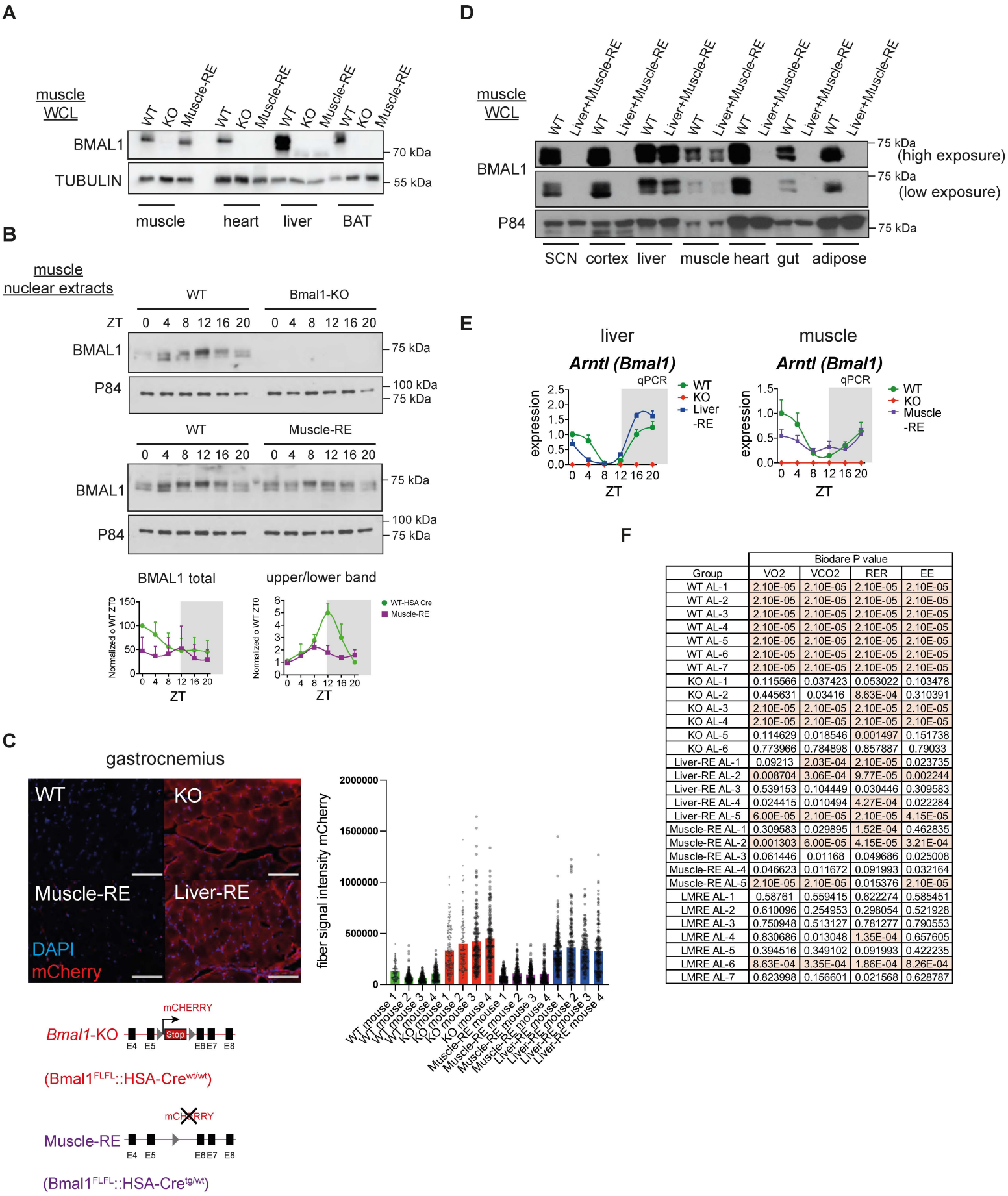
SUPPLEMENTARY FIGURE 1



F

	Biodare P value			
Group	VO2	VCO2	RER	EE
WT AL-1	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT AL-2	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT AL-3	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT AL-4	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT AL-5	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT AL-6	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT AL-7	2.10E-05	2.10E-05	2.10E-05	2.10E-05
KO AL-1	0.115566	0.037423	0.053022	0.103478
KO AL-2	0.445631	0.03416	8.63E-04	0.310391
KO AL-3	2.10E-05	2.10E-05	2.10E-05	2.10E-05
KO AL-4	2.10E-05	2.10E-05	2.10E-05	2.10E-05
KO AL-5	0.114629	0.018546	0.001497	0.151738
KO AL-6	0.773966	0.784898	0.857887	0.79033
Liver-RE AL-1	0.09213	2.03E-04	2.10E-05	0.023735
Liver-RE AL-2	0.008704	3.06E-04	9.77E-05	0.002244
Liver-RE AL-3	0.539153	0.104449	0.030446	0.309583
Liver-RE AL-4	0.024415	0.010494	4.27E-04	0.022284
Liver-RE AL-5	6.00E-05	2.10E-05	2.10E-05	4.15E-05
Muscle-RE AL-1	0.309583	0.029895	1.52E-04	0.462835
Muscle-RE AL-2	0.001303	6.00E-05	4.15E-05	3.21E-04
Muscle-RE AL-3	0.061446	0.01168	0.049686	0.025008
Muscle-RE AL-4	0.046623	0.011672	0.091993	0.032164
Muscle-RE AL-5	2.10E-05	2.10E-05	0.015376	2.10E-05
LMRE AL-1	0.58761	0.559415	0.622274	0.585451
LMRE AL-2	0.610096	0.254953	0.298054	0.521928
LMRE AL-3	0.750948	0.513127	0.781277	0.790553
LMRE AL-4	0.830686	0.013048	1.35E-04	0.657605
LMRE AL-5	0.394516	0.349102	0.091993	0.422235
LMRE AL-6	8.63E-04	3.35E-04	1.86E-04	8.26E-04
LMRE AL-7	0.823998	0.156601	0.021568	0.628787

Figure S1. Characterization of muscle and liver+muscle clock reconstituted mice. Related to Figure 1.

(A) Whole-cell lysates (WCL) collected at zeitgeber time (ZT) 4. ZT0 = lights on, ZT12 = lights off. BAT – brown adipose tissue; muscle – gastrocnemius muscle. WT is WT-*Hsa*-Cre.

(B) Top, nuclear protein extracts from gastrocnemius muscle collected at the indicated time points. WT is WT-*Hsa*-Cre. Bottom, densitometry quantification of BMAL1, n=3. Total BMAL1 was normalized to p84 loading control.

(C) RFP staining for mCherry in transverse sections of gastrocnemius muscle. Left- representative images, red – mCherry, blue – DAPI. Scale bar- 100μm. Right- signal intensity of mCherry within each fibre, minimum 100 fibers counted per mouse. Individual mice, n = 4 (WTs #1 and #4 are WT-*Alfp*-Cre, WTs #2 and #3 are WT-*Alfp*+*Hsa*-Cre).

(D) Whole-cell lysates (WCL) collected at ZT4. SCN – suprachiasmatic nucleus enriched tissue punch from hypothalamus. WT is WT-*Alfp*+*Hsa*-Cre.

(E) Quantitative PCR of *Bmal1* expression confirming loss of expression in *Bmal1* KO mice, n=3-6. Liver WT is WT-*Alfp*-Cre. Muscle WT is WT-*Hsa*-Cre.

(F) Rhythmicity analysis of metabolic cage parameters using BioDare2. Values are p-value for each parameter for each mouse for a ~24 h period rhythm. Orange-highlighted = $p<0.01$. WT-*Alfp*-Cre = 2; WT-*Hsa*-Cre = 1; WT-*Alfp*-Cre and WT-*Hsa*-Cre = 4. VO2 – oxygen consumption rate; VCO2 – carbon dioxide release rate; EE – energy expenditure; RER – respiratory exchange ratio.

