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Supplemental information

Defining the mammalian coactivation of hepatic 12-h clock and lipid metabolism

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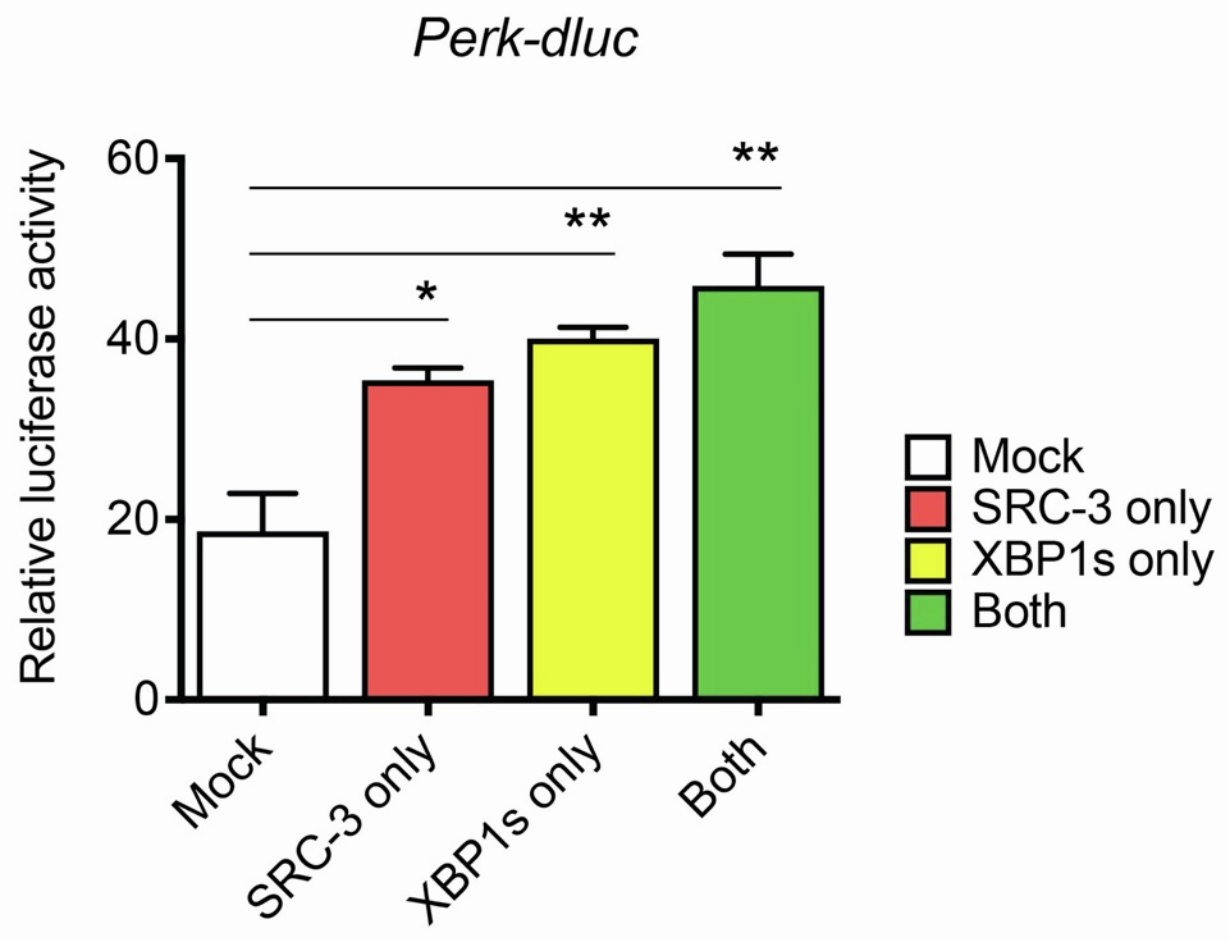


Figure S1. SRC-3 coactivates XBP1 transcription *in vitro*. Related to Figures 1. Transient luciferase assay using WT (XBP1s binding motif CACGTC) Eif2ak3-dluc vector with mock (white), SRC-3 (red), XBP1 (yellow) expression vectors and both SRC-3 and XBP1 expression vectors (green) in mouse embryonic fibroblast (MEFs) (n = 4). Relative luciferase activity is shown. Unpaired Student's t-test was performed with p value indicated. Data are graphed as the mean ± SEM. *p < 0.05, **p < 0.005, ***p < 0.001, ****p < 0.0001.

