Detailed Supplementary Material and methods

Animal housing

Animal studies were conducted in accordance with the regulations of the veterinary office of the State of Geneva. Mice were maintained under standard animal housing conditions (12h-light–12h-dark), with free access to food and water. *Caveolin1* knockout mice have been described previously [1, 2]. Wheel-running activities were recorded as previously described [3].

Construction of stable cell lines

For the construction of the PER2-TAP expression plasmid, we PCR-amplified full-length *Period2* cDNA (oligos mPer2_F_BglXho and mPer2_R_Bgl; see Table 1) from a cDNA clone kindly provided by S.A. Brown, Zürich. The PCR-product was digested (*Bgl*II) and ligated into the pCMV-C-term-TAP (*Bam*HI) ([4], kindly provided by E. Izaurralde), from which a part of the multicloning site had before been eliminated by opening the vector with *Xho*I/*Sal*I and religation. The final construction encoded an open reading frame for a PER2-TAP fusion protein. For the construction of the TAP-LUCIFERASE expression plasmid, firefly luciferase cDNA was PCR-amplified (oligos Fluc_F_Eco and Fluc_R_Hind) from plasmid pGL3 (Promega) and cloned 3' of the TAP-tag-encoding sequence of vector pCMV-C-term-TAP (cloning sites *Eco*RI/*Hin*dIII). For the generation of stably transfected cells, plasmids were linearized by *Apa*LI, gel-purified, and transfected into NIH3T3 cells using FuGENE6 (Roche). Three days after transfection, cells were selected at a final concentration of 1 mg/ml G418 (Gibco). Clonal colonies were expanded and transgene expression was

assessed by α–TAP-Western blotting from total cell extracts. We selected cell lines that expressed readily detectable levels of PER2-TAP and TAP-LUC for our studies.

Cells and cell culture

NIH3T3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal bovine serum (FBS) and 1% Penicilin-Streptomycin-glutamine (PSG, GIBCO). Primary fibroblasts were prepared from wild-type, homo- and heterozygous *Caveolin1* knockout mice with the same genetic background using Liberase TM (Roche) following the manufacturer's instructions. Primary cells were grown in DMEM supplemented with 20% FBS, 1% PSG, 8 mM non-essential amino acids (Sigma), 1 mM Na-Pyruvate (GIBCO), 0.006 mM β-mercaptoethanol, and 18 mM HEPES (pH 7.0) and cultured at 37°C under low oxygen (2%). Cells were synchronized with 100 nM dexamethasone (Brunschwig), 10 mM forskolin (Alexis), 1 μM PMA (Sigma) or 5.6 nM glucose and real-time bioluminescence was recorded as described [5]. For serum synchronization, culture medium was replaced by medium containing 50% horse serum (GIBCO) for 2h hours before bioluminescence recording.

TAP-tag purification of PER2 complexes and identification of PER2-associated proteins

NIH3T3 cells stably expressing the PER2-TAP construct were treated with 50% horse serum for two hours followed by two hours of serum-free medium, and then harvested. The TAP-tag purification was performed from NUN total cell extract [6] from a 5 ml cell pellet essentially as described [7]. Control cells expressing TAP-LUCIFERASE were submitted to the same procedure. The proteins of the purified complexes were separated on an 8-16% SDS-polyacrylamide gel. Silver-stained protein bands were excised, reduced, alkylated and digested in the gel pieces with trypsin (Promega, WI) as described [8]. After extraction from

the gel pieces, the peptides were dried down, dissolved in 10 μl 5% formic acid and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Peptides were separated using reversed phase chromatography with an Agilent 1100 nanoflow system (Agilent, CA). Tandem mass spectrometry was performed with a 7-tesla hybrid linear ion trap-Fourier transform (LTQ FT) mass spectrometer (Thermo Scientific, Germany). The data sets were searched against the NCBI protein database using the search engine Mascot (Matrix Science, UK) using carbamidomethyl cysteine as a fixed modification and oxidized methionine as a variable modification. Mass tolerances were set to 5 ppm for the precursor ions and 0.5 Da for the fragment ions. The data sets were interpreted using Mascot (Matrix Science, UK).

Plasmids and transfections

The following plasmids were used: Bmal1-luciferase [5], Dbp-luciferase [9]; pCMV-BD, pCMV-BD-PER2, pCMV-BD-V5-PER2, pCMV-AD, pCMV-AD-CRY2, pCMV-BD-Y14, pCMV-AD-Magoh, pFR-Luc (original plasmid: Stratagene) and pRL-control [10]; PKCδ-DN (Addgene); pCMV-V5-PER2, pCMV-PER2-TAP, and pCMV-TAP-LUCIFERASE; pET42TEV-CAVIN-3; pCI-HA and modified vector encoding mouse *Cavin-1*, *Cavin-2*, *Cavin-3, Cavin-3-mut* (generated by site-directed mutagenesis using oligos CAVIN-3-mut F and CAVIN-3-mut R) and *Gadd45gip1* cDNAs. shRNAs were cloned into a modified pSuper-GFP/neo vector (Oligoengine), where the puromycin cassette has replaced the neomycin. For primer sequences, see Table S1. Transient transfections of NIH3T3 cells were carried out with FuGENE 6 Transfection Reagent (Roche) according to the manufacturer's instructions.

Lentivirus production and transduction

The Bmal1-luciferase and HA-CAVIN-3 lentivector particles were produced by calcium

phosphate co-transfection with the two packaging constructs PAX2 and MD2G in HEK293T cells as described [11]. The lentivector-containing supernatants were collected after 72h and filtered through a 0.45-μm pore-sized polyethersulfone membrane. About 60'000 primary tail fibroblasts of each genotype or NIH3T3 cells were plated in 35 mm dishes and transduced with 1 ml of the lentivirus suspension. Blasticidin (Invitrogen) selection was applied from days 3 to 9 after transduction.

Co-immunoprecipitation and immunoblotting

Two days after transfection with the indicated plasmids, cells were synchronized with 50% horse serum. Two hours later, medium was replaced with normal growth medium and cells where harvested two hours later. Proteins were extracted using RIPA buffer (150 mM NaCl, 1% NP40, 0.5% Na-deoxycholate, 0.1% SDS, 50 mM Tris-HCl pH 8.0, protease inhibitors). Cell pellets were homogenized in three volumes of RIPA and incubated on ice for 20 min. Insoluble material was removed by centrifugation (20 min, 20,000***g***, 4°C). Extracts were incubated for 6h at room temperature with the indicated antibodies and protein G magnetic beads (Invitrogen). Beads were washed (3x with TBS, 0.1% NP40, 1x with TBS, 0.1% NP40, 0.4 M NaCl, 1x with TBS, 0.1% TBS, 0.1% NP40), SDS-PAGE sample buffer was added, samples were heated (95°C, 5 min) and separated by SDS-PAGE. For endogenous PER2: CAVIN-3 interaction, four 10 cm dishes of *Per2::Luc* and CMV-luc cells were grown to confluence and synchronized with horse serum and RIPA extracts were prepared as described above. Extracts were incubated for 2.5 hours at room temperature with BSA pre-blocked protein G agarose beads (Roche) together with 150 µl of pre-immune or anti-CAVIN-3 serum. Beads were washed as described above, and luciferase activity was determined in a plate reader using a luciferase assay system (Promega).

