**Supplemental Table 1. Clinical characteristics of study subjects.**

| Parameter | Value |
|---|---|
| n | 17 |
| Age [years] | 46.4 ± 2.4 |
| Body weight [kg] | 99.8 ± 1.9 |
| BMI [kg/m$^2$] | 32.9 ± 0.5 |
| Waist circumference [cm] | 111.2 ± 1.7 |
| Waist to hip ratio | 1.01 ± 0.01 |
| Total body fat [%] | 33.1 ± 1.2 |
| Fasting glucose [mg/dl] | 90.4 ± 1.4 |
| Fasting insulin [mU/l] | 12.8 ± 1.7 |
| HOMA-IR | 2.89 ± 0.40 |

Data are shown as mean± SEM

**Supplemental Table 2. Primers used for real-time qPCR and promoter mutagenesis**

| Gene | Primer name | Sequence |
|---|---|---|
| **Human qPCR primers** | | |
| *NR1D1 (REV-ERBα)* | NR1D1_F1 | 5’-CCAGCAATGTCGCTTCAAGAA-3’ |
| | NR1D1_R1 | 5’-TCTGCATCTCAGCAAGCATCC-3’ |
| *NR1D2 (REV-ERBβ)* | NR1D2_F | 5’-CAGATGTCAGCAATGTCGCTTC-3’ |
| | NR1D2_R | 5’-GCATCCTCTGTTTTTCACGCTT-3’ |
| *PER1* | PER1_F | 5’-ATTCCGCCTAACCCCGTATGT-3’ |
| | PER1_R | 5’-CCGCGTAGTGAAAATCCTCTTG-3’ |
| *PER2* | PER2_F | 5’-AGCAGGTGAAAGCCAATGAAG-3’ |
| | PER2_R | 5’-AGGTAACGCTCTCCATCTCCTC-3’ |
| *PER3* | PER3_F | 5’-GTCCAAGCCTTACAAGCTGGTTT-3’ |
| | PER3_R | 5’-GACCGTCCATTTGTTGGCAT-3’ |
| *CLOCK* | CLOCK_F | 5’-ATTCCACAAGGCATGTCCCA-3’ |
| | CLOCK_R | 5’-TTTGCTTCTATCATGCGTGTCC-3’ |
| *BMAL1* | BMAL1_F | 5’-CATTAAGAGGTGCCACCAATCC-3’ |
| | BMAL1_R | 5’-CAAAAATCCATCTGCTGCCC-3’ |
| *CRY1* | CRY1_F | 5’-GGGACCTGTGGATTAGTTGGG-3’ |
| | CRY1_R | 5’-GCTCCAATCTGCATCAAGCAA-3’ |
| *CRY2* | CRY2_F | 5’-TGCATCTGTTGACACTCATGATTC-3’ |
| | CRY2_R | 5’-GGTACTCCCCCAGCCCAG-3’ |

| | | |
|---|---|---|
| *RORA* | RORA_F | 5’-ACAACCAGCGGGAGGTGA-3’ |
| | RORA_R | 5’-GTTTGGCAAACTCCACCACA-3’ |
| *DBP* | DBP_F | 5’-GAAAAATCCAGGTGCCGGA-3’ |
| | DBP_R | 5’-CGTTGTTCTTGTACCGCCG-3’ |
| *TEF* | TEF_F | 5’-AACCGTGTCCAGCACAGAATCT-3’ |
| | TEF_R | 5’-GGTCCGGATTGAAGTTCACATC-3’ |
| *RPLP0* | QT00075012 | QuantiTect Primer Assay (Qiagen) |
| **Mouse qPCR primers** | | |
| *Bmal1* | QT00101647 | QuantiTect Primer Assay (Qiagen) |
| *Clock* | QT00197547 | QuantiTect Primer Assay (Qiagen) |
| *Cry1* | QT00117012 | QuantiTect Primer Assay (Qiagen) |
| *Cry2* | QT00168868 | QuantiTect Primer Assay (Qiagen) |
| *Dbp* | QT00103089 | QuantiTect Primer Assay (Qiagen) |
| *NR1D1* | QT00164556 | QuantiTect Primer Assay (Qiagen) |
| *Per1* | QT00113337 | QuantiTect Primer Assay (Qiagen) |
| *Per2* | QT00198366 | QuantiTect Primer Assay (Qiagen) |
| *c-Fos* | QT00147308 | QuantiTect Primer Assay (Qiagen) |
| 36B4 | fw36B4 | TCATCCAGCAGGTGTTTGACA |
| | rv36B4 | GGCACCGAGGCAACAGTT |
| **Promoter mutagenesis primers** | | |
| mPer2-CRE | mPer2-CRE-MUT fw | CCAGAACAATGTAGCCACCATTGAATTCAATGTAAGCGAGGAAAC |
| | mPer2-CRE-MUT rv | GTTTCCTCGCTTACATTGAATTCAATGGTGGCTACATTGTTCTGG |
| mPer2-E-BOX | mPer2-E-BOX fw | GGGCGGGCTCAGCGCGCGCGGTGCTAGTTTCCACTATGTGACAGCGGAGG |

| | | |
|---|---|---|
| | mPer2-E-BOX rv | CCTCCGCTGTCACATAGTGGAAACTAGCACCGCGCGCGCTGAGCCCGCCC |
| mPer2-NFY | fw_NFY_mut | GGCACTCCGACCAAAGGCGCGCGCA |
| | rv_NFY_mut | TGCGCGCGCCTTTGGTCGGAGTGCC |
| mPer2-SP1 | fw_SP1_mut | CAATGGCGCGCGCAGGTTTGGGCTCAGCGCGCGCG |
| | rv_SP1_mut | CGCGCGCGCTGAGCCCAAACCTGCGCGCGCCATTG |
| mPer2-NFY-SP1 | fw_NFY_SP1_mut | GCACTCCGACCAAAGGCGCGCGCAGGTTTGGGCTCAGCGCGCGCG |
| | rv_NFY_SP1_mut | CGCGCGCGCTGAGCCCAAACCTGCGCGCGCCTTTGGTCGGAGTGC |

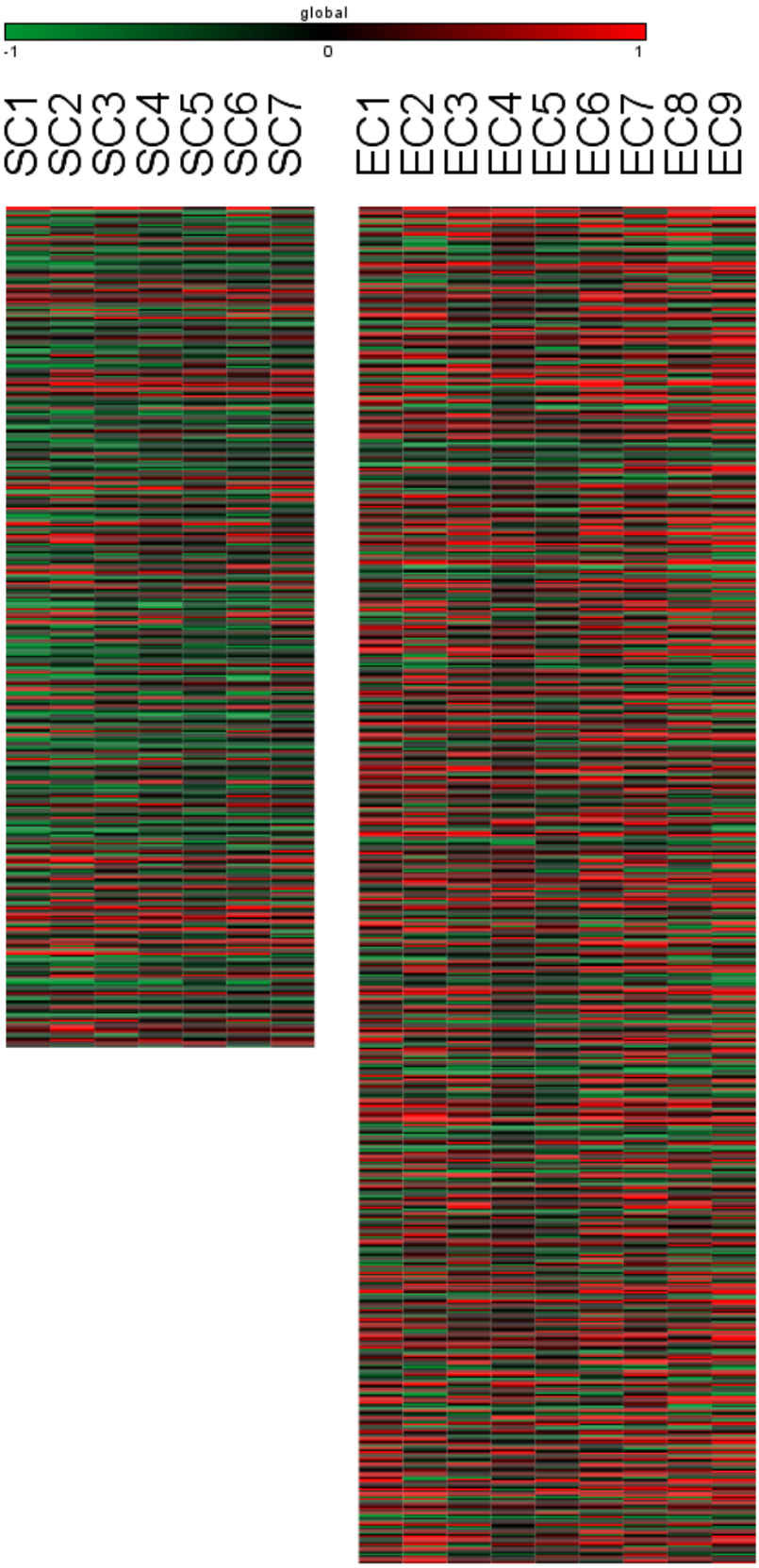
**Supplemental Table 3. Signaling pathways affected in adipose tissue upon the saline infusion and the euglycemic hyperinsulinemic clamp**