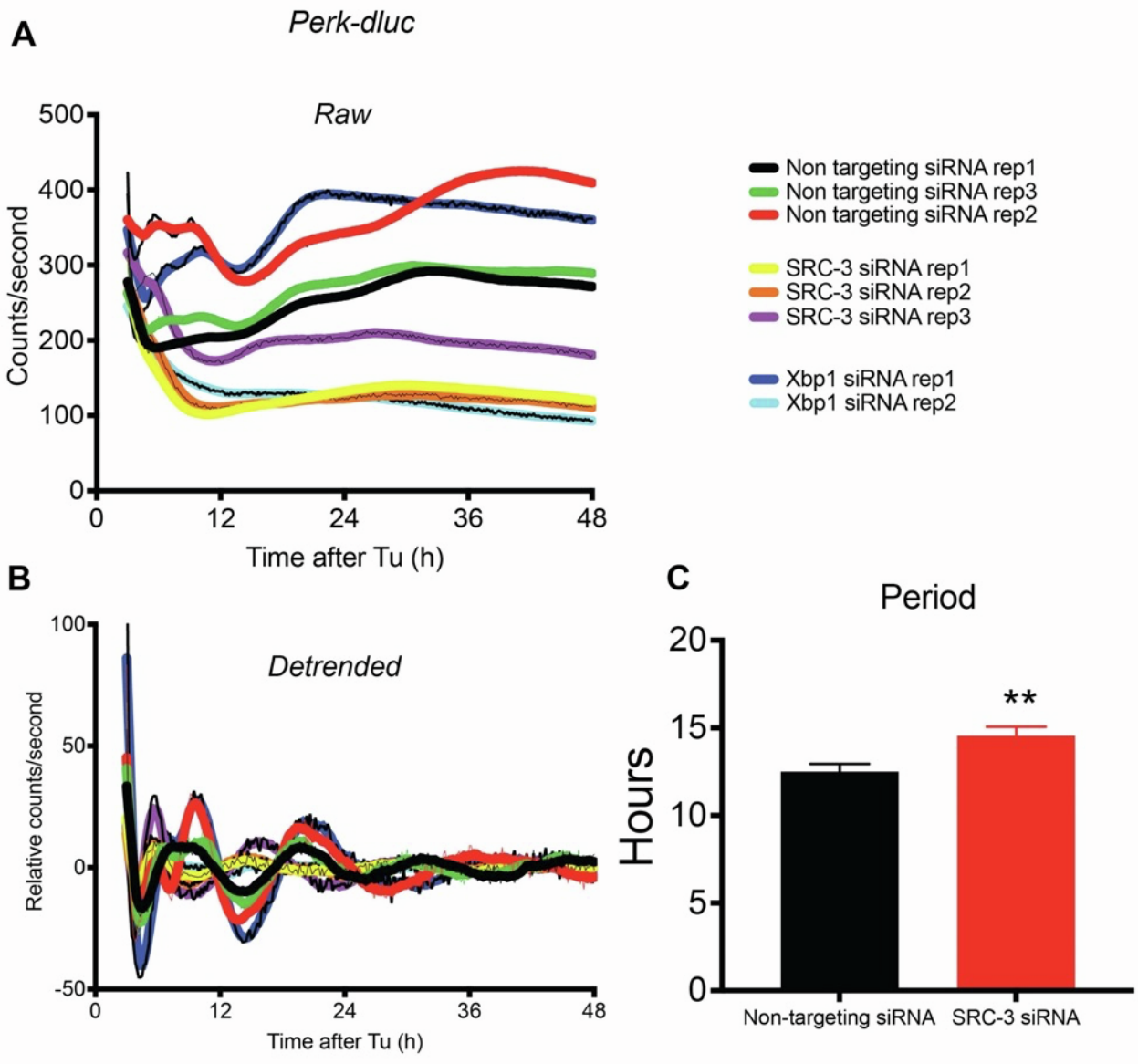


Figure S2. Real-time luminescence recording of non-targeting, SRC-3 siRNA- or Xbp1 siRNA-transfected Eif2ak3-dluc MEFs in response to tunicamycin (Tu) treatment. Related to Figures 1. (**A** & **B**) Raw (**A**) and detrended (**B**) graphs are provided. For Tu treatment, 2-3 independent experiments are shown. Eif2ak3-dluc MEFs were transfected with different siRNAs and then shocked with a Tu treatment and real-time luciferase were recorded by Lumicycle for a total of 48 hours. (**C**) Eigenvalue parameter periods of the Real-time luminescence of non-targeting siRNA and SRC-3 siRNA are shown, n=3. Unpaired Student's t-test was performed with p value indicated. Data are graphed as the mean ± SEM. *p < 0.05, **p < 0.005, ***p < 0.001, ****p < 0.0001.