SUPPLEMENTARY FIGURE 2

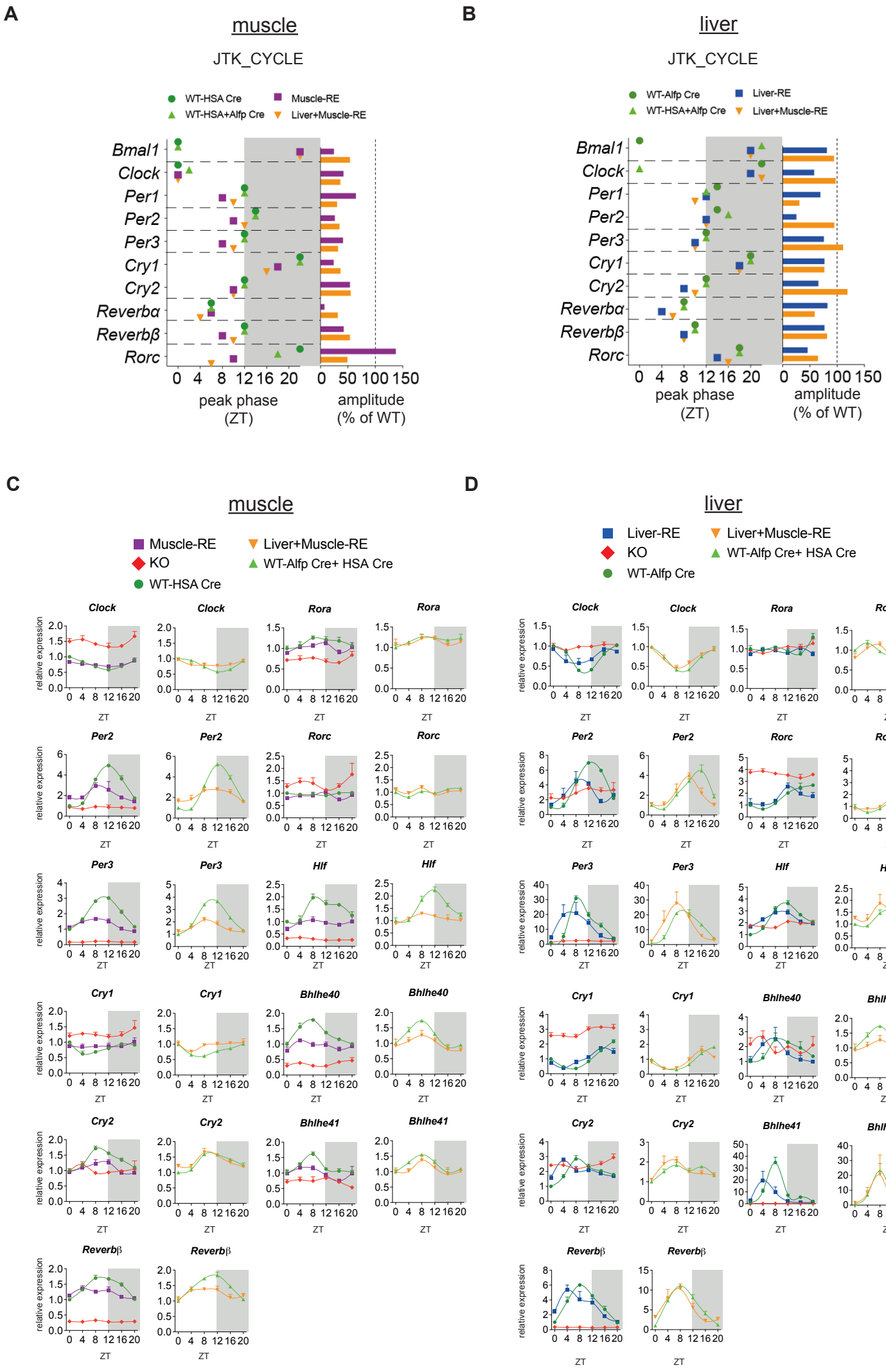


Figure S2. Oscillation of clock genes in single and double RE mice. Related to Figure 2.

(A-D) RNA sequencing of gastrocnemius muscle (A) and liver (B) harvested around the clock under 12 h light/12 h dark conditions, n=3. ZT – zeitgeber time; ZT0 = lights on; ZT12 = lights off. Data are normalized to WT ZT0 to facilitate comparisons across all genotypes.

(A and B) Amplitude and phase of clock genes as determined by JTK_CYCLE.

(C and D) Expression of clock genes.

SUPPLEMENTARY FIGURE 3

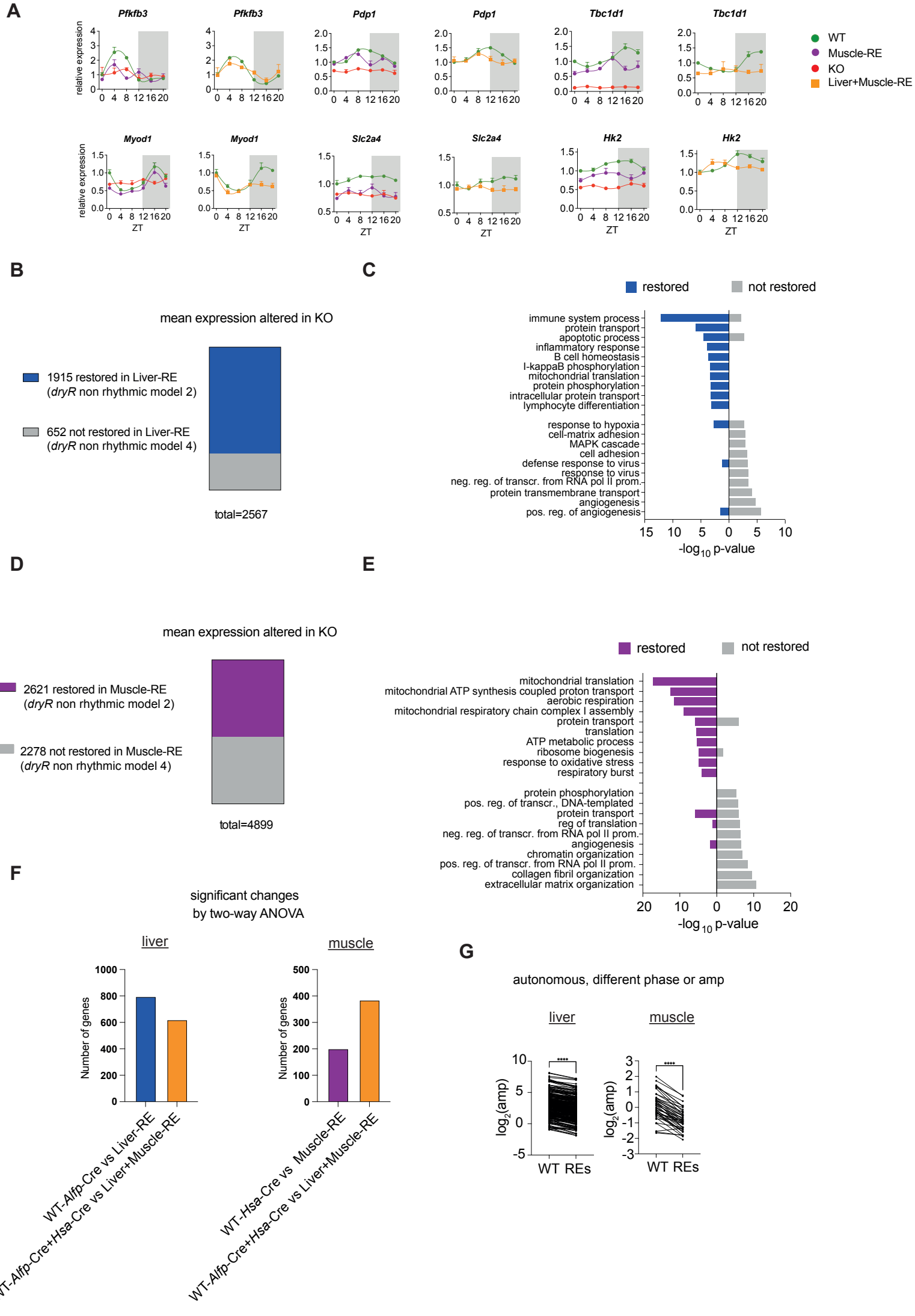


Figure S3. Analysis of diurnal transcriptomes in liver and muscle. Related to Figure 3.

(A) Example genes in muscle involved in glucose metabolism (*Pfkfb3, Pdp1, Slc2a4, Tbc1d1, Hk2*) or muscle development (*Myod1*).

(B and D) *dryR* mean models were used to compare daily average expression of genes that did not oscillate in any genotype. See also Tables S3 and S4.

(C and E) Pathway enrichments for non-oscillating genes with daily average expression changes which were either restored or not restored in single RE mice.

(F) Genome wide changes in single RE and double RE mice in muscle and liver as assessed using two-way ANOVA ($p<0.01$) via Nitecap analysis.

(G) Amplitude comparison of genes oscillating autonomously with altered phase or amplitude. Muscle data, two-tailed paired *t*-test, ****$p < 0.0001$. Liver data, Wilcoxon test (non-normal distribution), ****$p < 0.0001$.