| KEGG ID | Pathway name | Gene number (total) | % | P-value | Genes |
|---|---|---|---|---|---|
| **Saline infusion** | | | | | |
| hsa04310 | Wnt signaling pathway | 11 (141) | 7.8 | 0.0007 | CXXC4, AXIN2, FZD10, BAMBI, CACYBP, TCF7, SOX17, PRICKLE2, WNT11, NFATC1, DAAM1 |
| hsa04934 | Cushing syndrome | 10 (152) | 6.6 | 0.0043 | ATF4, AXIN2, AGTR1, FZD10, TCF7, ITPR1, CDK2, LDLR, WNT11, GNAI3 |
| hsa05200 | Pathways in cancer | 22 (499) | 4.4 | 0.0054 | ZBTB16, JAK2, AXIN2, AGTR1, FZD10, EDNRB, JAK3, IL12RB1, NOTCH1, SMO, TCF7, FGF18, CDK2, BCL2L11, TGFB2, HSP90AB1, RASSF5, WNT11, GNAI3, GNG7, HES5, RARA |
| hsa05203 | Viral carcinogenesis | 11 (193) | 5.7 | 0.0081 | HIST1H2BB, YWHAB, ATF4, YWHAQ, HIST1H2BG, HDAC6, JAK3, MAD1L1, YWHAB, CDK2, HIST1H2BD, REL |
| hsa04390 | Hippo signaling pathway | 9 (151) | 6.0 | 0.0122 | YWHAB, YWHAQ, AXIN2, CCN2, FZD10, YWHAB, SMAD7, TCF7, TGFB2, WNT11 |
| hsa05202 | Transcriptional misregulation in cancer | 10 (180) | 5.6 | 0.0135 | ZBTB16, CEBPB, PER2, PLAU, FUT8, SIN3A, HHEX, CDK14, REL, RARA |
| hsa04659 | Th17 cell differentiation | 7 (106) | 6.6 | 0.0156 | JAK2, NFKBIE, JAK3, IL12RB1, HSP90AB1, NFATC1, RARA |
| hsa05217 | Basal cell carcinoma | 5 (62) | 8.1 | 0.0181 | AXIN2, FZD10, SMO, TCF7, WNT11 |
| hsa03060 | Protein export | 3 (23) | 13.0 | 0.0183 | SEC61B, SRPRB, SEC63 |
| hsa04658 | Th1 and Th2 cell differentiation | 6 (89) | 6.7 | 0.0224 | JAK2, NFKBIE, JAK3, IL12RB1, NOTCH1, NFATC1 |
| hsa04933 | AGE-RAGE signaling pathway in | 6 (95) | 6.3 | 0.0297 | JAK2, AGTR1, EDN1, PLCD4, TGFB2, NFATC1 |

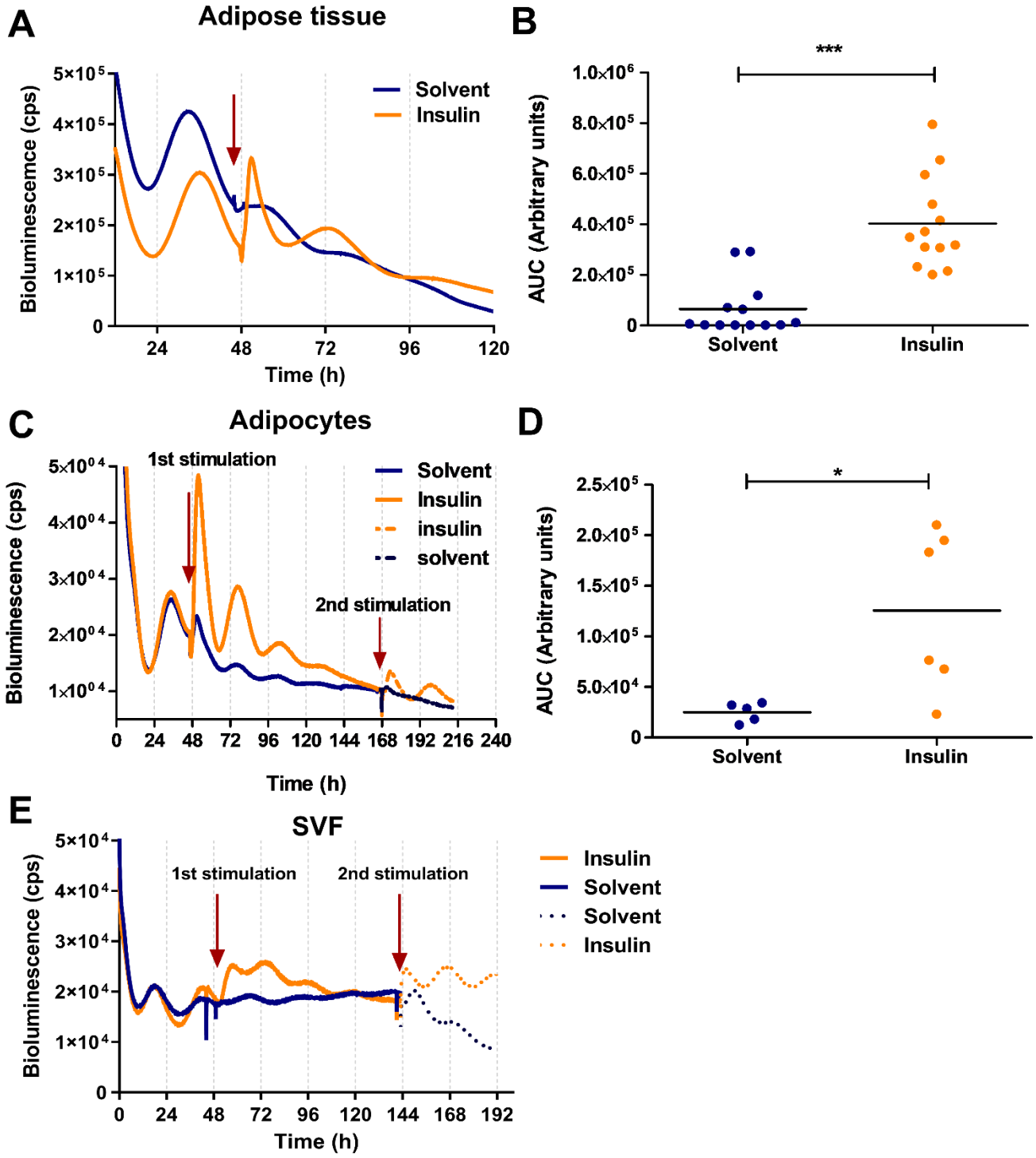
| | | | | | |
|---|---|---|---|---|---|
| | diabetic complications | | | | |
| hsa04916 | Melanogenesis | 6 (98) | 6.1 | 0.0339 | FZD10, EDN1, EDNRB, TCF7, WNT11, GNAI3 |
| hsa04710 | Circadian rhythm | 3 (30) | 10.0 | 0.0370 | PER2, NR1D1, PER1, NR1D1 |
| hsa00533 | Glycosaminoglycan biosynthesis | 2 (13) | 15.4 | 0.0396 | FUT8, B3GNT2 |
| hsa05206 | MicroRNAs in cancer | 8 (157) | 5.1 | 0.0403 | DDIT4, FOXP1, RDX, PLAU, NOTCH1, SOX4, BCL2L11, TGFB2, SOX4, |
| hsa04915 | Estrogen signaling pathway | 7 (130) | 5.4 | 0.0417 | FKBP5, ATF4, HSPA8, ITPR1, HSP90AB1, GNAI3, RARA |
| hsa04550 | Signaling pathways regulating pluripotency of stem cells | 7 (134) | 5.2 | 0.0478 | JAK2, ID3, AXIN2, FZD10, JAK3, WNT11, LIFR, LIFR |
| **Euglycemic hyperinsulinemic clamp** | | | | | |
| hsa05202 | Transcriptional misregulation in cancer | 18 (180) | 10.0 | $1.08 \times 10^{-5}$ | ZBTB16, GADD45B, CEBPB, BCL11B, PER2, CEBPE, SP1, CEBPA, MAF, CEBPB, HHEX, HOXA9, LMO2, BCL6, SIX1, CEBPA, SIN3A, HOXA10, GRIA3, RARA |
| hsa04713 | Circadian entrainment | 10 (93) | 10.8 | 0.0006 | PER2, PLCB1, PER1, ITPR1, CAMK2G, PER3, RASD1, GNAI3, PLCB1, GNG7, GRIA3 |
| hsa05200 | Pathways in cancer | 28 (499) | 5.6 | 0.0016 | MSH2, ZBTB16, GLI1, RAC3, GADD45B, PIK3R1, AXIN2, PTGER4, PTGER2, NKX3-1, FZD10, NCOA3, SP1, CEBPA, IL7R, FGF18, NOTCH1, PLCB1, FZD4, LAMA2, FGF18, CAMK2G, FZD5, HSP90AB1, RASSF5, CEBPA, GNAI3, PLCB1, GNG7, HES5, RARA |
| hsa04750 | Inflammatory mediator regulation of TRP channels | 9 (93) | 9.7 | 0.0023 | PLA2G4A, MAP2K3, PIK3R1, PTGER4, PTGER2, TRPV1, PLCB1, ITPR1, CAMK2G, PLCB1 |
| hsa04915 | Estrogen signaling pathway | 11 (130) | 8.5 | 0.0023 | FKBP4, PIK3R1, CREB5, NCOA3, SP1, PLCB1, GPER1, ITPR1, HSP90AB1, GNAI3, CREB5, CREB5, PLCB1, CREB5, RARA |
| hsa04152 | AMPK signaling pathway | 10 (118) | 8.5 | 0.0036 | SREBF1, PIK3R1, CREB5, RPTOR, PPP2R3B, PFKFB3, IRS2, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | PFKFB3, CREB5, CAB39L, CREB5, TBC1D1, PPP2R1B, CREB5 |
| hsa04310 | Wnt signaling pathway | 11 (141) | 7.8 | 0.0044 | RAC3, AXIN2, FZD10, CTNNBIP1, PLCB1, FZD4, CACYBP, CAMK2G, FZD5, PRICKLE2, PLCB1, NKD1 |
| hsa05224 | Breast cancer | 11 (141) | 7.8 | 0.0044 | GADD45B, PIK3R1, AXIN2, FZD10, NCOA3, SP1, FGF18, NOTCH1, FZD4, FGF18, FZD5, HES5 |
| hsa04725 | Cholinergic synapse | 9 (110) | 8.2 | 0.0071 | KCNJ14, PIK3R1, CREB5, PLCB1, ITPR1, CAMK2G, KCNJ12, GNAI3, CREB5, CREB5, PLCB1, GNG7, CREB5 |
| hsa04730 | Long-term depression | 6 (56) | 10.7 | 0.0076 | PLA2G4A, PLCB1, ITPR1, GNAI3, PLCB1, PPP2R1B, GRIA3 |
| hsa04934 | Cushing syndrome | 11 (152) | 7.2 | 0.0077 | AXIN2, CREB5, FZD10, SP1, PLCB1, FZD4, ITPR1, CAMK2G, FZD5, RASD1, GNAI3, CREB5, CREB5, PLCB1, CREB5 |
| hsa04070 | Phosphatidylinositol signaling system | 8 (96) | 8.3 | 0.0098 | PI4KB, PIK3R1, PIK3C2B, IP6K2, PLCD3, PLCB1, ITPR1, PIK3C3, PLCB1 |
| hsa05217 | Basal cell carcinoma | 6 (62) | 9.7 | 0.0123 | GLI1, GADD45B, AXIN2, FZD10, FZD4, FZD5 |
| hsa04924 | Renin secretion | 6 (63) | 9.5 | 0.0132 | PTGER4, PTGER2, ADRB1, PLCB1, ITPR1, GNAI3, PLCB1 |
| hsa04710 | Circadian rhythm | 4 (30) | 13.3 | 0.0133 | PER2, NR1D1, PER1, NR1D1, PER3 |
| hsa05221 | Acute myeloid leukemia | 6 (64) | 9.4 | 0.0142 | ZBTB16, PIK3R1, PER2, CEBPE, CEBPA, CEBPA, RARA |
| hsa00051 | Fructose and mannose metabolism | 4 (32) | 12.5 | 0.0166 | GMPPB, MPI, SORD, PFKFB3, PFKFB3, SORD |
| hsa04728 | Dopaminergic synapse | 9 (128) | 7.0 | 0.0181 | CREB5, PPP2R3B, PLCB1, ITPR1, CAMK2G, GNAI3, CREB5, CREB5, PLCB1, PPP2R1B, GNG7, GRIA3, CREB5 |
| hsa04024 | cAMP signaling pathway | 12 (195) | 6.2 | 0.0184 | GLI1, RAC3, PIK3R1, PTGER2, CREB5, SOX9, SSTR2, ADRB1, PDE4B, CAMK2G, GNAI3, CREB5, CREB5, GRIA3, CREB5 |
| hsa04390 | Hippo signaling pathway | 10 (151) | 6.6 | 0.0192 | YWHAQ, AXIN2, SCRIB, PRKCI, CCN2, FZD10, FZD4, FZD5, PPP2R1B, NKD1 |