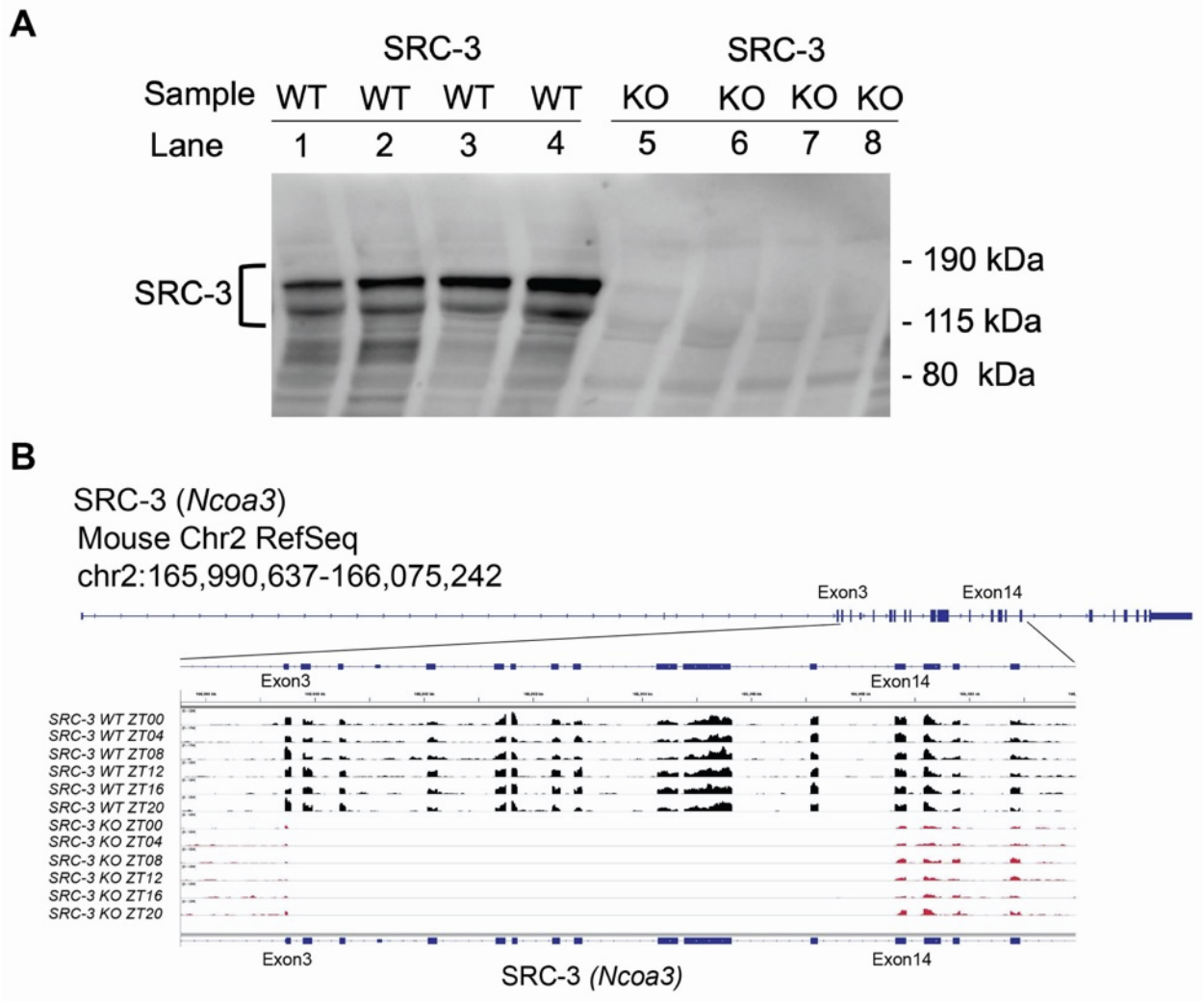


Figure S3. Deletion of SRC-3 in mice. Related to Figures 1-5. (**A**) Immunoblot analysis of total hepatic SRC-3 in the SRC-3 wildtype (WT) and knockout (KO) mice. (**B**) The strategy to delete SRC-3 in mice and the SRC-3 WT and KO RNA-Seq results at the SRC-3 (*Ncoa3*) mRNA level. Mouse hepatic mRNA views of all RNA-Seq data at the SRC-3 *(Ncoa3)* locus are shown.

