethynyl-2'-deoxyuridine (EdU) (carbosyth) by APCs across the genotypes, EdU was administered intraperitoneally (1 mg at 5mg/mL in sterile PBS) into *Per3*$^{-/-}$ and WT littermates and the number of EdU^{+} APCs was quantified by FACS within 4 hours of this dose, confirming there was not a

significant difference across the genotypes. EdU was then injected for 3 consecutive days. At the end of the EdU chase period (2 weeks), subcutaneous adipose depots were harvested and the mature adipocytes were purified by centrifugation as previously described (Wong et al., 2016). A Click-iT EdU Alexa Fluor 488 Flow Cytometry Assay Kit (Molecular Probes) was used to label the EdU^+ nuclei. DAPI was added to nuclei immediately prior to FACS analysis. EdU gating was based on samples isolated from mice that were not injected with EdU.

Adipogenesis Assays
APCs were grown to confluence for 48 hrs and then stimulated with media containing 1 µmol/L dexamethasone, 0.5 µmol/L 3-isobutyl-1-methyl-xanthine, and 100 nmol/L insulin for 2 days, after which the medium was replaced with growth medium containing 100 nmol/L insulin for 8 days. For spontaneous adipogenesis assays, confluent primary cultures of APCs were maintained in growth media (described above in Cell Culture section).The level of adipogenesis was evaluated by RT-qPCR measuring the expression levels of markers of adipogenesis and by visualizing the cells with microscopy after Oil Red O staining (Life Line Cell Technology).

For infection with adenovirus, cells were cultured to confluence and infected with adenovirus generated from pAV.ExSiAd-Per3 and/or pAV.ExSiAd-Klf15 vectors (Cyagen) as previously described (Wong et al., 2016). Cells were infected with Ad-GFP for a comparison control. Adipogenesis was induced as described above.

Plasmid Constructs and Vectors
E-box 1 and 2 containing DNA fragments were subcloned into pGL4 luciferase reporter constructs (Promega). Full length *Per3* cDNA was subcloned into a pEF6/V5-His expression plasmid (Life Technologies).pCMV6-Bmal1-Myc construct was purchased from Origene.
PCR based site-directed mutagenesis was performed using the Quick Change Site Directed Mutagenesis kit (Agilent Technologies) following the manufacturer's protocol. shRNA against mBmal1 was purchased from SigmaAldrich. For shRNA adenoviruses against mKlf15, the hairpin sequence (5′ - 3′) GCGGTAAGATGTACATCAAACGTGTGCTGTCCGTTTGGTGTACATCTTGCTGC was cloned into the pEQ-shRNA adenoviral vector (Welgen). sh-Klf15 and sh-scrambled adenoviruses were amplified and purified (Welgen).

Transient Transfections and Reporter Assays
Reporter plasmids were co-transfected into NIH-3T3 cells with expression plasmids or control plasmids in 24-well plates using Lipofectamine 3000 (Invitrogen). Luciferase assays were performed using the Luciferase Assay kit (Promega) according to the manufacturer's protocol.

Co-Immunoprecipitation (Co-IP)
NIH-3T3 cells were transfected with Per3 and/or Bmal1-Myc plasmid constructs and co-immunoprecipitation (co-IP) was performed using the nuclear complex co-IP kit (Active Motif) according to the manufacturer's instructions using antibodies against c-MYC (Cell Signaling Technologies, 2276) or PER3 (Hybridoma clone 8C7). SDS-PAGE fractionation and immunoblot analysis was performed using anti-c-MYC (Cell Signaling Technologies, 2276) or anti-PER3 (Hybridoma clone 5D1) antibodies.

References for Supplemental Experimental Procedures

Aggarwal, A., Prinz-Wohlgenannt, M., Tennakoon, S., Hobaus, J., Boudot, C., Mentaverri, R., Brown, E.M., Baumgartner-Parzer, S., and Kallay, E. (2015). The calcium-sensing receptor: A promising target for prevention of colorectal cancer. Biochim Biophys Acta *1853*, 2158-2167.

Wong, J.C., Krueger, K.C., Costa, M.J., Aggarwal, A., Du, H., McLaughlin, T.L., and Feldman, B.J. (2016). A glucocorticoid- and diet-responsive pathway toggles adipocyte precursor cell activity in vivo. Sci Signal *9*, ra103.