SDS-PAGE and immunoblot analysis were performed according to standard protocols

[12]. The following antibodies were used: rabbit anti-PER2 (kindly provided by J. Ripperger, Fribourg), mouse anti-HA (kindly provided by R. Loewith, Geneva), mouse anti-FLAG (SIGMA), mouse anti-V5 (Invitrogen), mouse anti-U2AF65 (Sigma), rabbit anti-NF-κB (Santa Cruz), rabbit anti-CAVIN-3 (see Supplementary Fig. 2), and mouse anti-Protein A (Sigma).

RNA analysis by quantitative real-time RT-PCR

RNA isolated from cultured cells: RNAs were extracted using Trizol (Invitrogen). cDNA was synthesized from total RNA using random hexamers and SuperScript II reverse transcriptase (Invitrogen) following the supplier's instructions. cDNAs were PCR-amplified (Lightcycler 480, Roche) using SYBR Green Master Mix (Roche). Mean levels were calculated from triplicate PCR assays (technical replicas) for each sample and normalized to the levels of *Cyclophilin A* or *Rps9* mRNAs. Primers and probes are listed in Table S1.

RNA isolated from liver tissue: C57BL/6 female mice were entrained under 12h light/12h dark conditions for 2 weeks. Mice were sacrificed at 4h intervals, and liver RNAs were extracted as described [13]. cDNA synthesis and quantitative PCR were performed as described above.

Immunostaining

Immunostaining was performed 2 days after lentiviral transduction as follow: cells growing on coverslips were fixed using 4% paraformaldehyde for 10 min at room temperature, permeabilized with 0.5% Triton X-100 in PBS for 15 min and washed twice for 5 min in PBS. Samples were blocked in 2% BSA in PBS supplemented with 0.05% Tween-20. Primary antibodies (mouse anti-HA) were incubated for 1h at room temperature, coverslips were washed three times for 5 min in PBS, 0.05% Tween-20 before incubation with Alexa 594 or

Alexa 488-conjugated secondary antibodies (Molecular Probes). Nuclei were stained with DAPI. Microscopic images were taken on a Leica SP2 confocal microscope.

Two-hybrid assay

Cells were transfected with the plasmids specified in the text and the figure legends. 24h after transfection, cells were synchronized by serum shock, and luciferase activity was recorded *in vivo* and in real-time as described [14]. For dual luciferase assays, cells were additionally transfected with a pRL-control [10], encoding Renilla luciferase. Samples were synchronized 24h after transfection and harvested 40h later. Dual luciferase measurements were carried out using the Dual Glo Luciferase Assay System (Promega), and firefly luciferase signals were normalized to Renilla luciferase signals after subtraction of background signals from empty wells.

Antibody production

CAVIN-3 full-length cDNA was inserted into vector pET42TEV (kindly provided by S. Thore, Geneva), a bacterial expression vector containing GST and 6xHis tags for N-terminal fusion proteins. After bacterial expression and preparation of a denaturing extract (8M urea), GST-His-CAVIN-3 was affinity-purified on a Ni-NTA matrix (Qiagen). Purified recombinant protein was dialyzed twice against 2000 volumes of 1xPBS and used for the generation of polyclonal antisera in rabbits (Charles River). Sera were used at 1:100 dilution for immunoblotting.

Legends to Supplementary Figures

Supplementary Figure 1: Characterization of the PER2-TAP cell line.

A. *Per2* expression levels in the PER2-TAP and TAP-LUC cell lines, as determined by quantitative real-time RT-PCR before and 2 hours after the serum shock. The *Per2*-SYBR green probe recognized both endogenous *Per2* mRNA and the *Per2-TAP* mRNA (see Table 1). The values were normalized to *Rps9* mRNA **B.** As indicated by the real-time bioluminescence recording, circadian gene expression is abolished in the PER2-TAP cell line.

Supplementary Figure 2: CAVIN-3 expression in *Per2::Luc* and CMV-luc cell lines.

RIPA extract from *Per2::luc* and CMV-luc cell lines were loaded on a 10% acrylamide gel. CAVIN-3 protein was detected using a CAVIN-3 antibody (see Supplementary Figure 3). U2AF65 antibody was used as a loading control.

Supplementary Figure 3: Specificity of rabbit anti-mouse CAVIN-3 antibody.

The specificity of the rabbit antiserum raised against bacterially expressed recombinant CAVIN-3 protein was assessed by immunoblotting. Recombinant CAVIN-3 purified from bacteria, and RIPA extracts from untransfected and GFP-CAVIN-3 transfected NIH3T3 cells were loaded on an 8% acrylamide gel. Rabbit bleeds were used at a 100-fold dilution for immunoblotting experiments.

Supplementary Figure 4: Subcellular localization of CAVIN-3 protein.

NIH3T3 cells were transfected with an HA-CAVIN-3 expression plasmid. Two days after transfection, cells were fixed with 4% paraformaldehyde, and the subcellular localization of CAVIN-3 was examined by immunofluorescence microscopy, using anti-HA antibodies. Z-

stacks of 3.7 μm depth were acquired on a Leica SP2 confocal microscope and are depicted in 16 consecutive frames from top to bottom of the cell.

Supplementary Fig. 5: *Cavin-3* mRNA and protein expression is arrhythmic in NIH3T3 cells and in liver.

A. *Cavin-3* mRNA measured by qPCR in in two independent experimental series (dark and light gray, respectively) of NIH3T3 cells harvested every 4h over 40h after serum synchronization. Within each series, average expression over all time points was arbitrarily set to 1. **B.** Oscillating *Per2* and *Bmal1* mRNA profiles from the same samples as in A. **C.** *Cavin-3* mRNA profile in mouse liver. The data represent the mean ± SD (n=3). **D.** CAVIN-3 protein expression levels in serum-stimulated NIH3T3 cells examined by immunoblotting. PER2 served as a rhythmic control and U2AF65 as a loading control.

Supplementary Figure 6: Temporal accumulation of *Cavin-1*, *Cavin-2* and *Caveolin1* mRNAs in synchronized cells and mouse liver.

A. The temporal accumulation of *Cavin-1* and *Cavin-2* mRNA in serum-synchronized NIH3T3 cells was analyzed by quantitative real-time RT-PCR. Two independent experiments are shown. Within each series, average expression over all time points was arbitrarily set to 1. **B.** *Cavin-1*, *Cavin-2, Caveolin1, Per2* and *Bmal1* mRNA expression profiles determined by quantitative real-time RT-PCR in livers of C57BL/6 females. The data represent the mean ± SD (n=3). *Per2* and *Bmal1* mRNA profiles were used as controls for expression patterns of circadianly expressed transcripts.

Supplementary Figure 7: CAVIN-3 loss-of-function phenotype using the DBP-luc reporter.

NIH3T3 cells were transiently co-transfected with the DBP-luciferase reporter and either an shRNA (*Cavin-3* hp1) targeting *Cavin-3* mRNA (blue lines) or the empty plasmid (black lines). Two days after transfection, cells were synchronized by a forskolin shock followed by real-time bioluminescence recording. Data from duplicate transfections are shown.

Supplementary Figure 8: CAVIN-3 loss-of-function phenotype.