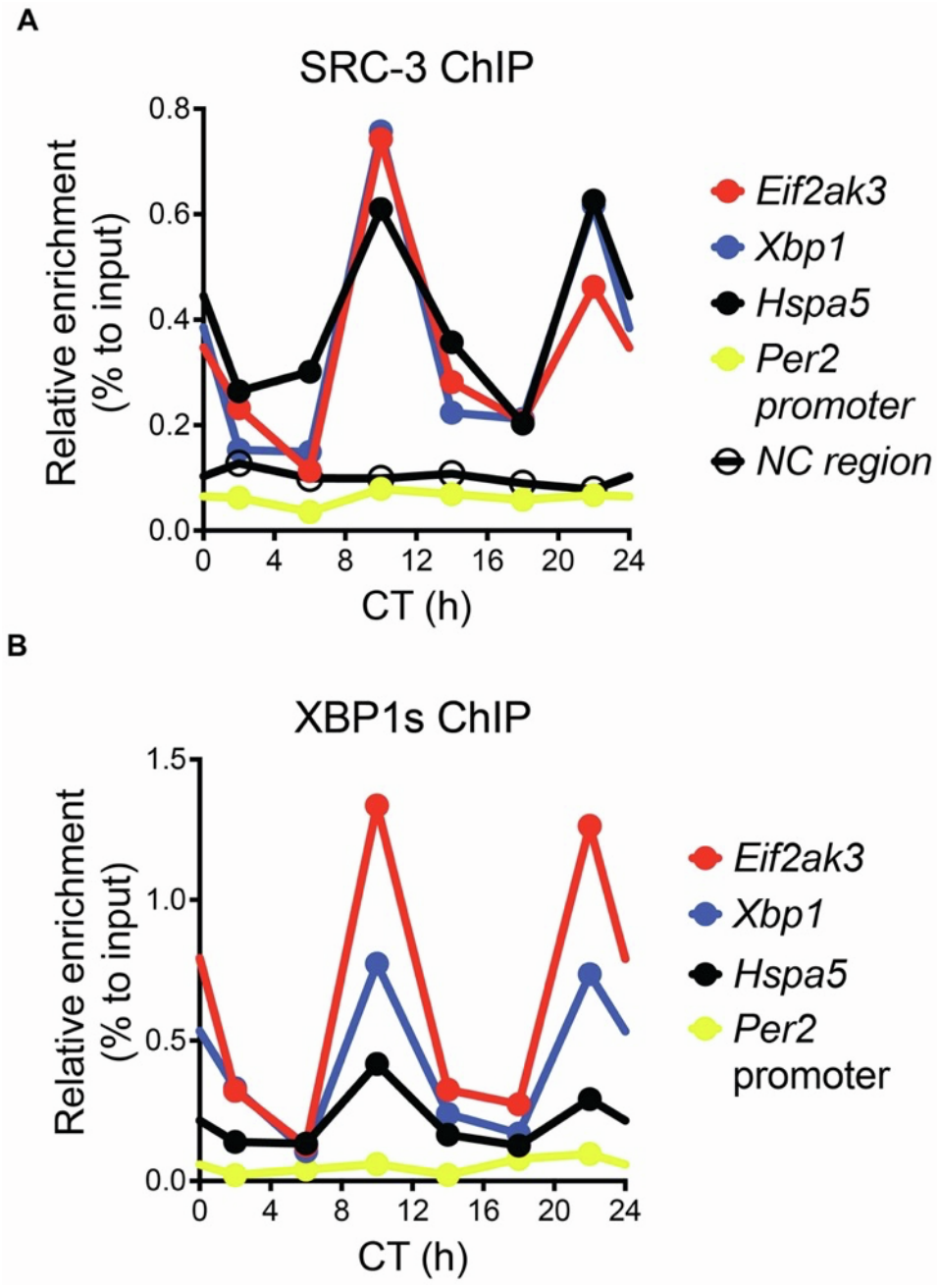


Figure S4. ChIP-qPCR of hepatic SRC-3 (**A**) and XBP1s (**B**) on selective 12-hour cycling gene promoters (*Eif2ak3*, *Xbp1* and *Hspa5*), 24-hour gene *Per2* promoter, and noncoding (NC) region every 4 CT hours for 24 hours in total (n = 3 mouse livers per time point). Related to Figures 1.