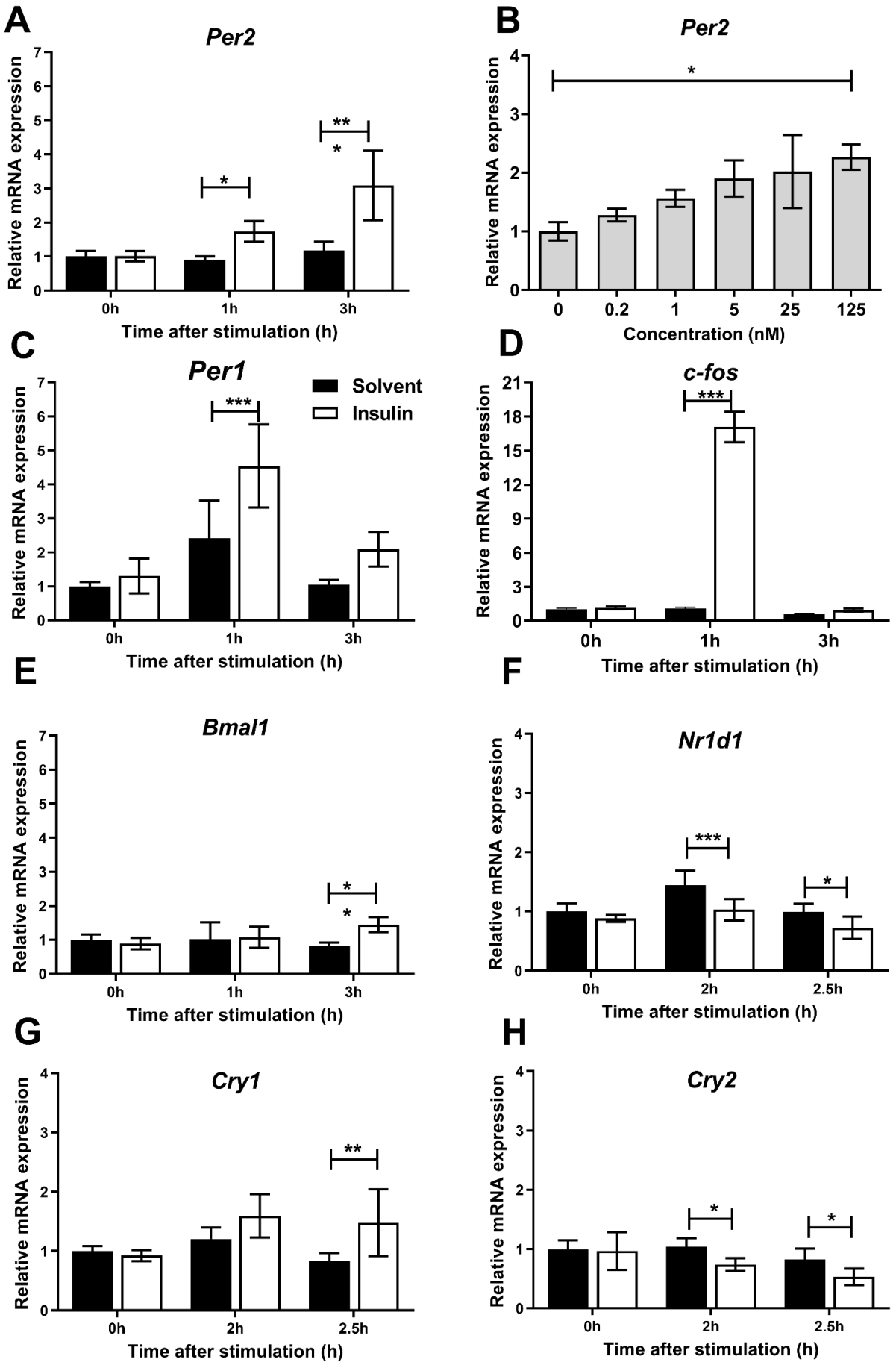
| | | | | | |
|---|---|---|---|---|---|
| hsa04550 | Signaling pathways regulating pluripotency of stem cells | 9 (134) | 6.7 | 0.0237 | PIK3R1, AXIN2, FZD10, SMARCAD1, JARID2, FZD4, POU5F1, FZD5, LIFR |
| hsa04916 | Melanogenesis | 7 (98) | 7.1 | 0.0327 | FZD10, EDN1, PLCB1, FZD4, CAMK2G, FZD5, GNAI3, PLCB1 |
| hsa05165 | Human papillomavirus infection | 16 (315) | 5.1 | 0.0363 | PIK3R1, AXIN2, PTGER4, CREB5, SCRIB, PRKCI, FZD10, ITGA11, PPP2R3B, NOTCH1, FZD4, LAMA2, NFX1, FZD5, CREB5, ITGA11, CREB5, PPP2R1B, CREB5, HES5 |
| hsa04150 | mTOR signaling pathway | 9 (146) | 6.2 | 0.0382 | DDIT4, PIK3R1, FZD10, SESN2, RPTOR, FZD4, FZD5, FLCN, CAB39L |
| hsa04928 | Parathyroid hormone synthesis, secretion and action | 7 (102) | 6.9 | 0.0394 | AKAP13, CREB5, SP1, PLCB1, PDE4B, ITPR1, AKAP13, AKAP13, GNAI3, CREB5, CREB5, PLCB1, CREB5 |
| hsa04664 | Fc epsilon RI signaling pathway | 5 (64) | 7.8 | 0.0484 | PLA2G4A, MAP2K3, RAC3, PIK3R1, MS4A2 |