Period lengths (in hours) determined by experiments with expression vectors producing 7 different shRNAs (hp's) targeting *Cavin-3* mRNA, an empty expression vector (control), and an expression vector specifying an irrelevant shRNA (control). Data represent mean ± SD ($n \geq 5$ per conditions) and p-values are calculated relative to the irrelevant hp. Note that the majority of *Cavin-3* shRNAs had a tendency to generate short period phenotypes as well (although to different extents, possibly owing to inferior knockdown efficiencies).

Supplementary Fig. 9: CAVIN-3 gain-of-function phenotype in PER2::luc cells.

A. Bioluminescence rhythms measured from *Per2::luc* primary cells transduced with HA-CAVIN-3 (blue) or GFP (black, control) expressing lentiviral vectors. The data were filtered with a low-pass filter (filfilt function in Matlab). **B.** Period length of control and CAVIN-3 overexpressing PER2::luc cells. Two independent experiments are shown.

Supplementary Figure 10: The chemical nature of the phase-resetting cues influenced the strength of the period length phenotype.

A. Bmal1-luciferase real-time bioluminescence profiles of cells with reduced *Cavin-3* expression levels. Cells were co-transfected with the Bmal1-luciferase reporter and either a *Cavin-3* shRNA expression vector or an empty vector. They were then synchronized using dexamethasone, forskolin, serum, glucose, or PMA as described in Supplementary Materials

and Methods. **B.** Bmal1-luciferase real-time bioluminescence profiles as in A, but upon overexpression of HA-CAVIN-3. **C.** Changes in period length observed in loss- and gain-of-function experiments with cells whose oscillators were synchronized through different signaling pathways. Period length changes are shown relative to the control cells that had received the same synchronization stimulus. Data are mean ± SD (n≥5 per conditions).

Supplementary Figure 11: *Cavin-1, Cavin-2* and *Cavin-3* mRNA expression levels following different synchronization cues.

Accumulation of *Cavin-1* (**A**), *Cavin-2* (**B**), and *Cavin-3* (**C**) mRNAs after the treatment of cells with several synchronization cues, as measured by quantitative real-time RT-PCR. Data represent mean ± SD.

Supplementary Figure 12: Effects of CAVIN-1, CAVIN-2, and CAVIN-3 gain- and loss-of-function on PER2:CRY2 two hybrid signals.

A. Dual luciferase assay quantifying the effect of CAVIN-1, CAVIN-2 and CAVIN-3 overexpression on the PER2:CRY2 interaction. Cells were co-transfected with the two-hybrid system vectors (see text), together with expression plasmids encoding HA-CAVIN-1, HA-CAVIN-2, HA-CAVIN-3, and Renilla luciferase. Luciferase activities were determined in cell lysates 40h after the serum treatment. The values represent firefly to Renilla luciferase signal ratios (mean ± SD; n=10) Note that the scale on the x-axis is logarithmic. **B.** Effect of CAVIN-1, CAVIN-2 and CAVIN-3 depletion on PER2:CRY2 interactions. NIH3T3 cells were co-transfected with the two-hybrid system vectors together with shRNAs targeting either *Cavin-1* mRNA, *Cavin-2* mRNA, or *Cavin-3* mRNA. 40 hours after serum treatment, Renilla and firefly luciferase activities were determined. The values are given on a logarithmic scale and represent firefly luciferase signals normalized to Renilla luciferase signals (mean ± SD;

n=5).

Supplementary Figure 13: BD-V5-PER2 interacts avidly with AD-CRY2.

BD-V5-PER2:AD-CRY2 interaction measured *in vivo* and in real-time after treatment with 50% horse serum. As expected, CAVIN-3 co-expression (blue) strongly increases the V5-PER2:CRY2 interaction (black).

Supplementary Figure 14: CAVIN-3 affects BD-V5-PER2 and AD-CRY proteins abundance.

Quantification of the western blot shown in Figure 3F using ImageJ software.

Supplementary Figure 15: CAVIN-3 overexpression decreases endogenous PER2 levels.

A. Cells were transfected with HA-CAVIN-3 or the empty vector (control). Two days after transfections, cells were synchronized using 50% horse serum and samples were taken before (0), four (4) or seven (7) hours following the serum shock. Samples were loaded on a 10% polyacrylamide gel, and PER2 and CAVIN-3 were detected using PER2 and HA antibodies, respectively. U2AF65 antibody was used as a loading control. **B.** Quantification of the Western blot shown in A.

Supplementary Figure 16: Titration of transfected amounts of PER2:CRY2 two-hybrid-partners and effect of CAVIN-3 overexpression.

Dual luciferase assay quantifying the effect of CAVIN-3 overexpression on the PER2:CRY2 interaction. Cells were co-transfected with different amounts (from 10 to 400ng of plasmids) of the two-hybrid system vectors (see text) together with expression plasmids encoding pCI-HA (control) or HA-CAVIN-3 and Renilla luciferase. Luciferase activities were determined

in cell lysates 40h after serum treatment. The values represent firefly to Renilla luciferase signal ratios (mean ± SD; n=3) Note that the scale on the X-axis is logarithmic.

Supplementary Fig. 17: Schematic representation of CAVIN-3-mut construct.

The threonine and the two serines within the PKC binding domain of CAVIN-3 were mutated to alanines, as indicated.

Supplementary Figure 18: Locomotor activity of *Caveolin1* knockout mice.

A. Wheel-running activity was recorded for *Caveolin1*$^{+/+}$ mice (wild-type), *Caveolin1*$^{+/-}$ mice (heterozygous knockout), and *Caveolin1*$^{-/-}$ mice (homozygous knockout) in constant darkness (DD) as described in (Lopez-Molina et al., 1997). Before the mice were shifted to DD (marked by gray shading), they were phase-entrained by 12h-light-12h-dark cycles (LD). Representative double-plot actograms obtained for two animals of each genotype are shown. **B.** Average free-running period length of 4 animals per genotype, mean ± SD.

References

1. Parton RG, Simons K (2007) The multiple faces of caveolae. *Nat Rev Mol Cell Biol* **8:** 185-194
2. Drab M *et al* (2001) Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* **293:** 2449-2452
3. Lopez-Molina L, Conquet F, Dubois-Dauphin M, Schibler U (1997) The DBP gene is expressed according to a circadian rhythm in the suprachiasmatic nucleus and influences circadian behavior. *Embo J* **16:** 6762-6771
4. Goodier JL, Zhang L, Vetter MR, Kazazian HH, Jr. (2007) LINE-1 ORF1 protein localizes in stress granules with other RNA-binding proteins, including components of RNA interference RNA-induced silencing complex. *Mol Cell Biol* **27:** 6469-6483
5. Nagoshi E, Saini C, Bauer C, Laroche T, Naef F, Schibler U (2004) Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell* **119:** 693-705
6. Lavery DJ, Schibler U (1993) Circadian transcription of the cholesterol 7 alpha hydroxylase gene may involve the liver-enriched bZIP protein DBP. *Genes Dev* **7:** 1871-1884
7. Puig O, Caspary F, Rigaut G, Rutz B, Bouveret E, Bragado-Nilsson E, Wilm M, Seraphin B (2001) The tandem affinity purification (TAP) method: a general procedure of protein complex purification. *Methods* **24:** 218-229
8. Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem* **68:** 850-858
9. Brown SA, Ripperger J, Kadener S, Fleury-Olela F, Vilbois F, Rosbash M, Schibler U (2005) PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator. *Science* **308:** 693-696
10. Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* **125:** 1111-1124
11. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L (1998) A third-generation lentivirus vector with a conditional packaging system. *J Virol* **72:** 8463-8471
12. Sambrook J, Maniatis T, Fritsch EF (1989) *Molecular cloning : a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
13. Schmidt EE, Schibler U (1995) Cell size regulation, a mechanism that controls cellular RNA accumulation: consequences on regulation of the ubiquitous transcription factors Oct1 and NF-Y and the liver-enriched transcription factor DBP. *J Cell Biol* **128:** 467-483
14. Nagoshi E, Brown SA, Dibner C, Kornmann B, Schibler U (2005) Circadian gene expression in cultured cells. *Methods Enzymol* **393:** 543-557