SUPPLEMENTARY FIGURE 4

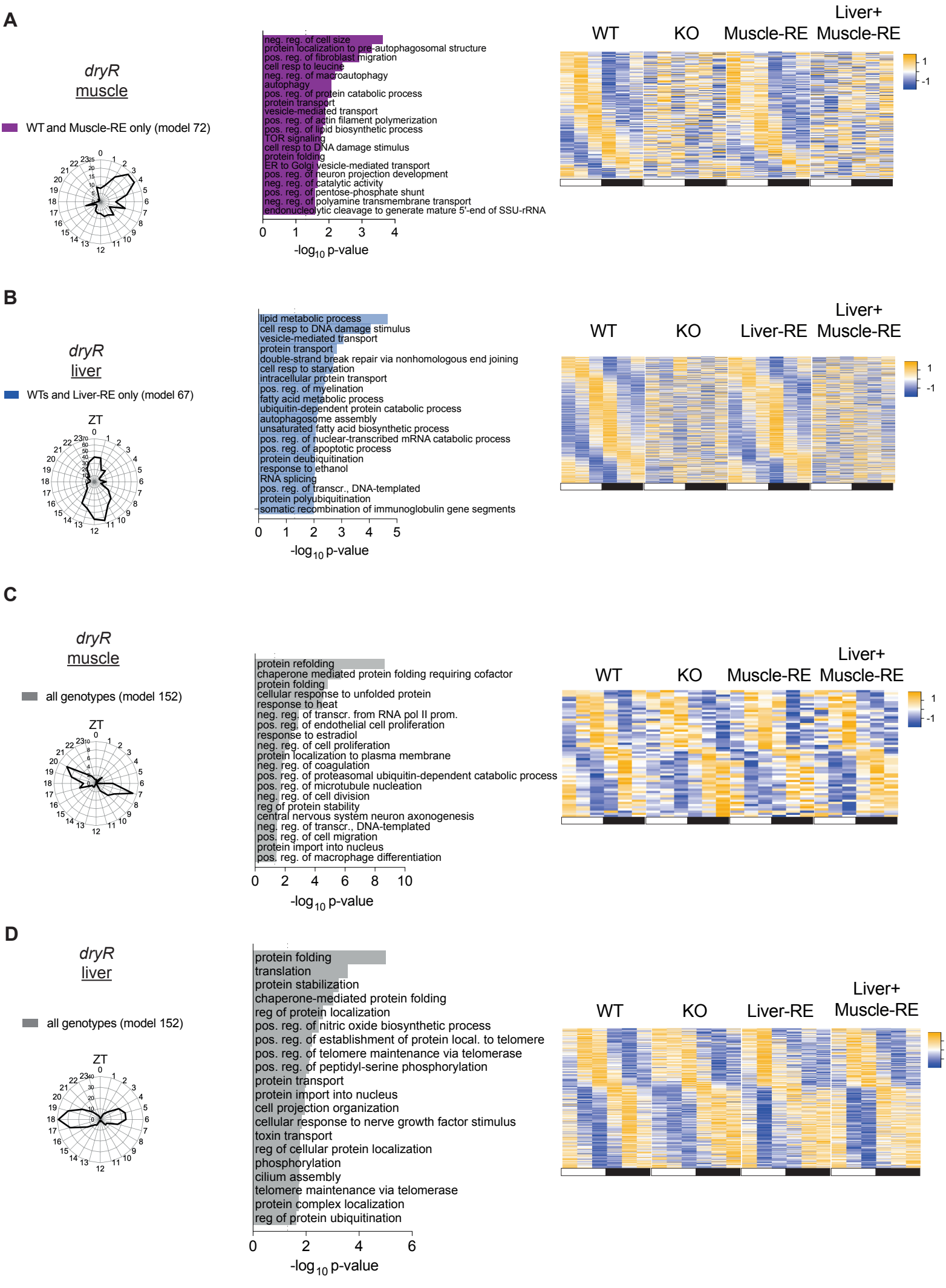


Figure S4. Comparison of oscillating genes across genotypes. Related to Figure 4.

(A-D) *dryR* analysis of RNA sequencing data. Left, polar histogram showing peak phases. Middle, DAVID biological process gene ontology enrichments. Right, heatmaps of group averages sorted by peak phase. Data from double Cre-positive WTs are presented.

(A and B) a *dryR* model of genes oscillating exclusively in WT and single, but not double, reconstituted mice.

(C and D) a *dryR* model of genes oscillating in all genotypes.

SUPPLEMENTARY FIGURE 5

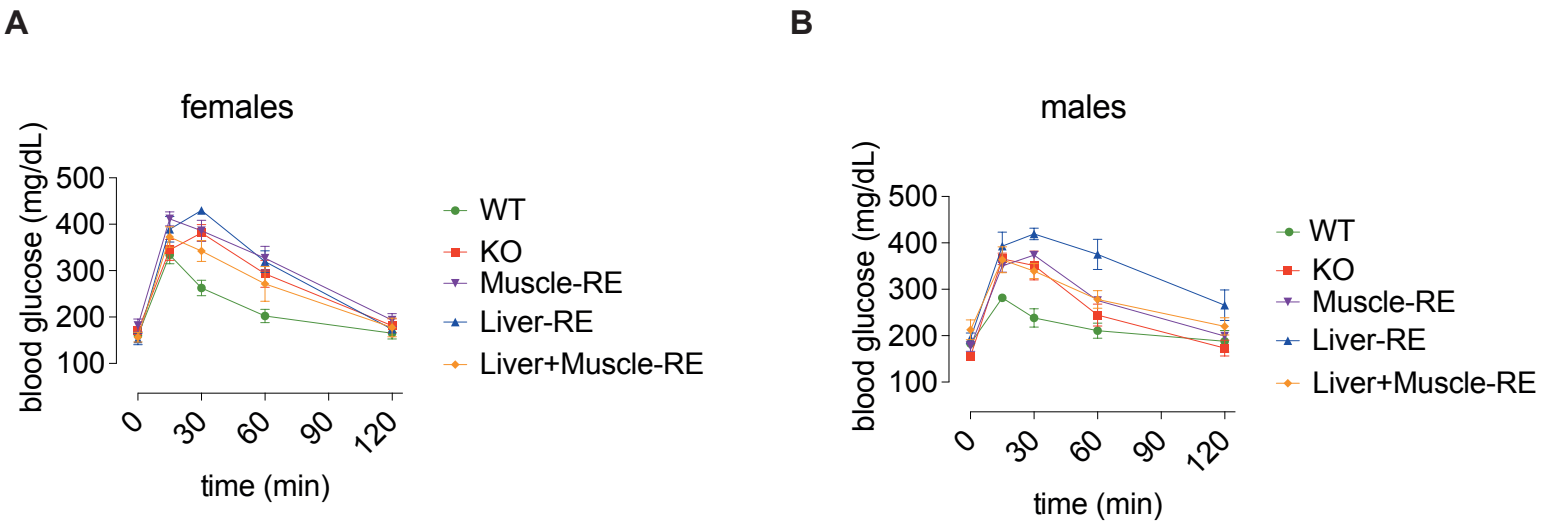
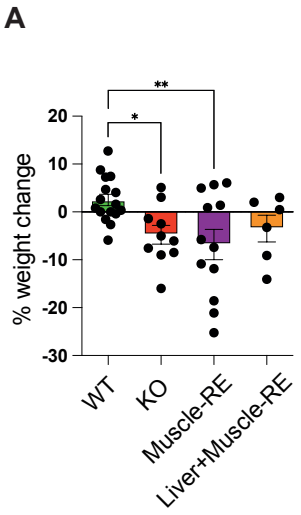
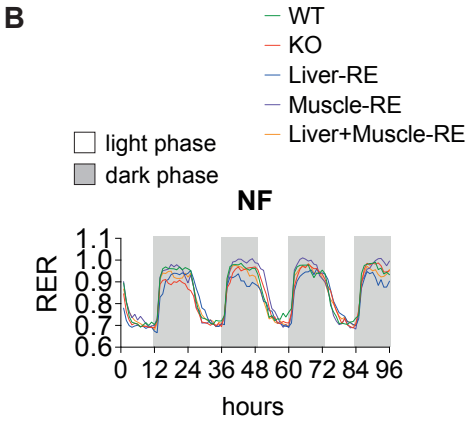


Figure S5. *Ad libitum* glucose tolerance tests. Related to Figure 5.

(A and B) Raw data for *ad libitum* oral glucose tolerance tests in female (left) and male (right) mice.