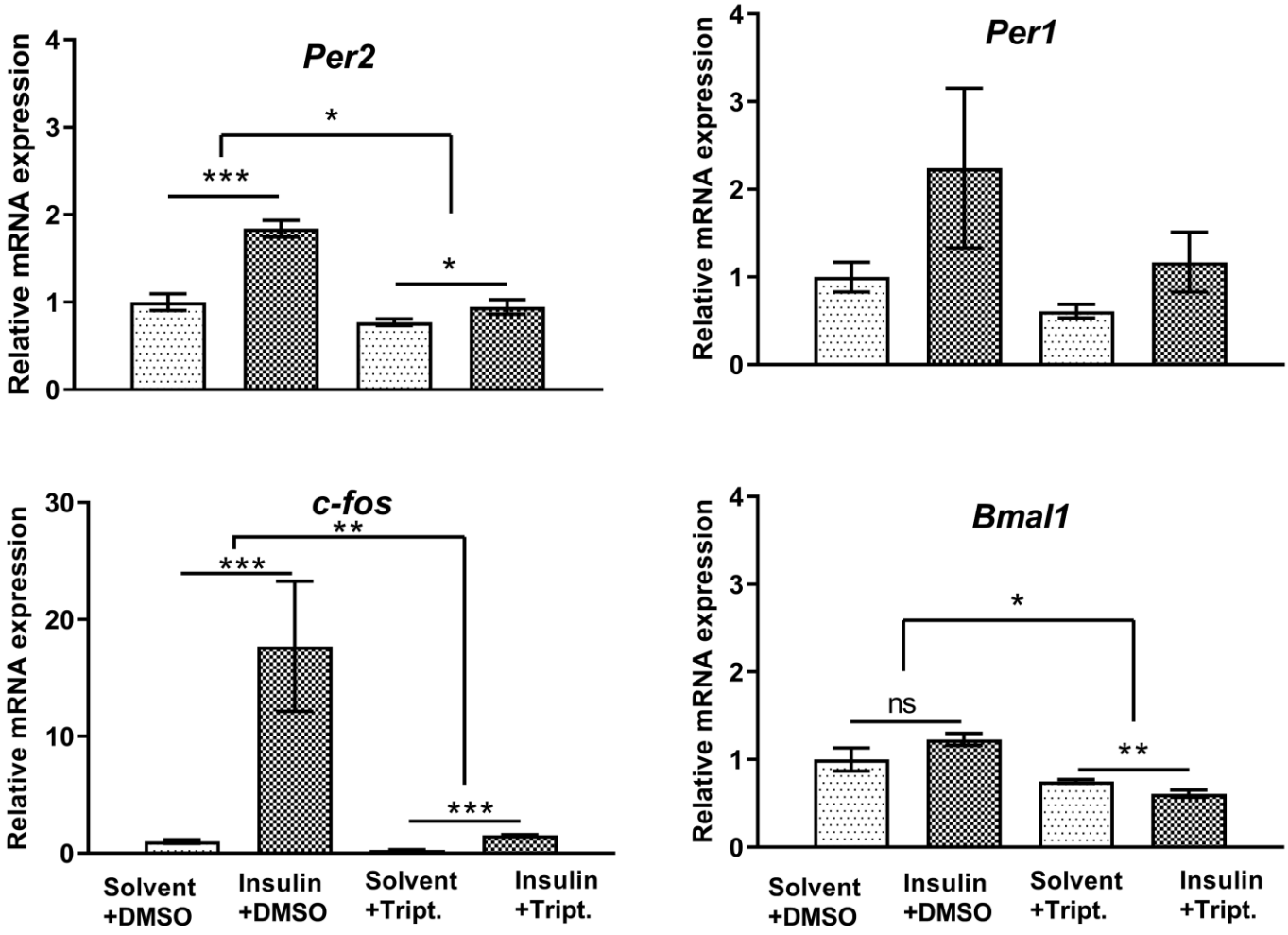

**Supplemental Figure 1. Heatmap representation of fold changes of all transcripts in SC and EC experiments depicted using a logarithmic color scale.** Expression decrease at 240 min in relation to -40 min value is shown in green and expression increase is shown in red. Heatmap was build using the GENE-E software (https://software.broadinstitute.org/GENE-E/).


A
Adipose tissue
Bioluminescemce (cps)
5×10⁵
4×10⁵
3×10⁵
2×10⁵
1×10⁵
0
24
48
72
96
120
Time (h)
Solvent
Insulin
B
***
AUC (Arbitrary units)
1.0×10⁶
8.0×10⁵
6.0×10⁵
4.0×10⁵
2.0×10⁵
0
Solvent
Insulin
C
Adipocytes
Bioluminescence (cps)
1st stimulation
2nd stimulation
Solvent
Insulin
insulin
solvent
0 24 48 72 96 120 144 168 192 216 240
Time (h)
D
*
AUC (Arbitrary units)
2.5×10⁵
2.0×10⁵
1.5×10⁵
1.0×10⁵
5.0×10⁴
0
Solvent
Insulin
E
SVF
Bioluminescence (cps)
5×10⁴
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
1st stimulation
2nd stimulation
0 24 48 72 96 120 144 168 192
Time (h)
Insulin
Solvent
Solvent
Insulin

**Supplemental Figure 2. Insulin leads to an increase in mPER2::LUC activity in adipose tissue and isolated adipocytes.** Real-time bioluminescence recordings of epididymal adipose tissues explants (**A-B**), adipose tissue derived adipocytes (**C-D**) and stromal vascular fraction (**E**) from mPer2$^{Luc}$ mice. **A and C:** Adipose tissue (**A**) and adipocytes (**C**) were stimulated with either 100 nM insulin or solvent. The second stimulation with insulin was applied to firstly solvent stimulated cells and vice versa (C). Time of stimulation is indicated by the red arrow. Results of one representative experiment are shown. **B and D:** The area under the induction curve induced by the stimulation were determined. (**B**) Adipose tissue stimulation. Overall, n=13 for solvent and insulin in eight independent experiments; h=hours; stimulations were performed 3-4 h (n=2), 8-9 h (n=2), 11-12 h (n=3), 15-16 h (n=3), 20-21 h (n=2), or 23-24 h (n=1) after peak. Data are shown as mean. (**D**) Adipocyte stimulation. Stimulations were performed 1-12 hours after peak. n=5 for solvent and n=6 for insulin. Data are shown as mean. *p<0.05, ***p<0.001 by the Mann-Whitney test. **E.** Stromal vascular fraction (SVF) derived from adipose tissue explants of PER2::LUC mice stimulated with 100nM insulin or solvent (red arrows).