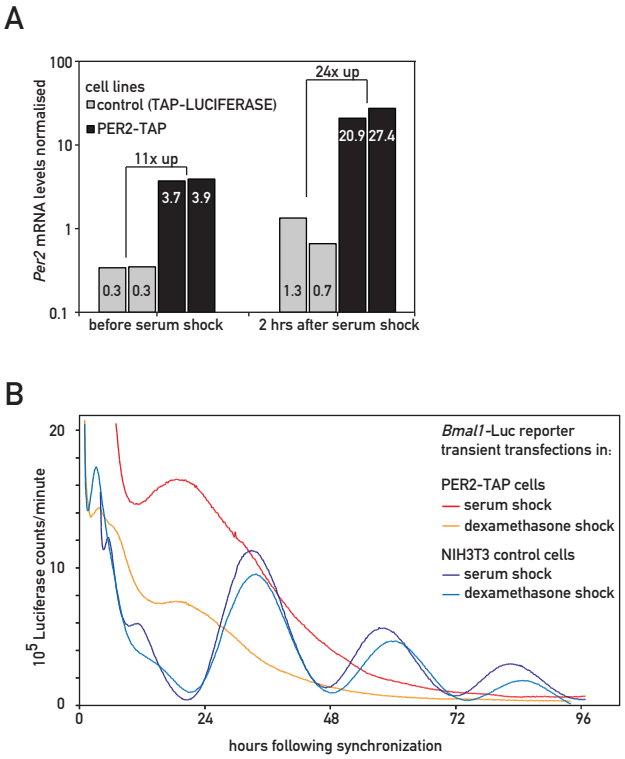
Table S1

Oligo name	oligo sequence
shRNAs	
CAVIN-3 hp1 upper	GATCCCCGAAACCCGTGCTTCAAATA TTCAAGAGA TATTTGAAGCACGGGTTTCTTTTTA
CAVIN-3 hp1 lower	AGCTTAAAAAGAAACCCGTGCTTCAAATATCTCTTGAATATTTGAAGCACGGGTTTCGGG
CAVIN-3 hp2 upper	GATCCCCGGTTTACAGAAAGTTCAAA TTCAAGAGA TTTGAACTTTCTGTAAACCTTTTTA
CAVIN-3 hp2 lower	AGCTTAAAAAGGTTTACAGAAAGTTCAAATCTCTTGAATTTGAACTTTCTGTAAACCGGG
CAVIN-3 hp3 upper	GATCCCCGTGACCTTGCTGGAGAAATTTCAAGAGAATTTCTCCAGCAAGGTCACTTTTTA
CAVIN-3 hp3 lower	AGCTTAAAAAGTGACCTTGCTGGAGAAATTCTCTTGAAATTTCTCCAGCAAGGTCACGGG
CAVIN-3 hp4 upper	GATCCCCGGCTCTTTCCAGTCGTAAATTCAAGAGATTTACGACTGGAAAGAGCCTTTTTA
CAVIN-3 hp4 lower	AGCTTAAAAAGGCTCTTTCCAGTCGTAAATCTCTTGAATTTACGACTGGAAAGAGCCGGG
CAVIN-3 hp5 upper	GATCCCC GCTGCGCCGCACTGGTTTA TCTCTTGAA TAAACCAGTGCGGCGCAGC TTTTTA
CAVIN-3 hp5 lower	AGCTTAAAAA GCTGCGCCGCACTGGTTTATTCAAGAGATAAACCAGTGCGGCGCAGC GGG
CAVIN-3 hp6 upper	GATCCCC GGAGGAGACTGAAATTCCA TCTCTTGAA TGGAATTTCAGTCTCCTCC TTTTTA
CAVIN-3 hp6 lower	AGCTTAAAAA GGAGGAGACTGAAATTCCATTCAAGAGATGGAATTTCAGTCTCCTCC GGG
CAVIN-3 hp7 upper	GATCCCC GGCTCTGCGGGAGCGGCAAGGCGAACCTTGCCGCTCCCGCAGAGCC TTTTTA
CAVIN-3 hp7 lower	AGCTTAAAAA GGCTCTGCGGGAGCGGCAAGGTTCGCCTTGCCGCTCCCGCAGAGCC GGG
CAVIN-3 hp8 upper	GATCCCC GCACGCCGACGCAGCCCAGGACGAATCCTGGGCTGCGTCGGCGTGC TTTTTA
CAVIN-3 hp8 lower	AGCTTAAAAA GCACGCCGACGCAGCCCAGGATTCGTCCTGGGCTGCGTCGGCGTGC GGG
CAVIN-2 hp1 upper	GATCCCC GCACACACTCCTGGATAAA TTCAAGAGA TTTATCCAGGAGTGTGTGC TTTTTA
CAVIN-2 hp1 lower	AGCTTAAAAA GCACACACTCCTGGATAAA TCTCTTGAA TTTATCCAGGAGTGTGTGC GGG
CAVIN-2 hp1 upper	GATCCCC GAAGAGAAGATGGAAGAAA TTCAAGAGA TTTCTTCCATCTTCTCTTC TTTTTA
CAVIN-2 hp2 lower	AGCTTAAAAA GAAGAGAAGATGGAAGAAA TCTCTTGAA TTTCTTCCATCTTCTCTTC GGG
CAVIN-1 hp1 upper	GATCCCCGCTGAGCCTTCTGGATAAA TTCAAGAGA TTTATCCAGAAGGCTCAGCTTTTTA
CAVIN-1 hp1 lower	AGCTTAAAAAGCTGAGCCTTCTGGATAAATCTCTTGAATTTATCCAGAAGGCTCAGCGGG
CAVIN-1 hp2 upper	GATCCCCGCCAGGCCGGCCAGATAAA TTCAAGAGA TTTATCTGGCCGGCCTGGCTTTTTA
CAVIN-1 hp2 lower	AGCTTAAAAAGCCAGGCCGGCCAGATAAATCTCTTGAATTTATCTGGCCGGCCTGGCGGG
PKCdelta hp1 upper	GATCCCC GGTCACATATCTCTTTAAA TTCAAGAGA TTTAAAGAGATATGTGACC TTTTTA
PKCdelta hp1 lower	AGCTTAAAAA GGTCACATATCTCTTTAAA TCTCTTGAA TTTAAAGAGATATGTGACC GGG
irrelevant hp lower	GATCCCCGGTCTGACAGTAGTTCTTATTCAAGAGATAAGAACTACTGTCAGACCTTTTTG
irrelevant hp upper	TTTTCAAAAAGGTCTGACAGTAGTTCTTATCTCTTGAATAAGAACTACTGTCAGACCGGG
Cloning	
CAVIN-3 pCI EcoR1 F	AAGAATTCatgggggagagcgcactggagcccgg
CAVIN-3 pCI Sal1 R	TTTGTCGACtcaggctgcgctctctatttgaagcac
CAVIN-1 pCI EcoR1 F	AAGAATTCatggaggatgtcacgctccatatc
CAVIN-1 pCI Xho R	TTCTCGAGtcagtcgctgtcgctcttgtcc
CAVIN-2 pCI MluI F	ACGACGCGTatgggagaggacgctgcaca
CAVIN-2 pCI Sal1 F	CGAGTCGACtcaggcagtctgatccacat
CRY2 pAD Not1 F	ATATGCGGCCGCCCACCatggcggcggctgctgtgg
CRY2 pAD Xho R	GACTCGAGtcaggagtccttgcttgctg
HA CAVIN-3 lenti F	ggggacaagtttgtacaaaaaagcaggctcgccaccgctagcatggcttctagctatcc
HA CAVIN-3 lenti R	ggggaccactttgtacaagaaagctgggtatcaggctgcgctctctattt
pAD FLAG Cry2 Not1 F	aatgcggccgcgtGATTACAAGGATGACGACGATAAGatggcggcggctgctgtggt
pAD FLAG Cry2 Xho1 R	aatCTCGAGtcaggagtccttgcttgctg
pBD BglII V5 BamH1 STOP F	gatctGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGggatccTGAa
pBD V5 BamH1 STOP BgII R	gatctTCAggatccCGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCa
Fluc_F_Eco	aggaattctATGGAAGACGCCAAAAACATAAAG
Fluc_R_Hind	tttaagcTTACACGGCGATCTTTCCGC
mPer2_F_BglXho	aacagatctcgagccaccatgaatggatacgtggacttc
mPer2_R_Bgl	GTTAGATCTcgtctgggcctctatcctgg
mPer2_UP	ATGCTCGCCATCCACAAGA
mPer2_DOWN	GCGGAATCGAATGGGAGAAT
rps9F	GACCAGGAGCTAAAGTTGATTGGA
rps9R	TCTTGGCCAGGGTAAACTTGA
CAVIN-3-mut F	gctgcgccgcgctGGTTTACAGAAAGTTCAAgctCTGAAAAGGGCTCTTgctagtcgtaaag
CAVIN-3-mut R	ctttacgactagcAAGAGCCCTTTTCAGagcTTGAACTTTCTGTAAACCagcgcggcgcagc
SYBR qPCR	
mPER2 F	ATGCTCGCCATCCACAAGA
mPER2 R	GCGGAATCGAATGGGAGAAT
mBmal1 F	CCAAGAAAGTATGGACACAGACAAA
mBmal1 R	GCATTCTTGATCCTTCCTTGGT
CAVIN-3 F	CACGTCCTGCTCTTCAAGGA
CAVIN-3 R	GAGCTCTGGGACTTTCTGGA
CAVIN-1 F	GTCAGCGTCAACGTGAAGAC
CAVIN-1 R	CCTCGTTGACCTCCAGTTTC
CAVIN-2 F	GCAGAAAAGTTCCAGCATCC
CAVIN-2 R	TCTCCTGGAGCATGTCTGTG
Cyclophilin F	GGAGATGGCACAGGAGGAA
Cyclophilin R	GCCCGGTAGTGCTTCAGCTT
Caveolin1 F	ccattctctccttcctgcac
Caveolin1 R	tctctttctgcgtgctgatg