SUPPLEMENTARY FIGURE 6





C

	Biodare P value			
Group	VO2	VCO2	RER	EE
WT NF-1	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT NF-2	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT NF-3	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT NF-4	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT NF-5	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT NF-6	2.10E-05	2.10E-05	2.10E-05	2.10E-05
WT NF-7	2.10E-05	2.10E-05	2.10E-05	2.10E-05
KO NF-1	1.52E-04	0.001914	2.10E-05	2.10E-05
KO NF-2	2.10E-05	2.10E-05	2.10E-05	2.10E-05
KO NF-3	2.10E-05	2.10E-05	2.10E-05	2.10E-05
KO NF-4	6.00E-05	0.003021	2.10E-05	2.10E-05
KO NF-5	9.04E-04	0.017574	2.10E-05	2.10E-05
Liver-RE NF-1	1.52E-04	0.001497	2.10E-05	2.10E-05
Liver-RE NF-2	0.102391	0.043624	0.020506	2.10E-05
Liver-RE NF-3	2.10E-05	1.86E-04	2.10E-05	2.10E-05
Liver-RE NF-4	2.10E-05	2.10E-05	4.17E-04	2.10E-05
Muscle-RE NF-1	2.10E-05	2.10E-05	2.10E-05	2.10E-05
Muscle-RE NF-2	2.10E-05	2.10E-05	2.10E-05	2.10E-05
Muscle-RE NF-3	2.10E-05	2.10E-05	2.10E-05	2.10E-05
Muscle-RE NF-4	0.005869	0.014207	2.10E-05	2.10E-05
Muscle-RE NF-5	0.00139	0.059169	2.10E-05	2.10E-05
LMRE NF-1	2.10E-05	2.35E-04	2.10E-05	2.10E-05
LMRE NF-2	2.35E-04	0.001479	2.10E-05	2.10E-05
LMRE NF-3	2.10E-05	2.10E-05	2.10E-05	2.10E-05
LMRE NF-4	0.060901	0.248166	2.10E-05	2.10E-05
LMRE NF-5	2.10E-05	6.00E-05	2.10E-05	2.10E-05
LMRE NF-6	2.10E-05	2.10E-05	2.10E-05	2.10E-05

D

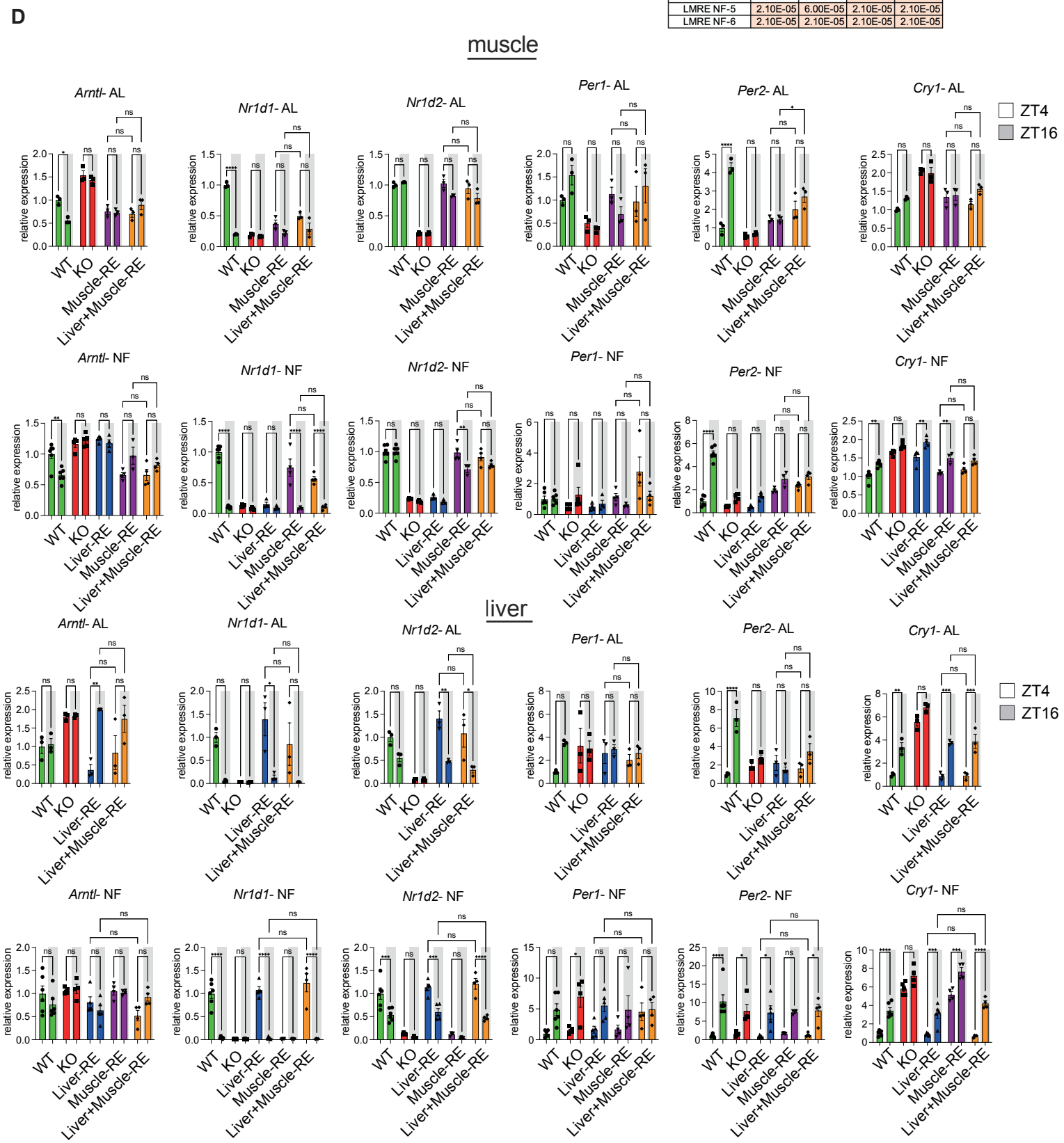


Figure S6. Effects of night feeding on RE mice. Related to Figure 6.

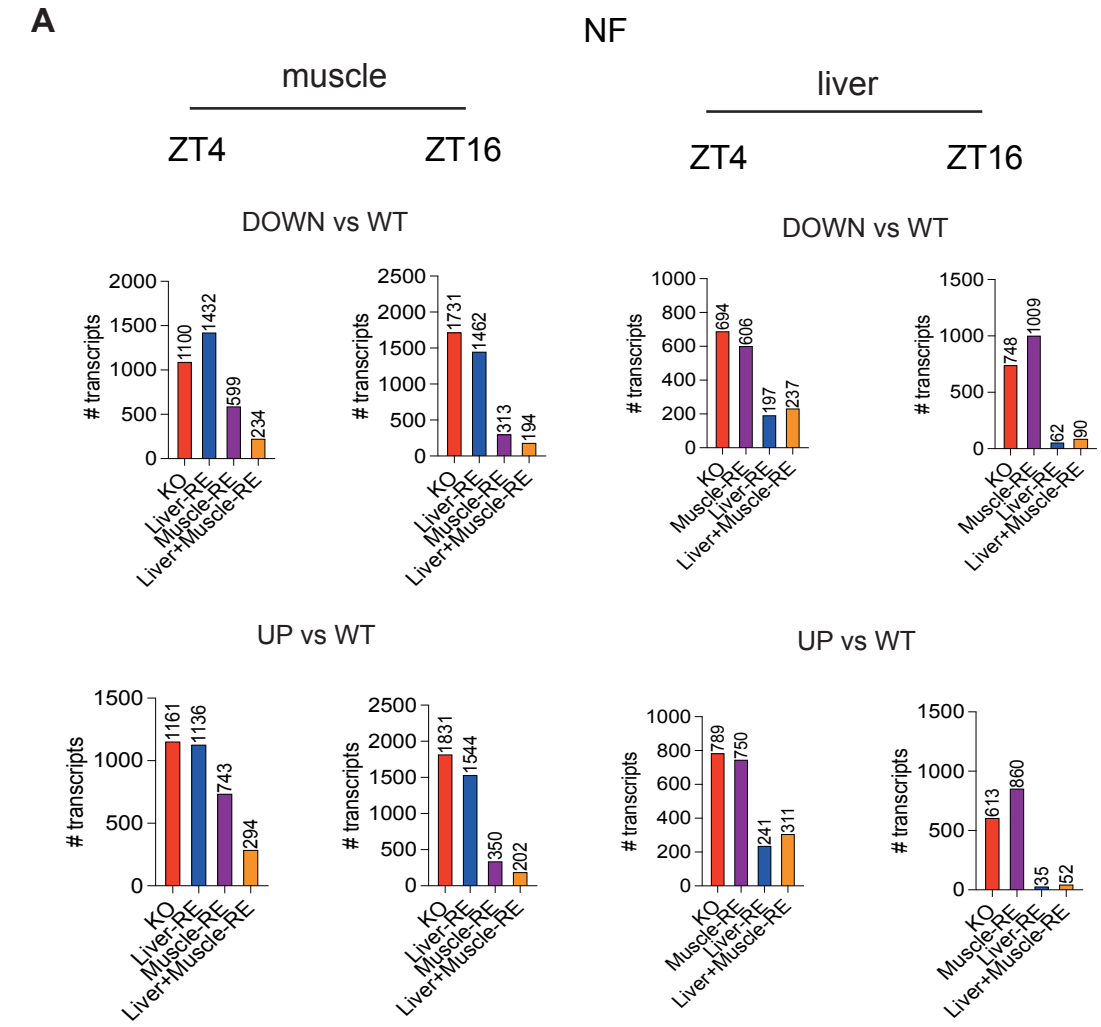
(A) Body weight change from *ad libitum* baseline through 2 weeks of night feeding, n=6-16 (WTs are 7 WT-*Hsa*-Cre, 1 WT-*Alfp*-Cre, 3 WT-*Alfp*+*Hsa*-Cre). One-way ANOVA with Fisher's LSD, * $p<0.05$, ** $p<0.01$.