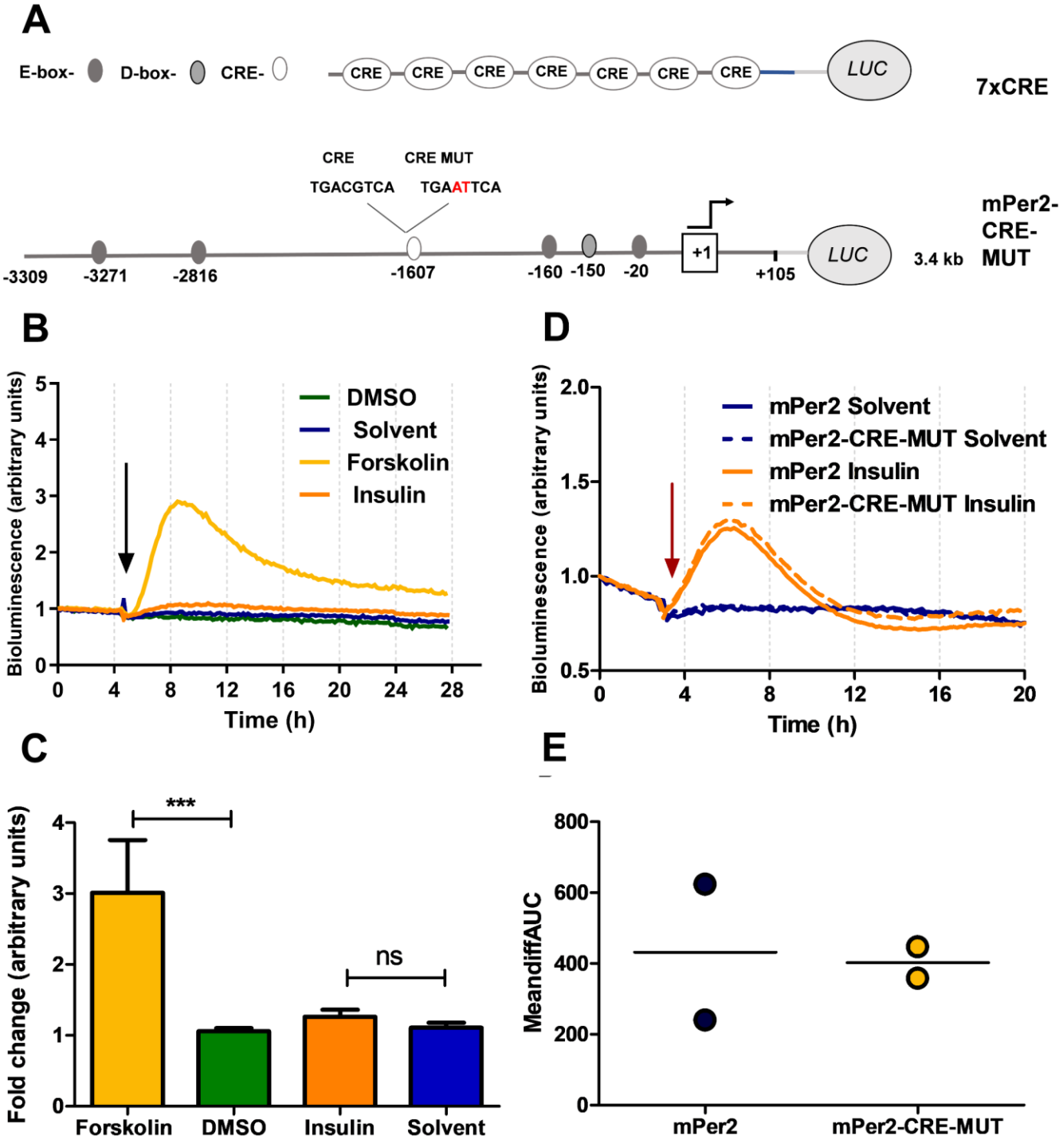

A
Per2
Relative mRNA expression
Time after stimulation (h)
0h
1h
3h
B
Per2
Relative mRNA expression
Concentration (nM)
0
0.2
1
5
25
125
C
Per1
Solvent
Insulin
Relative mRNA expression
Time after stimulation (h)
D
c-fos
Relative mRNA expression
Time after stimulation (h)
E
Bmal1
Relative mRNA expression
Time after stimulation (h)
F
Nr1d1
Relative mRNA expression
Time after stimulation (h)
2h
2.5h
G
Cry1
Relative mRNA expression
Time after stimulation (h)
H
Cry2
Relative mRNA expression
Time after stimulation (h)

**Supplemental Figure 3. Insulin transcriptionally regulates clock genes and immediate early genes in 3T3L1 adipocytes.** 3T3-L1 adipocytes were serum starved for 24h and subsequently stimulated with either solvent or 100nM insulin for 1 - 3h (**A, C-H**). Expression of mRNA for *Per2 (***A***), Per1 (***C***), c-fos (***D***), Bmal1 (***E***), Nr1d1 (***F***), Cry1* (**G**), and *Cry2* (**H**) was determined with qPCR. (**B**) Concentration-dependent regulation of the Per2 expression by insulin. Data show the results in mean±SD of six biological replicates (n=6). *p<0.05, **p<0.01, ***p<0.001 by two-way ANOVA and Bonferroni post-hoc test.

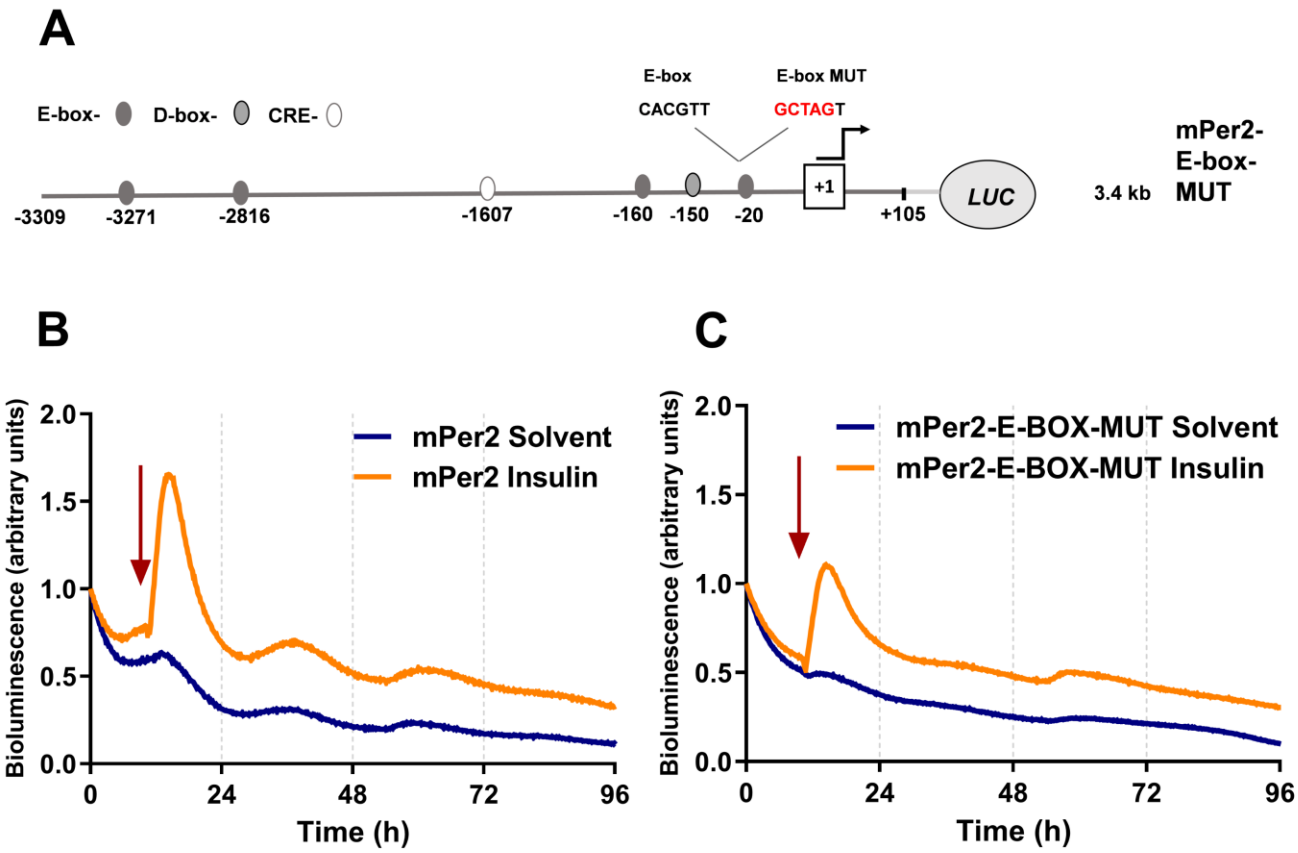



**Supplemental Figure 4. RNA polymerase II blocker attenuates the insulin-dependent induction of clock gene expression.**

3T3-L1 adipocytes were serum-starved for 24 hours and subsequently stimulated with either triptolide (Tript.) or its solvent DMSO during the treatment with 100 nM insulin or solvent for 1 hour (mPer1 and mc-fos) or 2 hours (mPer2 and mBmal1). The results are shown as mean ± SD of one experiment with three technical replicates (n=3). Data was normalized to the treatment with solvent + DMSO. *p<0.05, **p<0.01, ***p<0.001 by Student's t test.