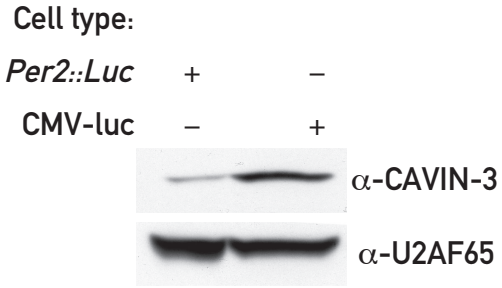
Table S1

Oligo name	oligo sequence
Genotyping	
Cav1 wt F	TGTTTAAACCCATTCCTGCTCT
Cav1 wt R	CTAAACCTTGATCCTGGTGAGG
Cav1 KO F	TCCTCCTATTGCGGTGTGT
Cav1 KO R	CCTGCGTGCAATCCATCTTGTTCAATG

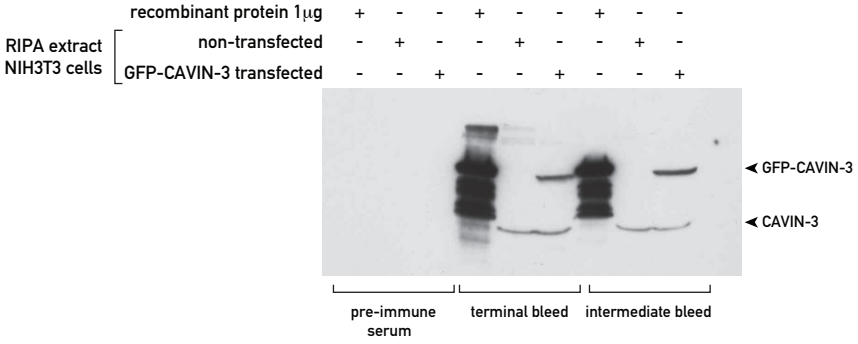
Supplementary Fig. 1



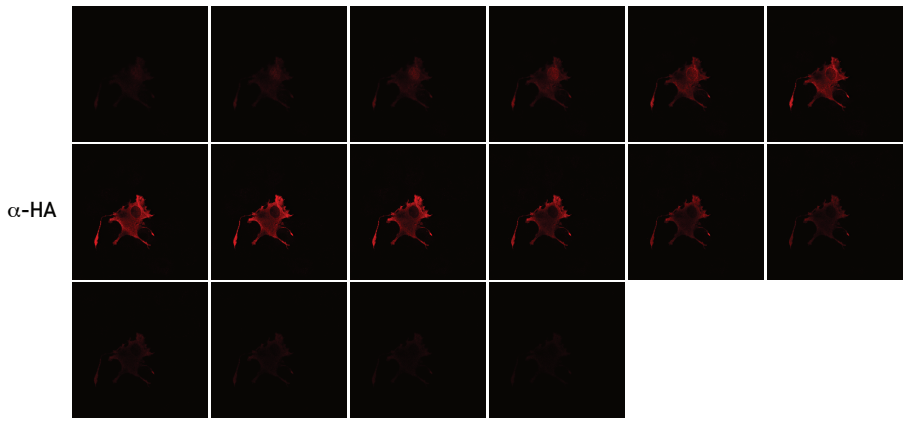