(B) Mean RER recorded after 2 weeks of night feeding, n=4-7 (WTs are 1 WT-*Hsa*-Cre, 2 WT-*Alfp*-Cre, 4 WT-*Alfp*+*Hsa*-Cre).

(C) Rhythmicity analysis of metabolic cage parameters using BioDare2. Values are p-value for each parameter for each mouse for a ~24 h period rhythm. Orange-highlighted = $p<0.01$. VO2 – oxygen consumption rate; VCO2 – carbon dioxide release rate; EE – energy expenditure; RER – respiratory exchange ratio.

(D) RNA-Seq data from AL and NF mice at ZT4 and ZT16 in gastrocnemius muscle (top) and liver (bottom), n = 3-6. For AL, single RE or KO mice were normalized to the *Alfp*-Cre- or *Hsa*-Cre- positive WT control, and double RE mice were normalized to the *Alfp*+*Hsa*-Cre-positive WT control. For clarity, only double Cre WT is shown in AL panel. For NF WTs (WT-*Alfp*-Cre = 2; WT-*Hsa*-Cre = 2; WT-*Alfp*+*Hsa*-Cre = 2). Nr1d1 is replicated from the main figure for comparison with liver here. Two-way ANOVA, Bonferroni's post-hoc test, *$p< 0.05$, ** $p<0.01$, **** $p<0.0001$, ns = not significant.

SUPPLEMENTARY FIGURE 7



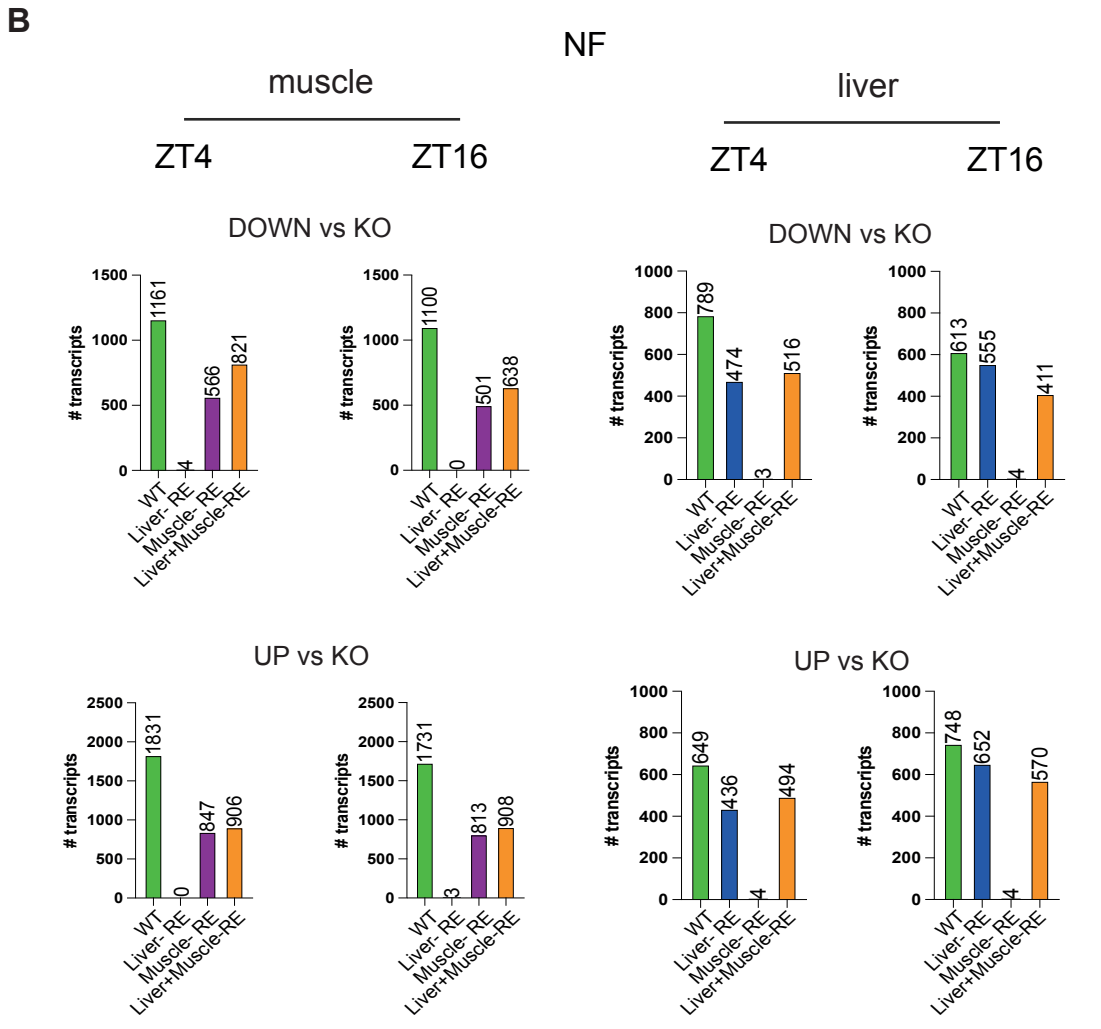
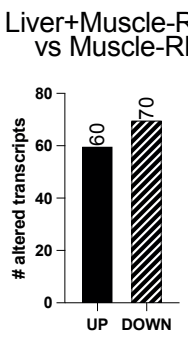


Figure S7. Transcriptional effects of night feeding in RE mice. Related to Figure 6.

(A and B) Genome-wide changes under night feeding at ZT4 and ZT16 in gastrocnemius muscle and liver versus WT. n= 3-6, WTs are 2 WT-*Alfp*-Cre, 2 WT-*Hsa*-Cre, 2 WT-*Alfp*+*Hsa*-Cre. Significant differences were calculated by DESeq2 (FDR<0.05).