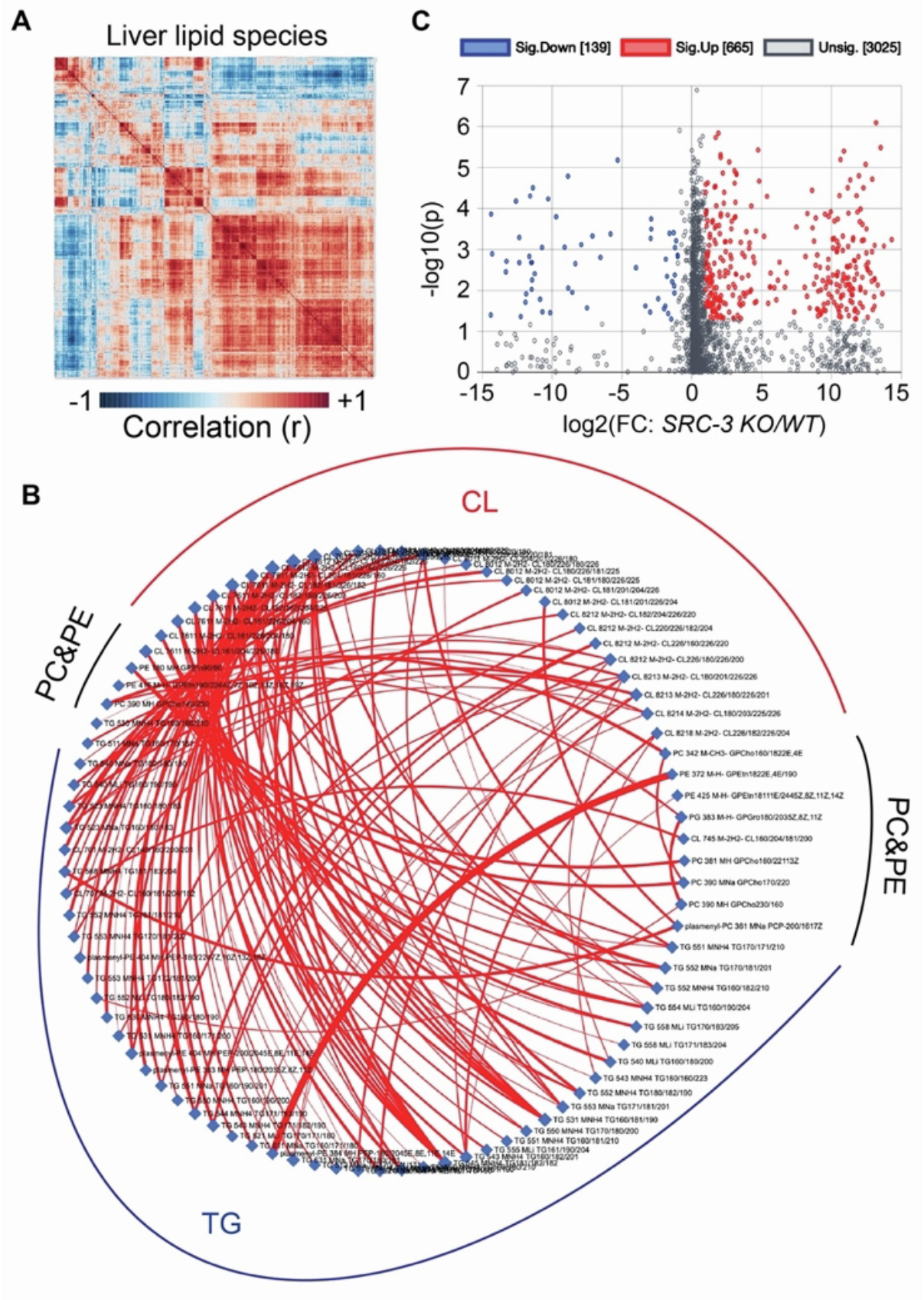