Supplementary Fig. 2



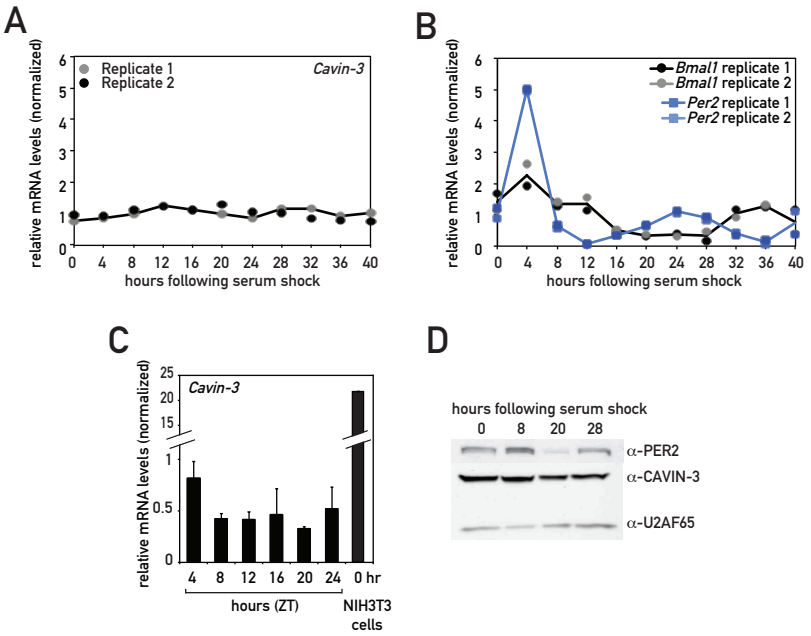
Supplementary Fig. 3



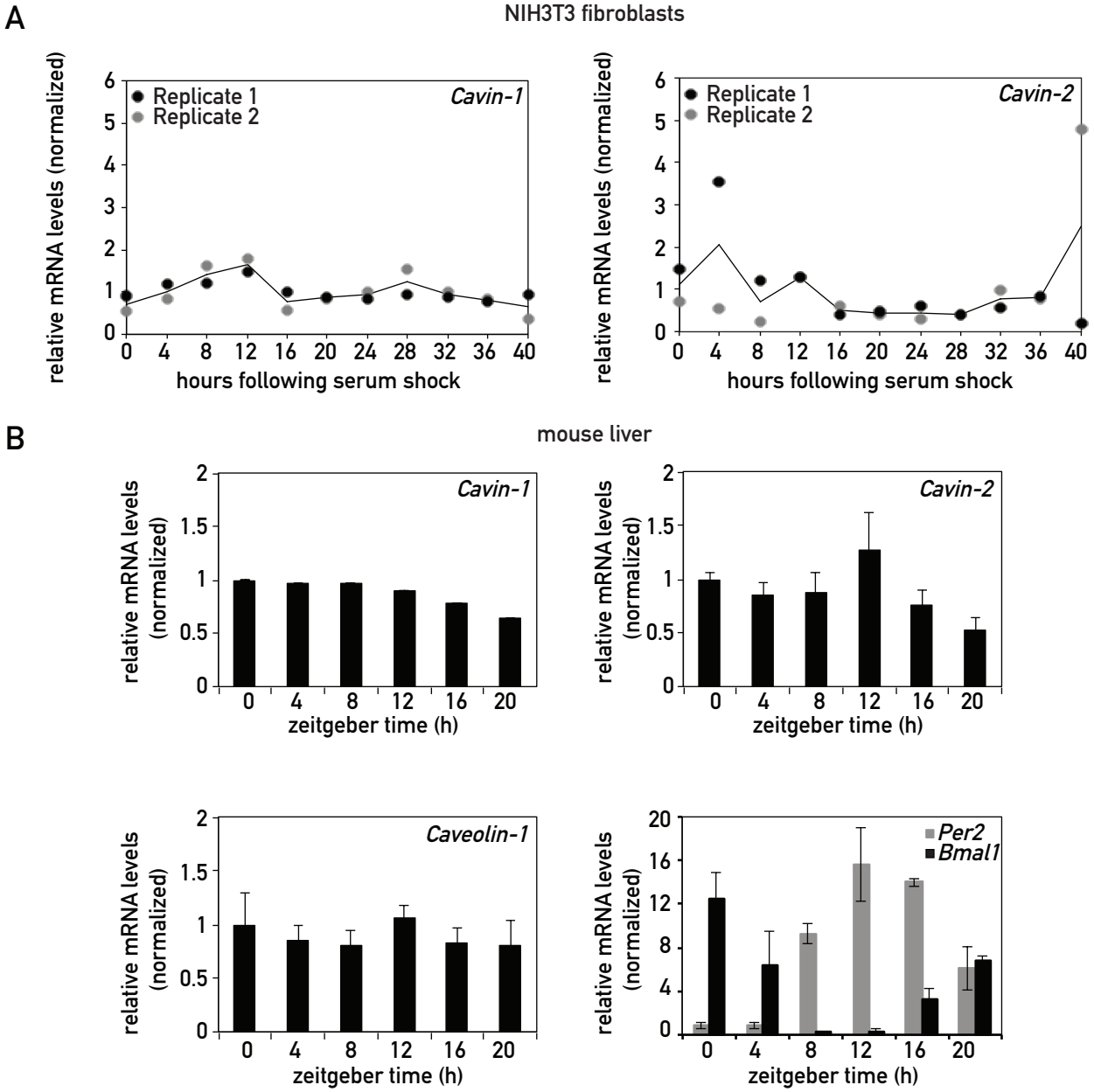
Supplementary Fig. 4



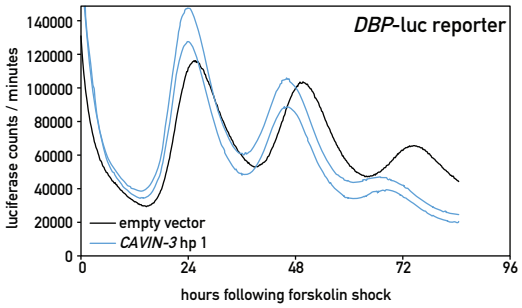
Supplementary Fig. 5



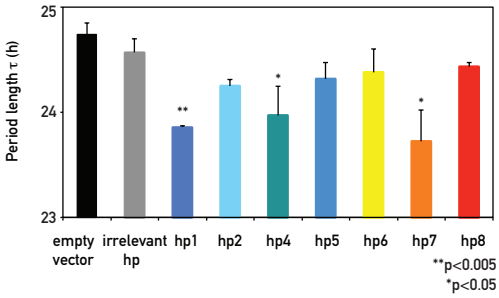
Supplementary Fig. 6



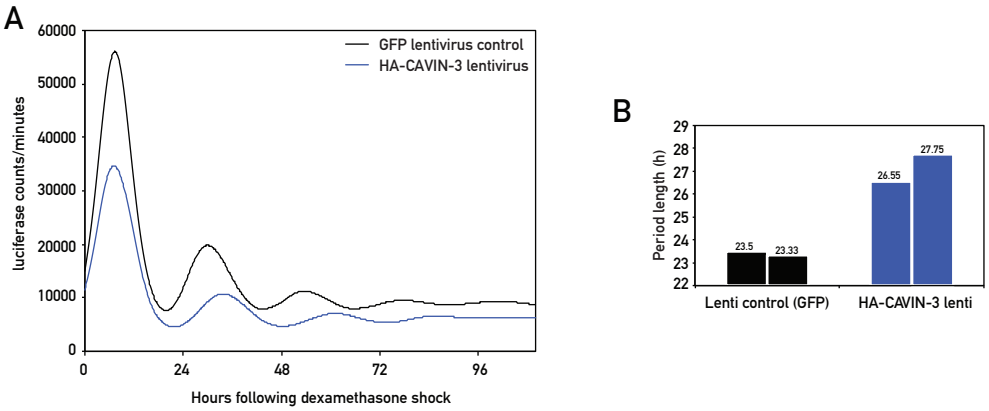