SUPPLEMENTARY FIGURE 8

A



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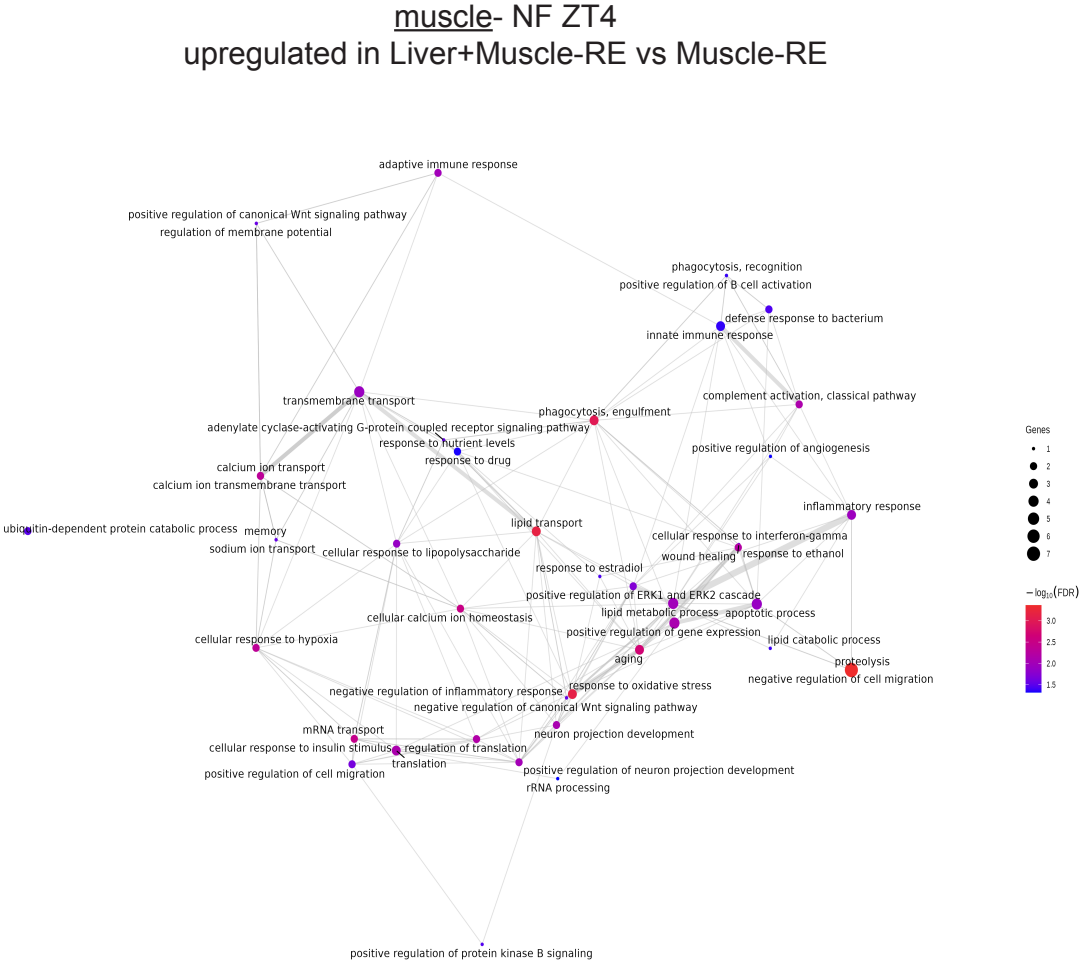


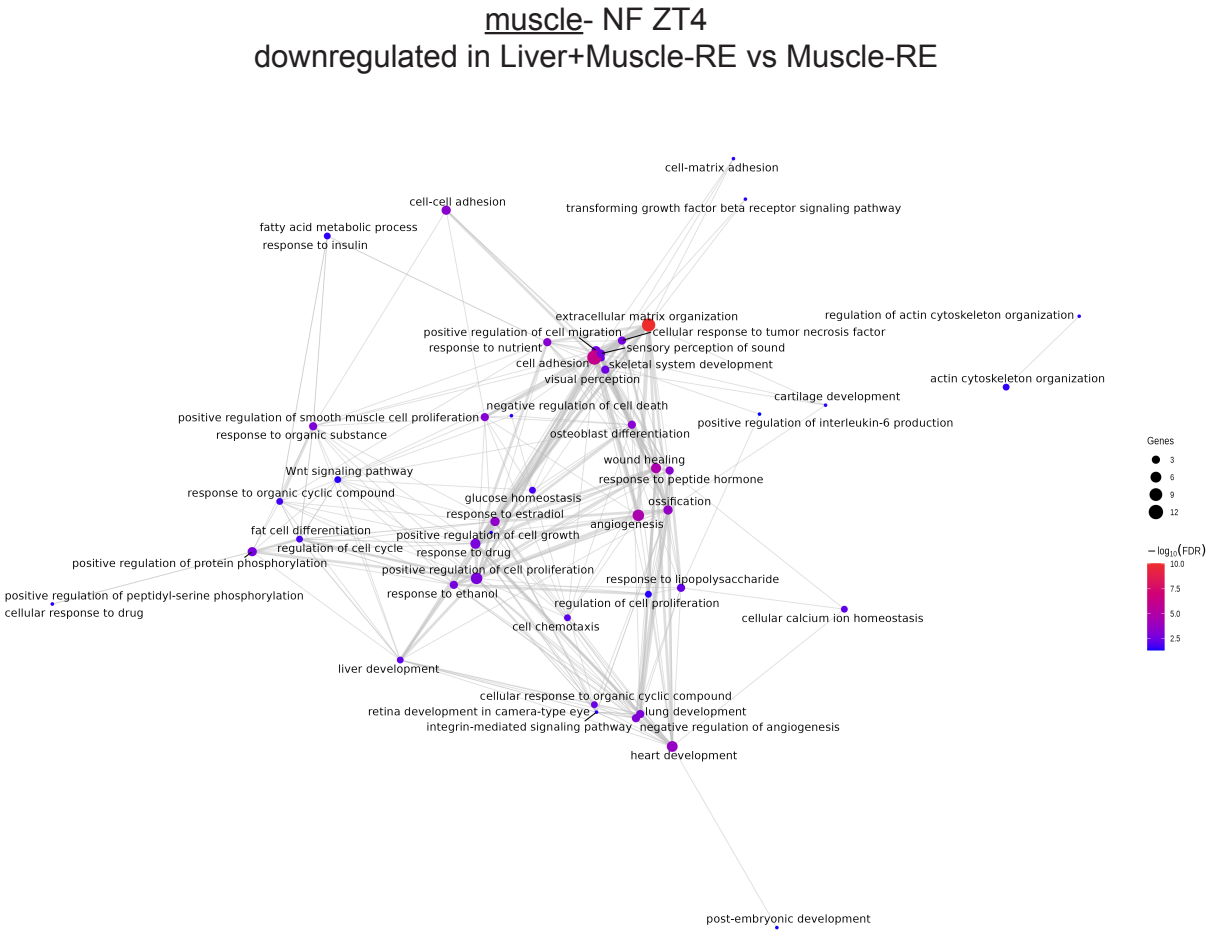


Figure S8. Transcriptional changes in Muscle-RE vs Liver+Muscle-RE muscle under night feeding. Related to Figure 6.

(A) Number of genes altered under night feeding at ZT4 in gastrocnemius Liver+Muscle-RE vs Muscle-RE. Significant differences were calculated by DESeq2 (FDR<0.05).

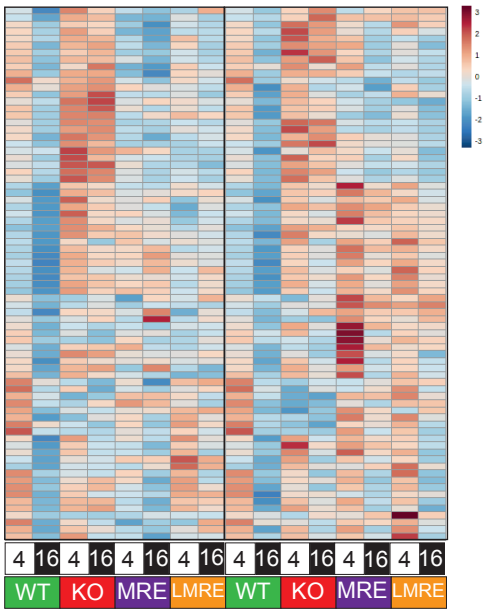
(B) Network gene ontology enrichment analysis for biological process of genes altered under night feeding at ZT4 in gastrocnemius Liver+Muscle-RE vs Muscle-RE. Single nodes without connections were excluded.

SUPPLEMENTARY FIGURE 9

A

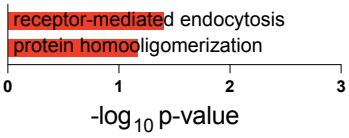
JTK_CYCLE- muscle

'WT only' circadian genes peaking at ZT4

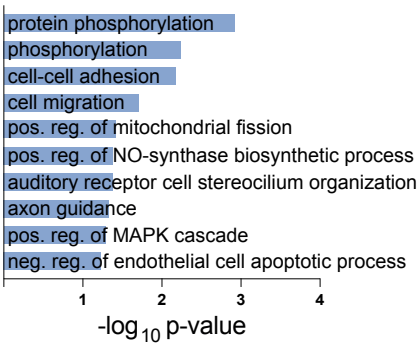


	WT	KO	MRE	LMRE
significant within genotype	56	5	15	8
significant ZT4 vs WT NF 4		26	23	13
significant ZT16 vs WT NF 16		57	23	22