A
E-box-
D-box-
CRE-
CRE
CRE
CRE
CRE
CRE
CRE
CRE
LUC
7xCRE
CRE
CRE MUT
TGACGTCA
TGAATTCA
-3309
-3271
-2816
-1607
-160
-150
-20
+1
+105
LUC
3.4 kb
mPer2-CRE-MUT
B
Bioluminescence (arbitrary units)
DMSO
Solvent
Forskolin
Insulin
Time (h)
D
Bioluminescence (arbitrary units)
mPer2 Solvent
mPer2-CRE-MUT Solvent
mPer2 Insulin
mPer2-CRE-MUT Insulin
Time (h)
C
Fold change (arbitrary units)
***
ns
Forskolin
DMSO
Insulin
Solvent
E
MeandiffAUC
mPer2
mPer2-CRE-MUT

**Supplemental Figure 5. Insulin-inducible expression from the mPer2 promoter is CRE independent. (A**) Schematic representation of the 7xCRE reporter construct containing seven repeats of the Cyclic AMP Responsive Element (CRE) upstream of a minimal promoter (blue) fused to a luciferase (LUC) (above) and of the 3.4 kb mPer2 promoter reporter construct (below). The CRE binding site was mutated using site directed mutagenesis (red font, mPer2-CRE-MUT). **B and C**: Bioluminescence recordings of 3T3-L1 preadipocytes harboring the 7xCRE luciferase reporter construct. (**B**) Cells were stimulated with 10 µM forskolin (n=2), DMSO (n=1), 100 nM insulin (n=3) or solvent (n=1) (time of stimulation indicated by the black arrow). Raw bioluminescence data are shown. (**C**). Fold change of luminescence after the stimulation (n=4). *p<0.05, **p<0.01, ***p<0.001 by Kruskal-Wallis test with Dunn´s multiple comparison test. **D and E:** Bioluminescence recordings of 3T3-L1 pre-adipocytes harboring the mPer2- or the mPer2-CRE-MUT promoter luciferase reporter construct. (**D**). Cells were stimulated with either 100 nM insulin (n=3) or solvent (n=2) (time of stimulation indicated by the red arrow). Raw bioluminescence data are shown. (**E**). Mean difference of the area under the induction curve (MeandiffAUC) determined for each construct (n=2).




**Supplemental Figure 6. Insulin-inducible expression from the mPer2 promoter is E-box -20 independent.** (**A**) Schematic representation of the 3.4 kb mPer2 promoter reporter construct. The E-box, 20 bp upstream was mutated using site directed mutagenesis (red font). LUC: luciferase, MUT: mutation, +1 represent the transcriptional starting site. **B and C:** Real-time bioluminescence recordings of 3T3-L1 cells. 3T3-L1 preadipocytes harboring the mPer2- (**B**) or the mPer2-E-box-MUT (**C**) promoter luciferase reporter construct were stimulated with either 100 nM insulin or solvent (time of stimulation indicated by the red arrow) (n=2 biological replicates for each construct and treatment).


5′
3′
125
E-box (-3271)
250
375
500
E-box (-2816)
625
750
875
1000
1125
1250
1375
1500
1625
1750
CRE (-1607)
1875
2000
2125
2250
2375
2500
2625
2750
2875
3000
3125
3250
E-box (-160)
D-box (-150)
3375
NFY (-58)
GC-box (-42)
E-box (-20)
TSS
3′
3475
5′
Met
Glu
Asp
luciferase

**Supplemental Figure 7. Mouse Per2 promoter sequence within a luciferase reporter construct.** Putative binding sites were screened using the MatInspector software and depicted using the SnapGene software (https://www.snapgene.com/). Per2 promoter sequence is indicated by the blue font and the translation start site of the luciferase gene is shown in red font. Binding sites are indicated using followind colors: green - E-box (5´-CACGTT-3´), dark green - D- box (5´-TTATGTAA-3´), blue - CRE (5´-TGACGTCA-3´), orange – NFY (5´-CCAAT-3´), and yellow – GC-box/SP1 binding site (5´-GGGCGG-3´). TSS (transcription start site) is indicated with a grey box.


5 ' TCGAGTGATCTCGGGTTTTGAGCATAGAAACGAACCATGATGGTGCGCACGGAGCACAAACAATGCCTTGAGATCAGGCTAAGGGGAGAGAAGGAAAAGGAATTGGATATAGCCTGTATTCTAGG
3 ' AGCTCACTAGAGCCCAAAACTCGTATCTTTGCTTGGTACTACCACGCGTGCCTCGTGTTTGTTACGGAACTCTAGTCCGATTCCCCTCTCTTCCTTTTCCTTAACCTATATCGGACATAAGATCC
125
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250
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1000
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1500
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1625
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E-box (-1257)
1750
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1875
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2125
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2250
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2375
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2625
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2750
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2875
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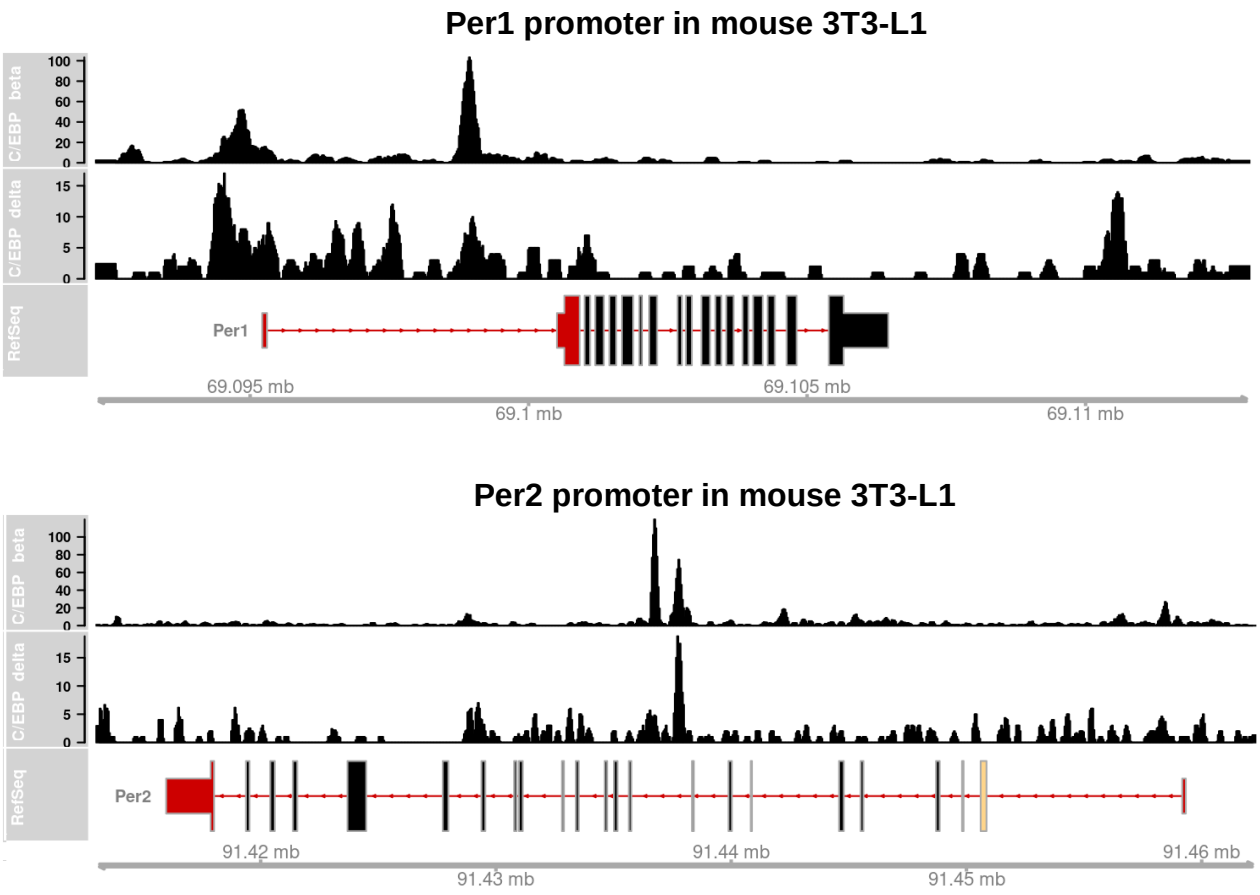
3000
GC-box (-60)
NFY (-29)
TSS
AAGTAAGCTTGGCATTCCGGTACTGTTGGTAAAGCCACCATGGAAGAC 3 '
3048
TTCATTCGAACCGTAAGGCCATGACAACCATTTCGGTGGTACCTTCTG 5 '
Met Glu Asp
luciferase

**Supplemental Figure 8. Mouse Per1 promoter sequence within a luciferase reporter construct.** Putative binding sites were screened using the MatInspector software and depicted using the SnapGene software (https://www.snapgene.com/). Per1 promoter sequence is indicated by the blue font and the translation start site of the luciferase gene is shown in red font. Binding sites are indicated using followind colors: green - E-box (5´-CACGTT-3´), dark green - D- box (5´-TTATGTAA-3´), blue - CRE (5´-TGACGTCA-3´), orange – NFY (5´-CCAAT-3´), and yellow – GC-box/SP1 binding site (5´-GGGCGG-3´). TSS (transcription start site) is indicated with a grey box.