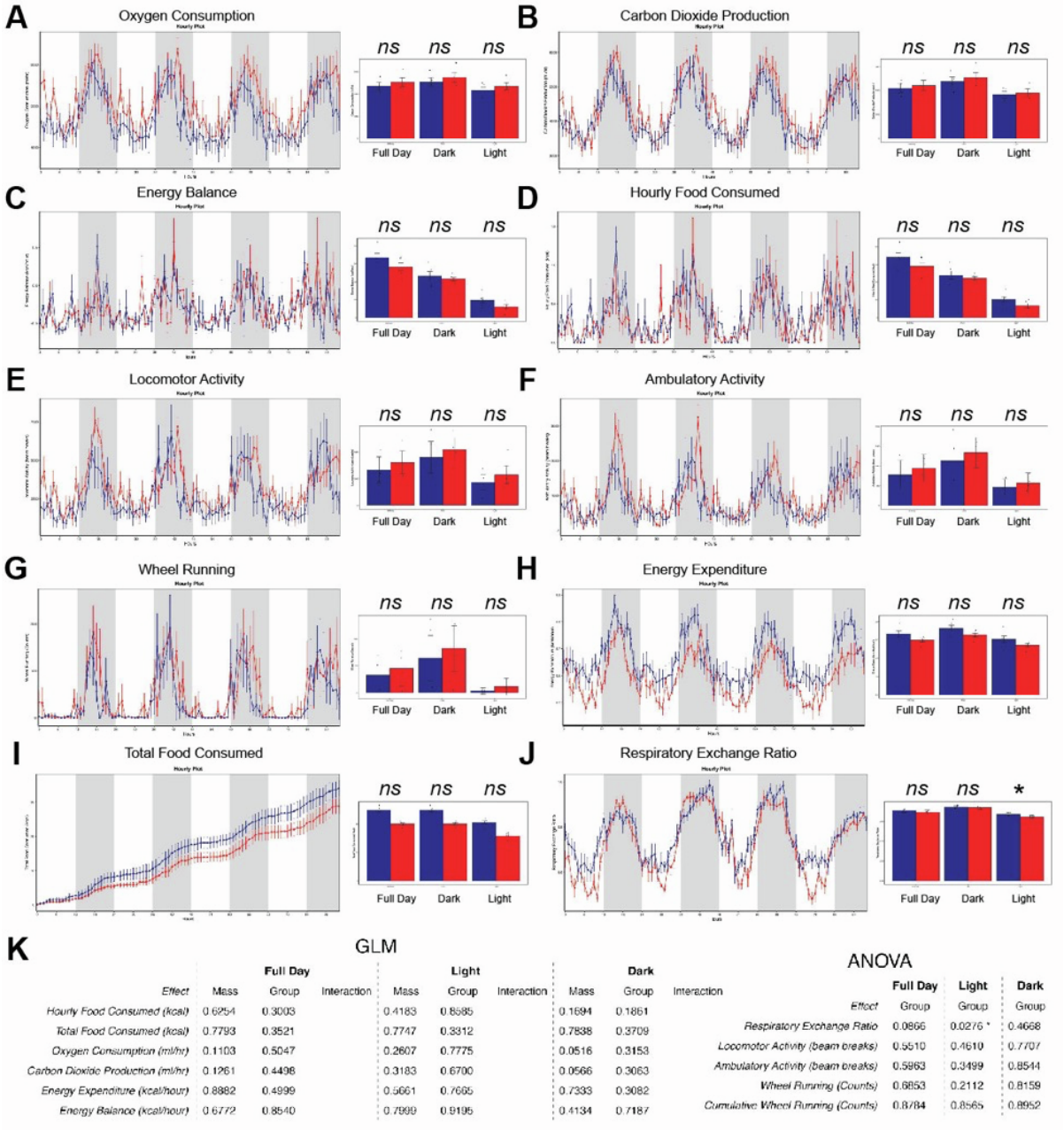