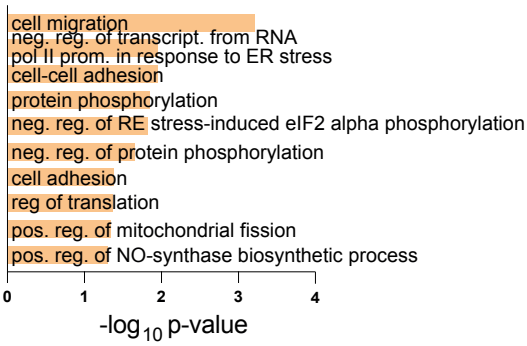
KO rescued under NF



Muscle-RE rescued under NF



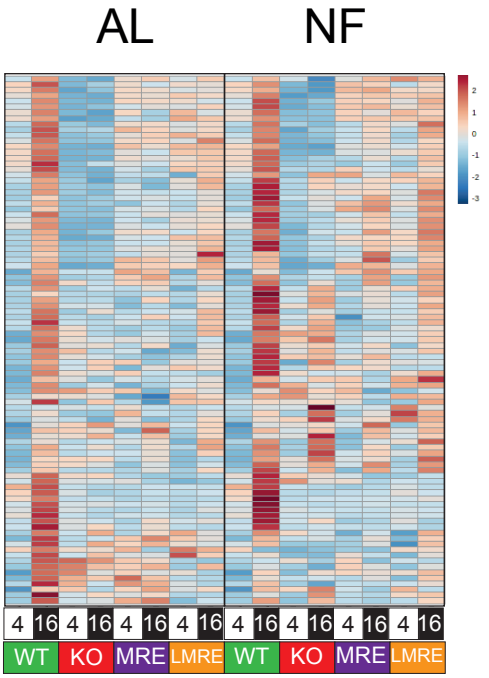
Liver+Muscle-RE rescued under NF



B

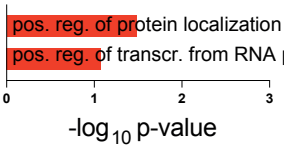
JTK_CYCLE- muscle

'WT only' circadian genes peaking at ZT16

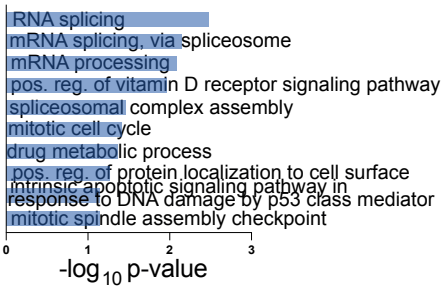


	WT	KO	MRE	LMRE
significant within genotype	76	13	15	12
significant ZT4 vs WT NF 4		33	15	9
significant ZT16 vs WT NF 16		51	36	20

KO rescued under NF



Muscle-RE rescued under NF



Liver+Muscle-RE rescued under NF

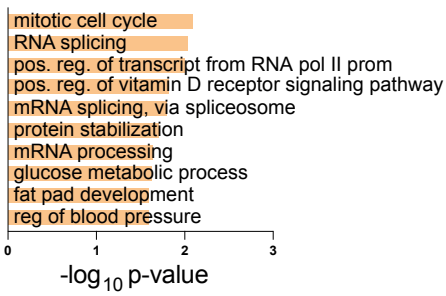


Figure S9. Transcriptional effects of night feeding on circadian and non-circadian genes in muscle RE mice. Related to Figure 6.

(A and B) Effect of night feeding on genes classified using JTK Venn diagram overlap approach (JTK $p < 0.01$ for classifications within each genotype) as only oscillating in WT gastrocnemius muscle (WT-*Hsa*-Cre and WT-*Alfp*+*Hsa*-Cre) under ad libitum feeding conditions and peaking between ZT2-6 (A) or ZT14-18 (B). To display on heatmap, single RE and KO mice under AL are normalized to single Cre WT control, double-RE mice under AL are normalized to double Cre WT control. For clarity, only double Cre WT is shown in AL panel. For both AL and NF, data is displayed as relative to WT controls within that feeding condition. Only genes detected as significantly different by DESeq2 (FDR<0.05) were included. DAVID gene ontology for biological process was performed on the subset of genes which were not significantly different from WT at each timepoint.

SUPPLEMENTARY FIGURE 10

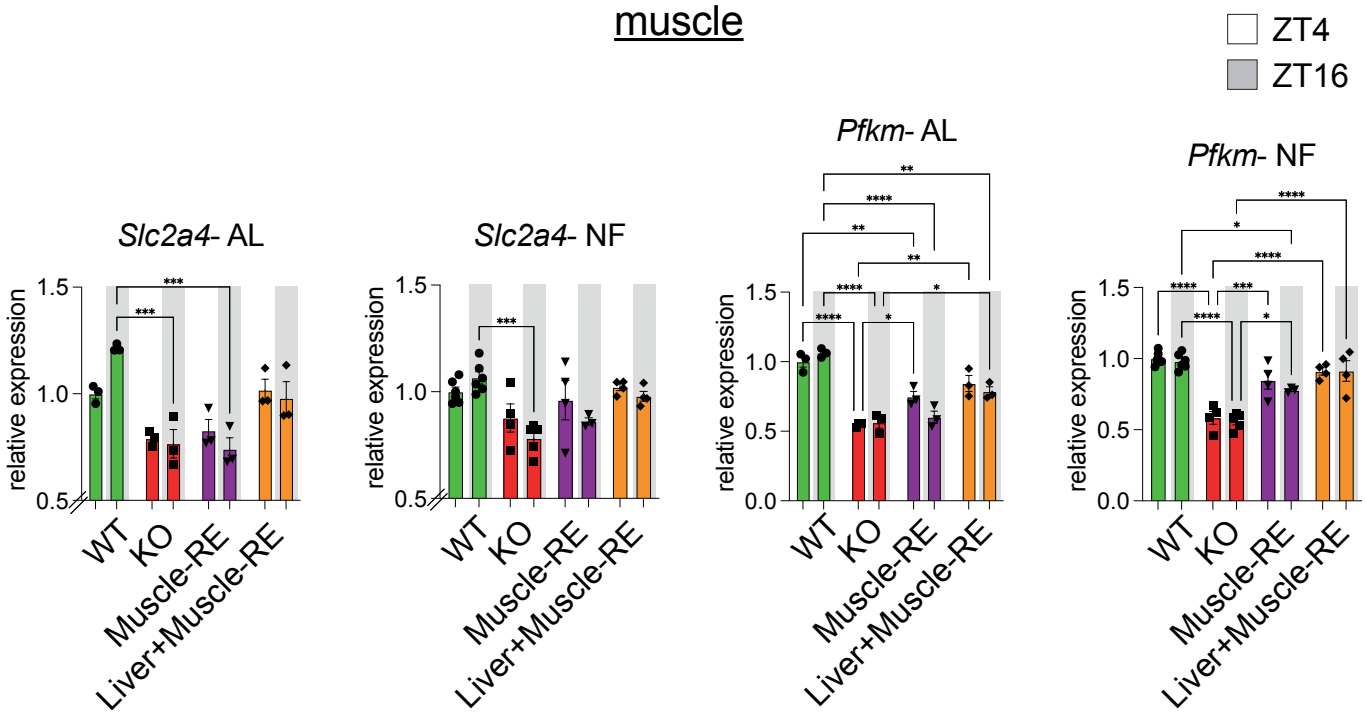


Figure S10. Example non-circadian genes in gastrocnemius muscle under night feeding. Related to Figure 6.

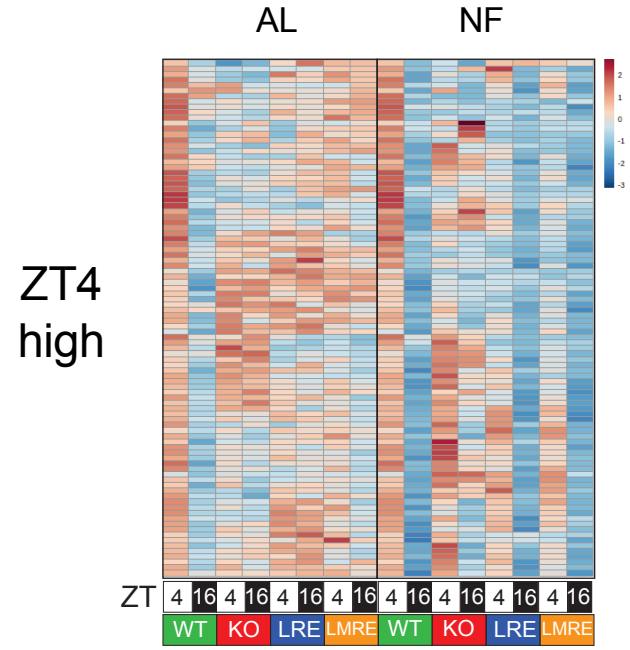
(A) RNA-Seq data from AL and NF mice at ZT4 and ZT16, n = 3-6. For AL, single RE and KO mice are normalized to single Cre WT control, double-RE mice are normalized to double Cre WT control. For clarity, only double Cre WT is shown in AL panel. Two-way ANOVA, Bonferroni's post-hoc test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