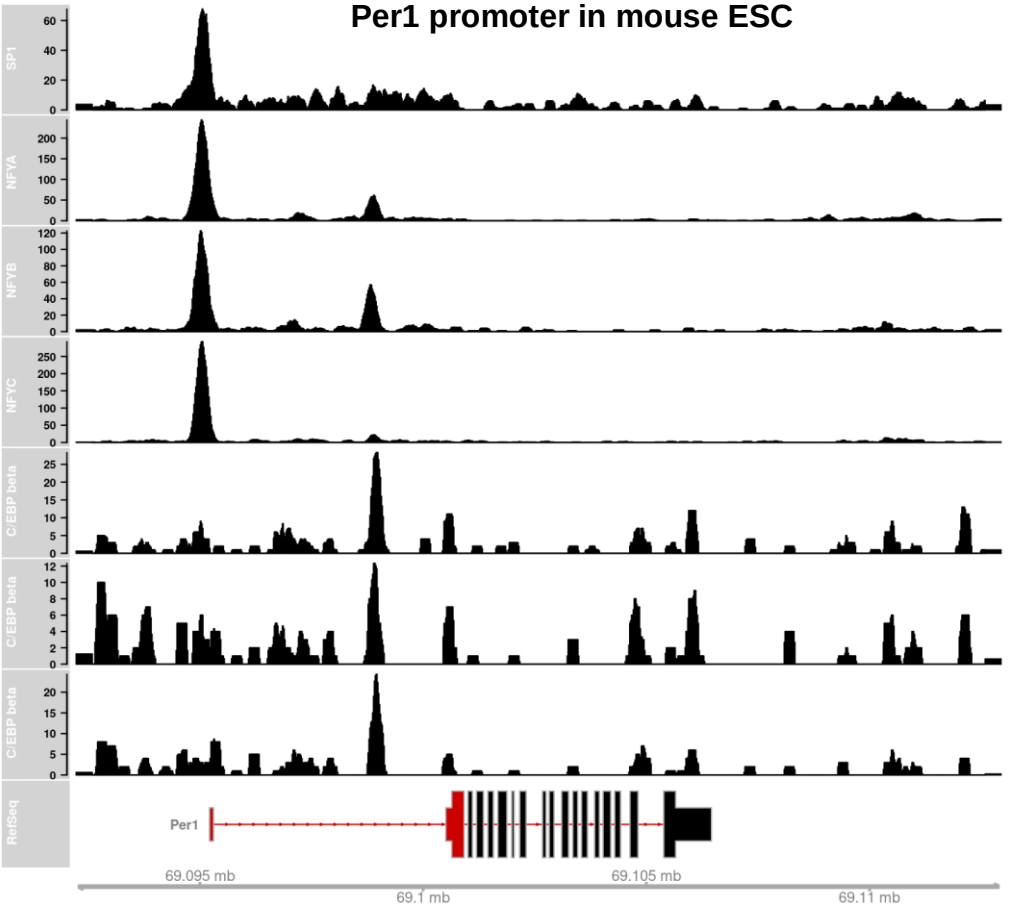
**A**

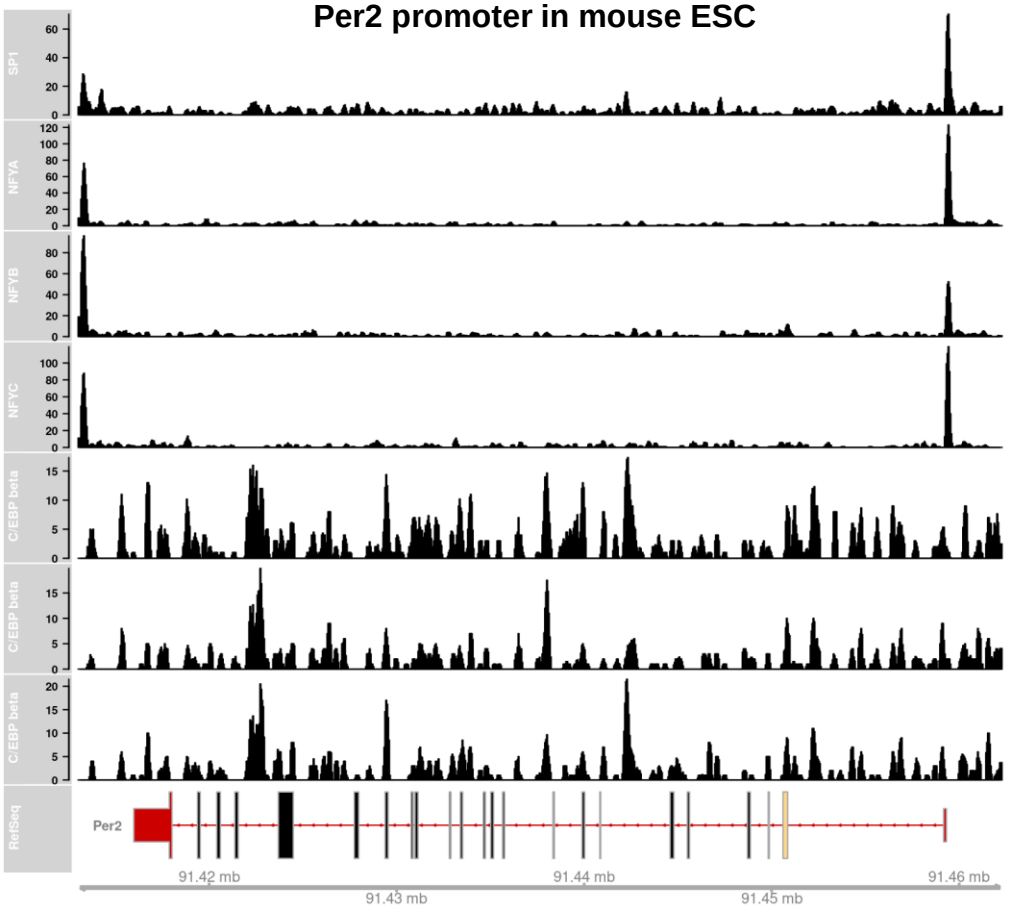
| Accession Number | Cell/Tissue | TFs |
|---|---|---|
| ENCFF659QAR | Hep2G | NFYA |
| ENCFF062IKU | Hep2G | NFYB |
| ENCFF117DNV | Hep2G | NFYC |
| ENCFF460NRJ | Hep2G | C/EBPalpha |
| ENCFF000XQN | Hep2G | C/EBPbeta |
| ENCFF616JYP | Hep2G | C/EBPdelta |
| ENCFF517LOQ | Hep2G | SP1 |
| GSE52496 | ESC | SP1 |
| GSE69099 | ESC | C/EBPbeta |
| GSE56839 | ESC | NFYA, NFYB, NFYC |
| GSE27826 | 3T3-L1 | C/EBPbeta, C/EBPdelta |