Supplementary Fig.7



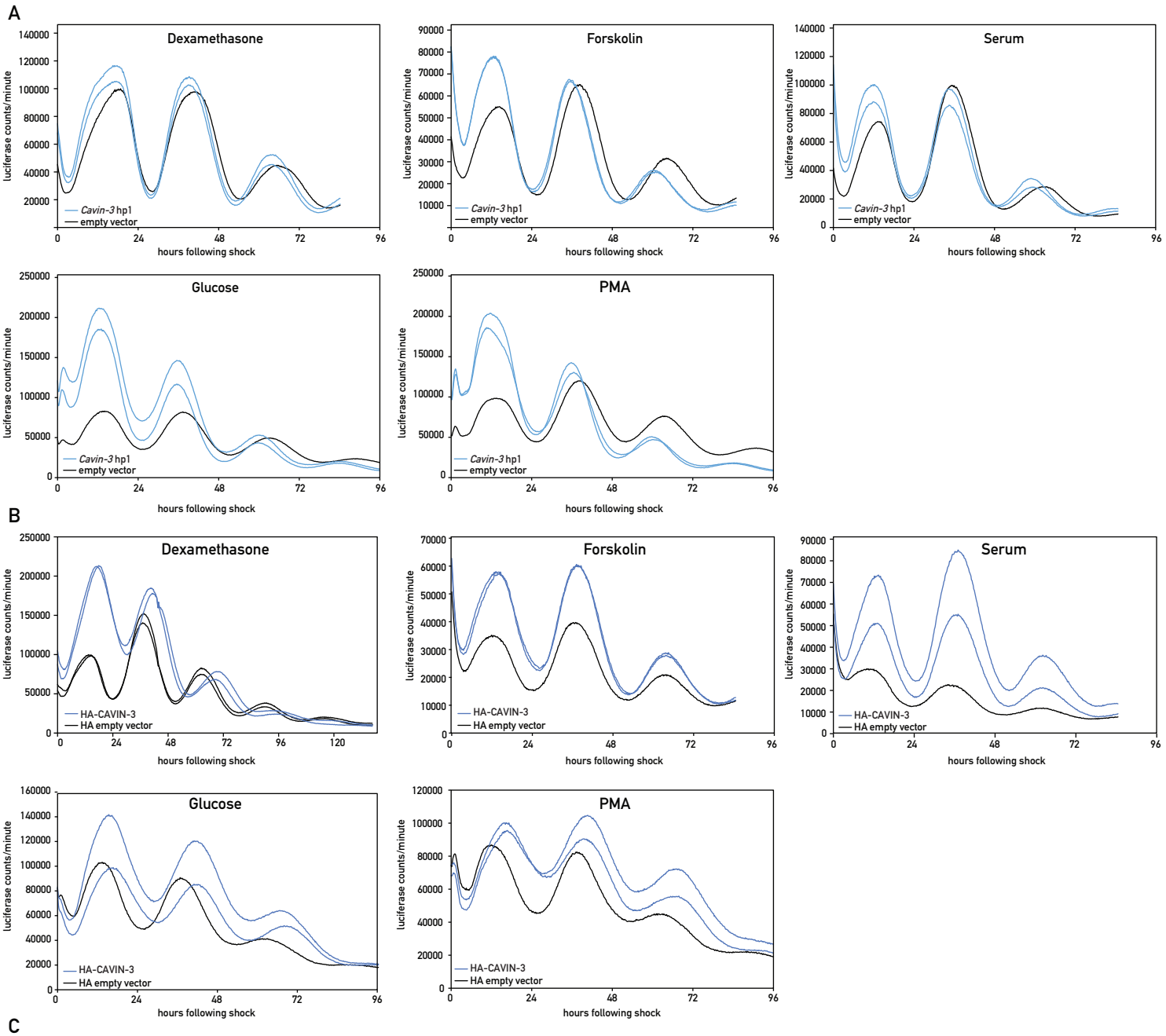
Supplementary Fig. 8



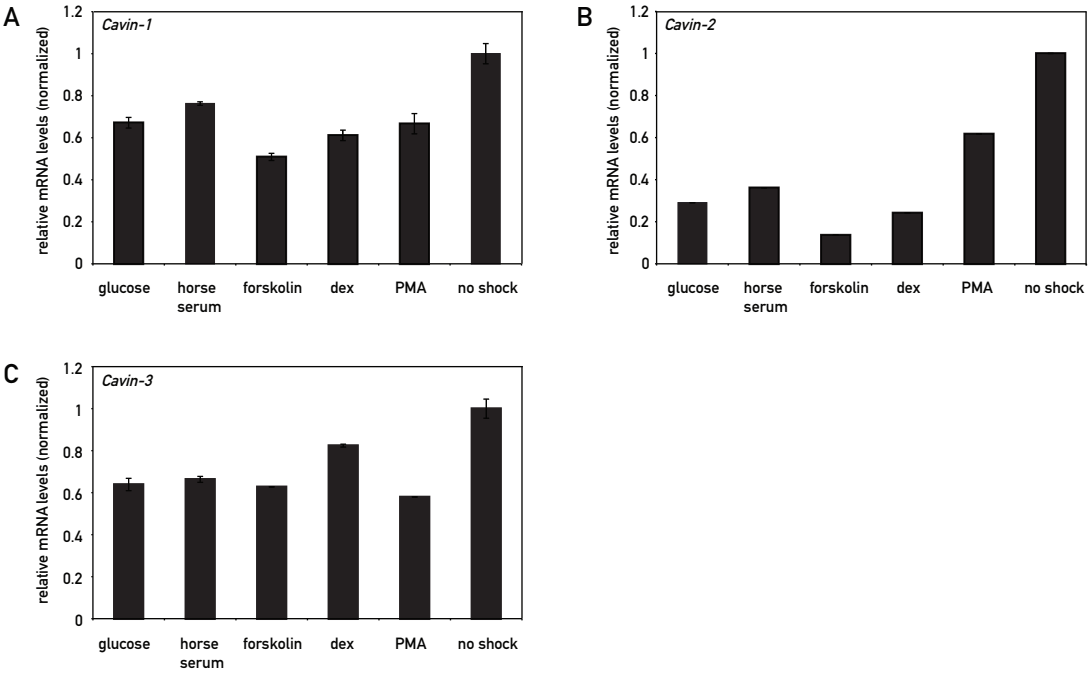
Supplementary Fig. 9



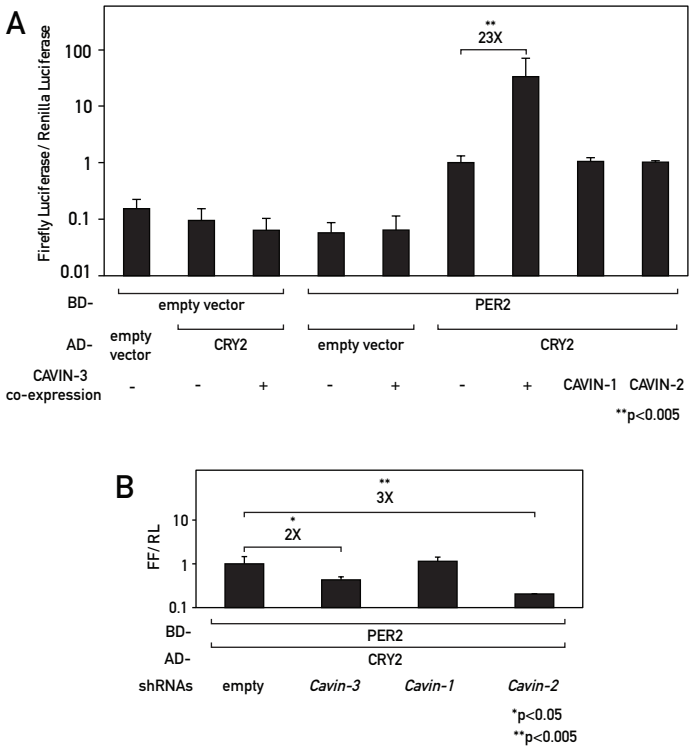
Supplementary Fig. 10



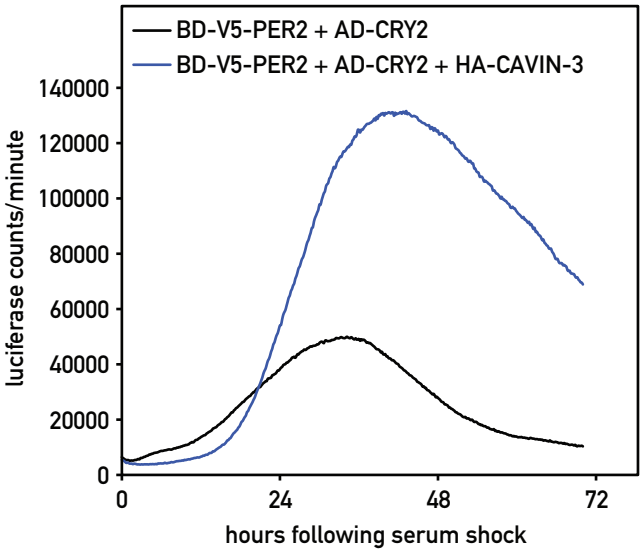
Supplementary Fig. 11