A
Liver lipid species
-1
+1
Correlation (r)
C
Sig.Down [139]
Sig.Up [665]
Unsig. [3025]
-log10(p)
log2(FC: SRC-3 KO/WT)
B
CL
PC&PE
PC&PE
TG

Figure S5. Overall correlation heatmap, network modeling and differential expression analysis of the liver lipidomic profiling. Related to Figures 4. (**A**) Overall correlation heatmap of the liver lipidomics profiling. Correlation analysis was used to visualize the overall correlations between different features of the lipidomics data from the SRC-3 WT and KO mice fed regular chow *ad libitum*. (**B**) Debiased sparse partial correlation (DSPC) network modeling and visualization of the lipidomic profiling. Four major classes of lipid species PC, PE, TG and CL were grouped to the same class as subnetworks. The DSPC module network was constructed by calculating the partial correlation coefficients and P-values for each pair of lipid metabolic features. The input lipid metabolites are shown as nodes, while the edges represent the correlation coefficients among the indicated lipid species. (**C**) Volcano plot representation of differential expression analysis of lipid species in the SRC-3 KO versus WT comparison. A combination of significant features at fold change (FC threshold 2) and t-tests (P-value threshold 0.05) in equal group variance was shown by volcano plot. The red (upregulated features) and blue (downregulated features) circles represent lipid species above the threshold with both fold changes and p values are log transformed.



K

GLM

	Full Day			Light			Dark		
Effect	Mass	Group	Interaction	Mass	Group	Interaction	Mass	Group	Interaction
Hourly Food Consumed (kcal)	0.6254	0.3003		0.4183	0.8585		0.1694	0.1861	
Total Food Consumed (kcal)	0.7793	0.3521		0.7747	0.3312		0.7838	0.3709	
Oxygen Consumption (ml/hr)	0.1103	0.5047		0.2607	0.7775		0.0516	0.3153	
Carbon Dioxide Production (ml/hr)	0.1261	0.4498		0.3183	0.6700		0.0566	0.3063	
Energy Expenditure (kcal/hour)	0.8882	0.4999		0.5661	0.7665		0.7333	0.3082	
Energy Balance (kcal/hour)	0.6772	0.8540		0.7999	0.9195		0.4134	0.7187	

ANOVA

	Full Day	Light	Dark
Effect	Group	Group	Group
Respiratory Exchange Ratio	0.0866	0.0276 *	0.4668
Locomotor Activity (beam breaks)	0.5510	0.4610	0.7707
Ambulatory Activity (beam breaks)	0.5963	0.3499	0.8544
Wheel Running (Counts)	0.6853	0.2112	0.8159
Cumulative Wheel Running (Counts)	0.8784	0.8565	0.8952

Figure S6. CLAMS analysis of the SRC-3 WT versus KO mice. Related to Figures 6. The relative real-time values (left panel) and full day, dark and light box plots (right panel) of oxygen consumption (**A**), carbon dioxide production (**B**), energy balance (**C**), hourly food consumed (**D**), locomotor activity (**E**), ambulatory activity (**F**), wheel running (**G**), energy expenditure (**H**), total food consumed (**I**) and respiratory exchange ratio (**J**) of the indicated mice (n = 4) housed under *ad libitum* feeding conditions. Averaged real-time values with error bars of the indicated mouse strains plotted against the indicated time points. (**K**) The integrated statistical analysis is shown for the indirect calorimetry experiments of the SRC-3 WT and KO mice (n = 4) housed under *ad libitum* feeding conditions. Generalized linear model (GLM) and One-way ANOVA analysis with Tukey's post-hoc analysis was performed with p value threshold 0.05. The GLM and ANOVA analyses were normalized by lean mass.