**B**

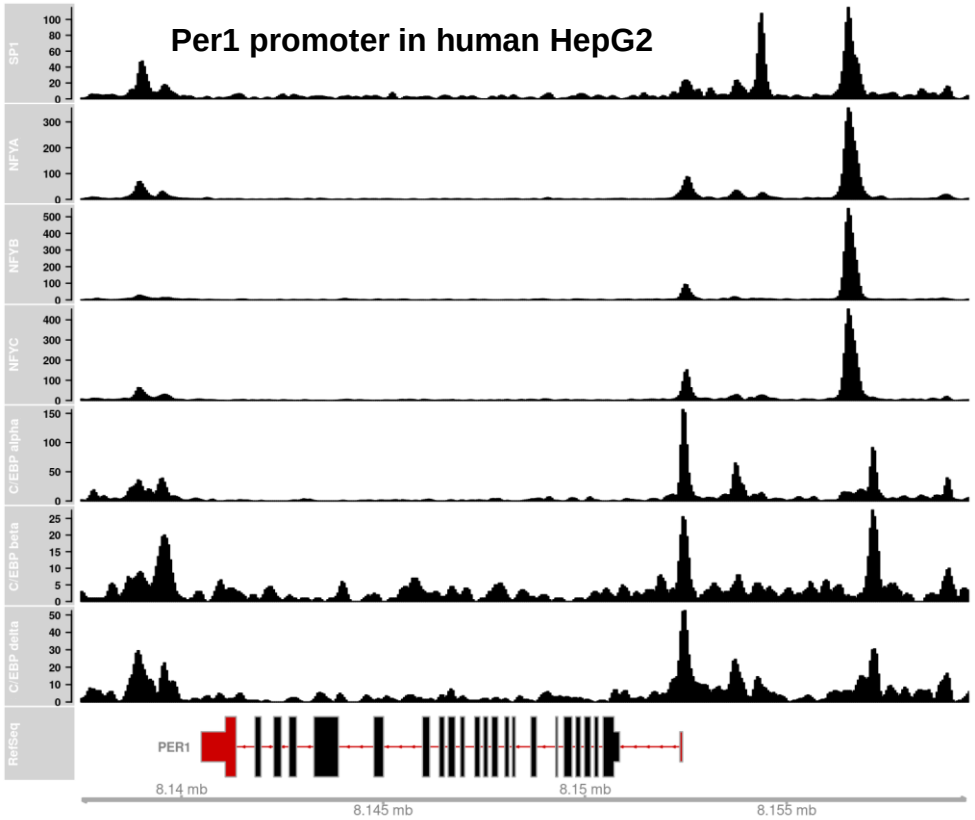


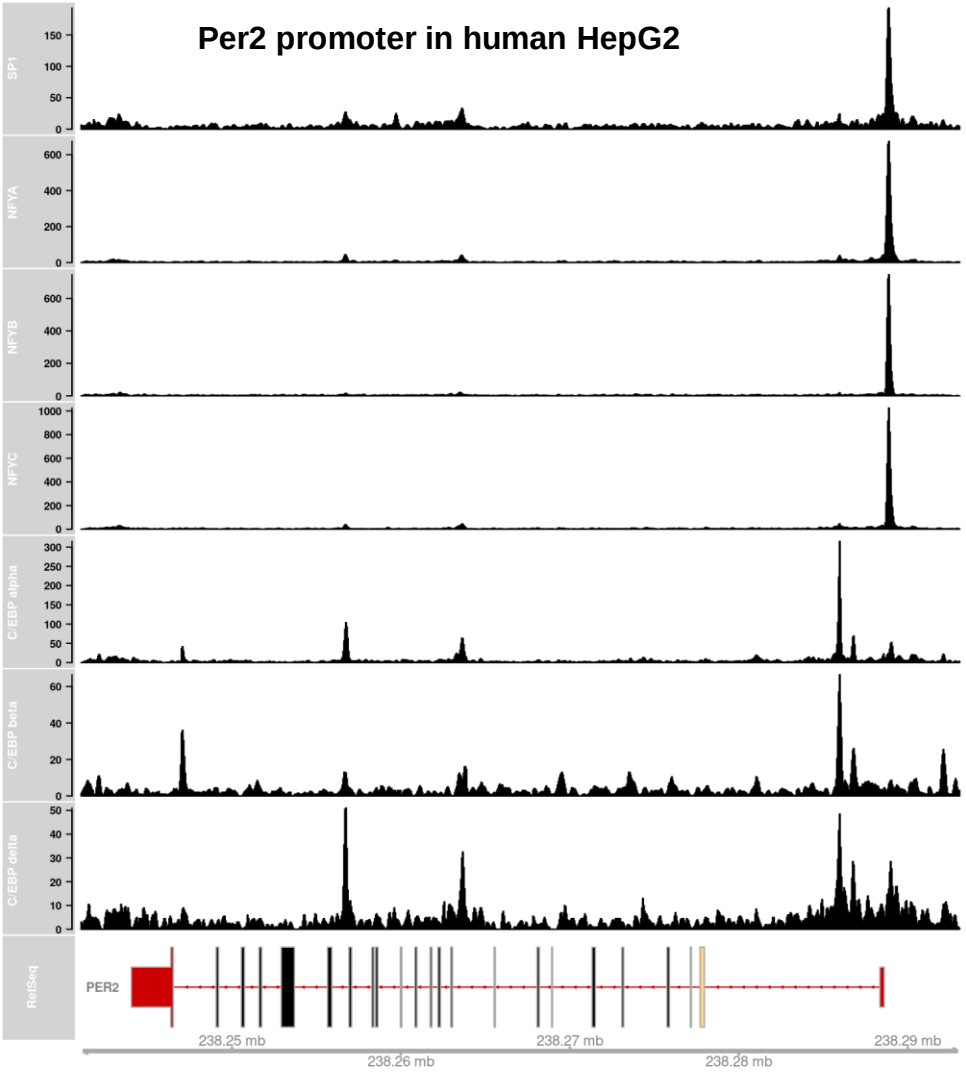
C


Per1 promoter in mouse ESC
SP1
NFYA
NFYB
NFYC
C/EBP beta
C/EBP beta
C/EBP beta
RefSeq
Per1
69.095 mb
69.1 mb
69.105 mb
69.11 mb

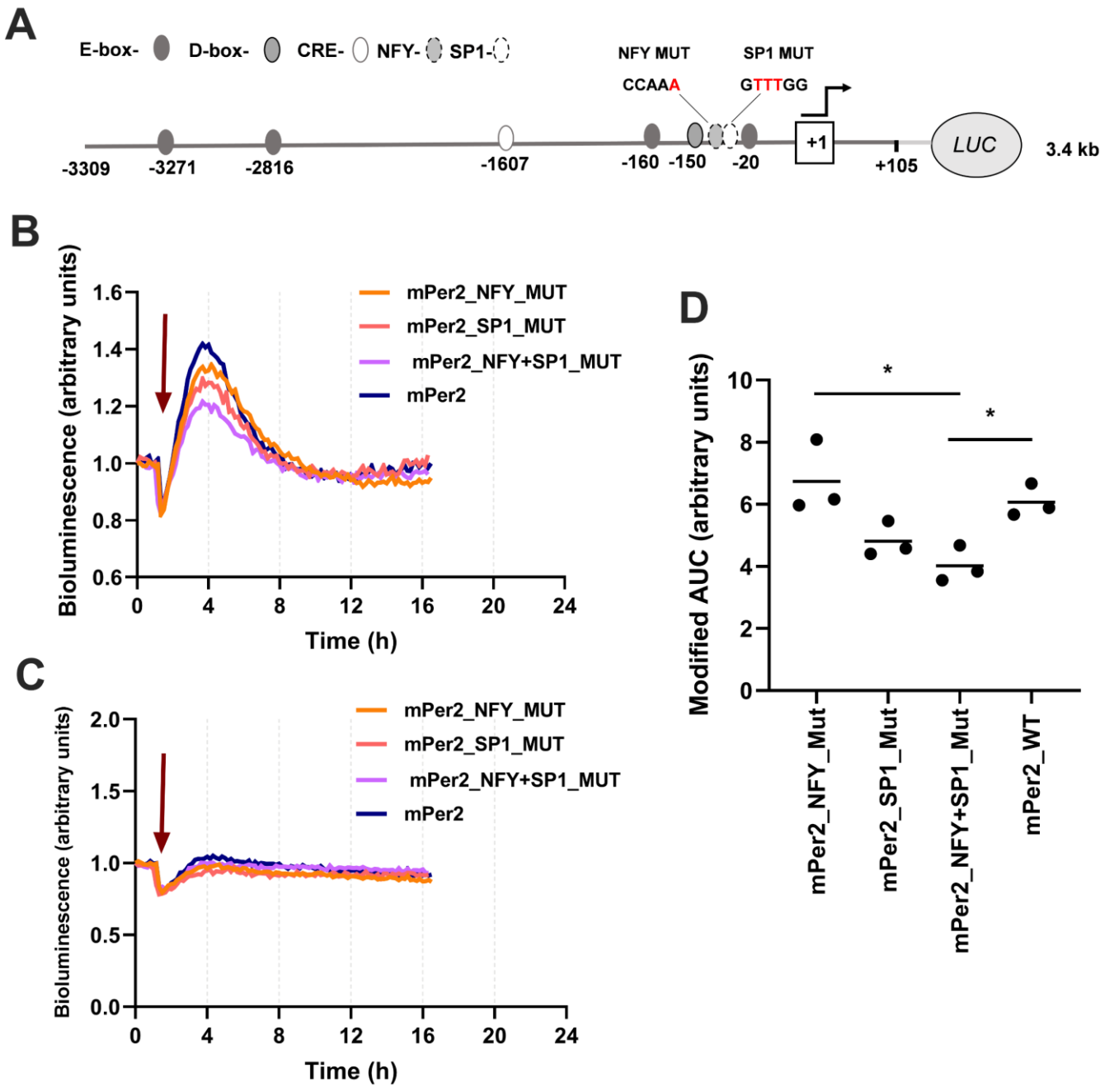

Per2 promoter in mouse ESC
SP1
NFYA
NFYB
NFYC
C/EBP beta
C/EBP beta
C/EBP beta
RefSeq
Per2
91.42 mb
91.43 mb
91.44 mb
91.45 mb
91.46 mb

D


Per1 promoter in human HepG2
SP1
NFYA
NFYB
NFYC
C/EBP alpha
C/EBP beta
C/EBP delta
RefSeq
PER1
8.14 mb
8.145 mb
8.15 mb
8.155 mb


Per2 promoter in human HepG2
SP1
NFYA
NFYB
NFYC
C/EBP alpha
C/EBP beta
C/EBP delta
RefSeq
PER2
238.25 mb
238.26 mb
238.27 mb
238.28 mb
238.29 mb

**Supplemental Figure 9. ChIP-Seq analysis of Per1 and Per2 promoter regions for NFY, SP1 and C/EBP transcription factors.** (**A**) Accession IDs from Encode (ENCFF) and GEO (GSE) databases for selected transcription factors. **(B**) Promoter analysis of mouse 3T3-L1 cells. **(C)** Promoter analysis of mouse embryonic stem cells (ESC). **(D**) Promoter analysis of human HepG2 cells.




**Supplemental Figure 10. Insulin-inducible expression from the mPer2 promoter is NFY and SP1 dependent. (A)** Schematic representation of the mPer2 promoter luciferase reporter construct with the potential binding sites for NFY (CCAAT) and SP1 (GGGCGG) are shown using a dashed line. LUC: luciferase,+1 represent the transcriptional starting site. **(B and C)** Bioluminescence recordings of 3T3-L1 pre-adipocytes harboring the mPer2- or the mPer2-NFY-MUT/mPer2-SP1-MUT/mPer2-NFY+SP1-MUT promoter luciferase reporter construct (one representative experiment is shown). Cells were stimulated with either **(B)** 100 nM insulin (n=3 technical replicates) or **(C)** solvent (n=3 technical replicates) (time of stimulation indicated by the red arrow). **(D)** Quantification of area under the induction curve (AUC) (n=3 biological replicates). *p<0.05, **p<0.01, ***p<0.001 by one-way ANOVA with multiple comparison test.