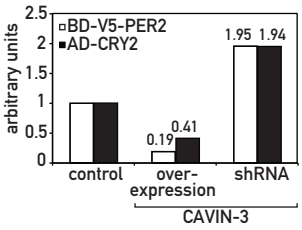
Supplementary Fig. 12



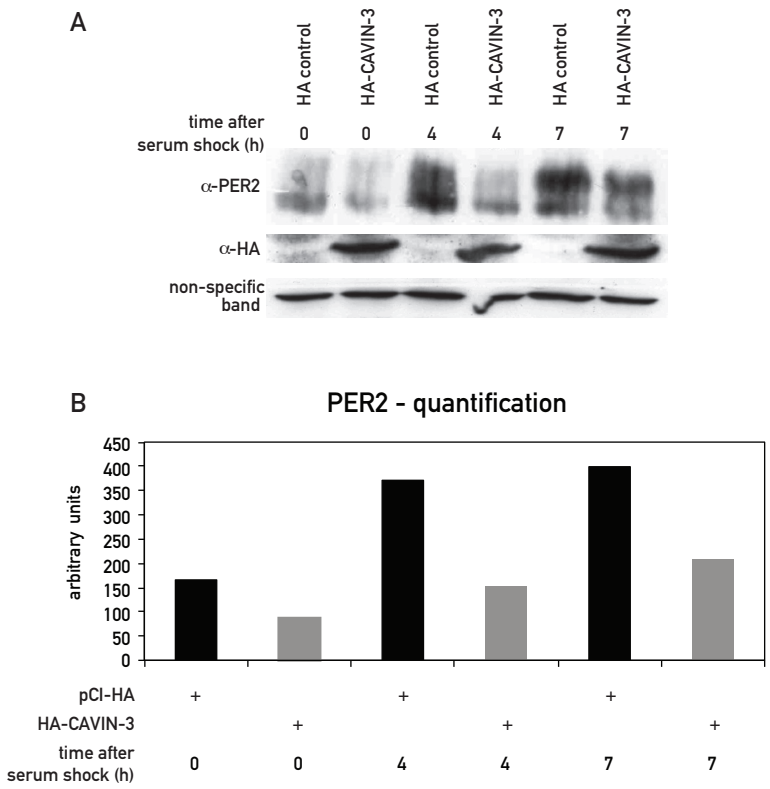
Supplementary Fig. 13



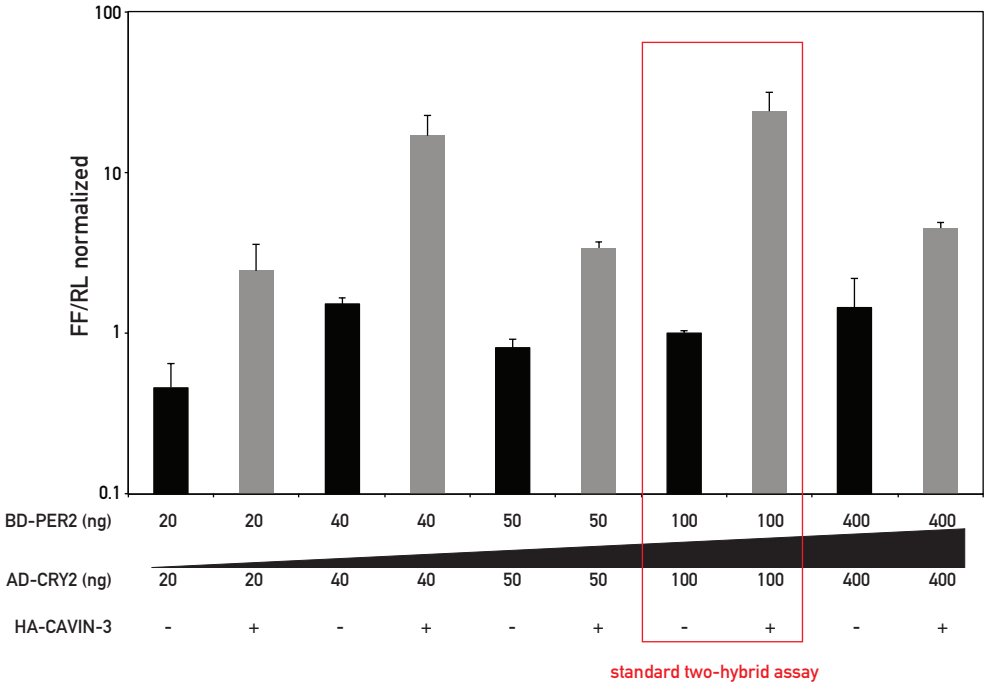
Supplementary Fig.14



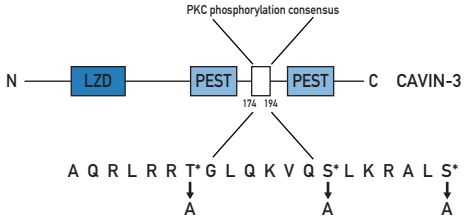
Supplementary Fig. 15



Supplementary Fig. 16



Supplementary Fig. 17



Supplementary Fig. 18