SUPPLEMENTARY FIGURE 11

A

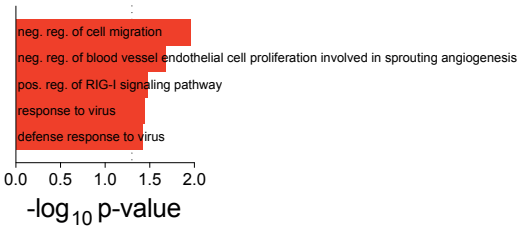
liver

dryR 'WT only' circadian genes

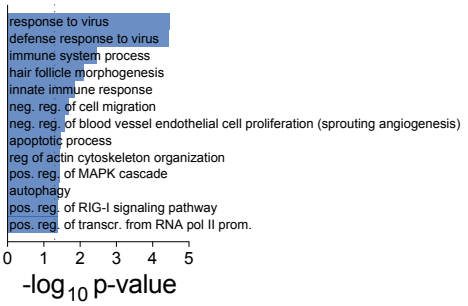


	WT	KO	LRE	LMRE
significant within genotype	94	13	30	30
significant ZT4 vs WT NF 4		15	9	6
significant ZT16 vs WT NF 16		15	2	0

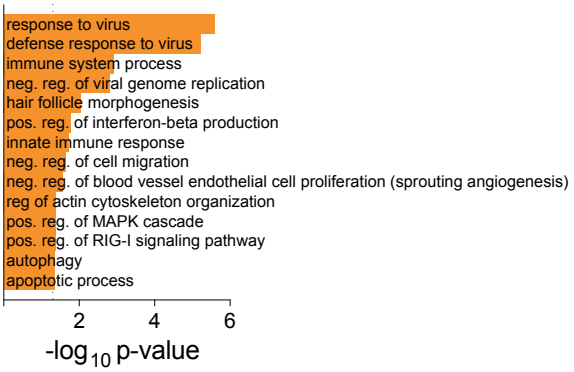
KO rescued under NF



Liver-RE rescued under NF



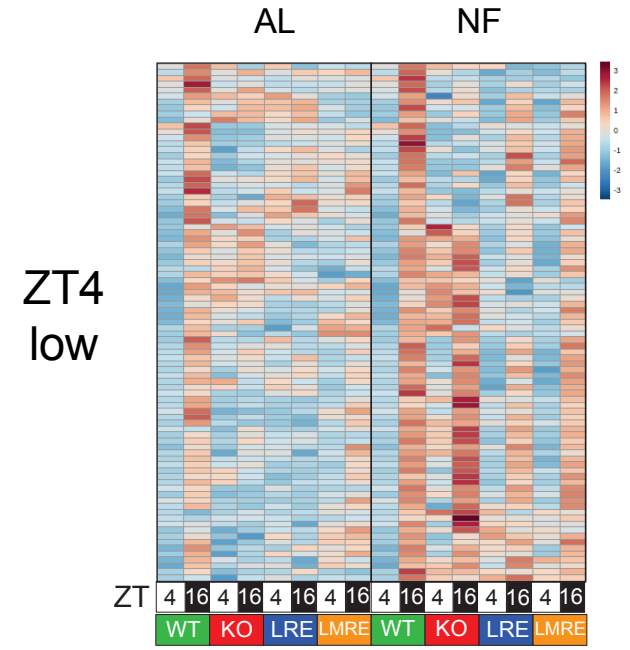
Liver+Muscle-RE rescued under NF



B

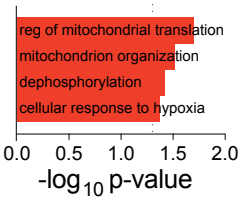
liver

dryR 'WT only' circadian genes

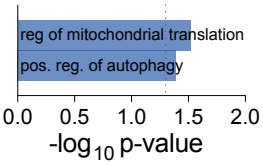


	WT	KO	LRE	LMRE
significant within genotype	87	16	38	30
significant ZT4 vs WT NF 4		18	2	5
significant ZT16 vs WT NF 16		19	5	4

KO rescued under NF



Liver-RE rescued under NF



Liver+Muscle-RE rescued under NF

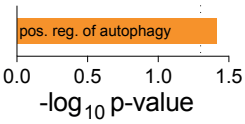


Figure S11. Transcriptional effects of night feeding on circadian and non-circadian genes in liver in RE mice using *dryR*. Related to Figure 6.

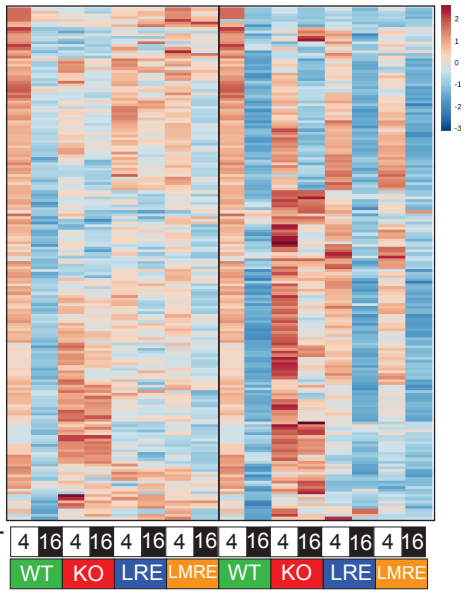
(A and B) Effect of night feeding on genes classified using DryR as only oscillating in WT liver (WT-*Alfp*-Cre and WT-*Alfp*+*Hsa*-Cre) under ad libitum feeding conditions. To display on heatmap, single RE and KO mice under AL are normalized to single Cre WT control, double-RE mice under AL are normalized to double Cre WT control. For clarity, only double Cre WT is shown in AL panel. For both AL and NF, data is displayed as relative to WT controls within that feeding condition. Only genes detected as significantly different by DeSeq2 (FDR<0.05) were included. DAVID gene ontology for biological process was performed on the subset of genes which were not significantly different from WT at each timepoint.

SUPPLEMENTARY FIGURE 12

A

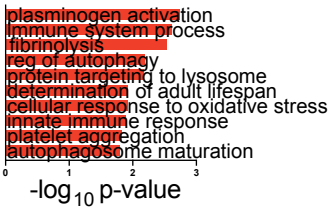
liver

JTK_CYCLE 'WT only' circadian genes peaking at ZT4

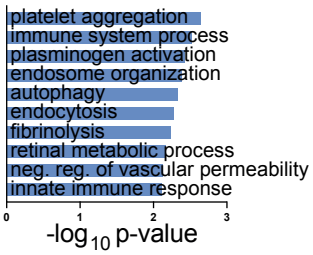


	WT	KO	LRE	LMRE	WT	KO	LRE	LMRE
significant within genotype	140	60	82	80				
significant ZT4 vs WT NF 4		30	6	4				
significant ZT16 vs WT NF 16		44	2	3				

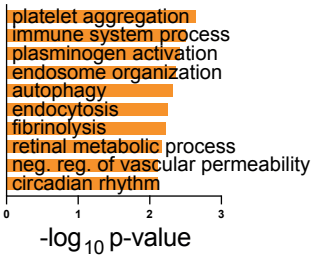
KO rescued under NF



Liver-RE rescued under NF



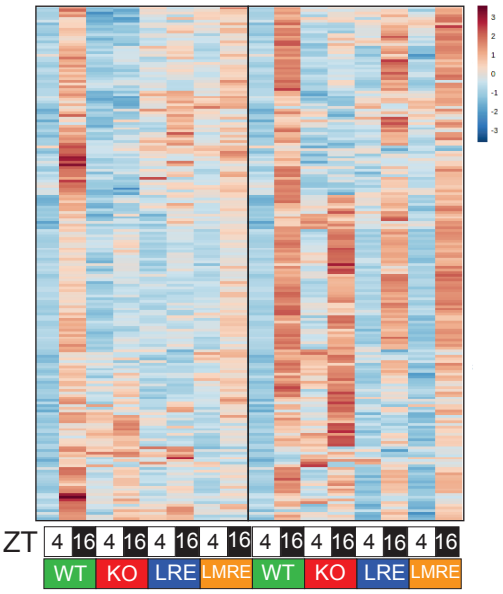
Liver+Muscle-RE rescued under NF



B

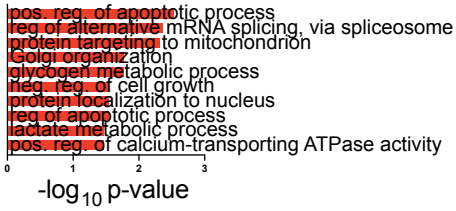
liver

JTK_CYCLE 'WT only' circadian genes peaking at ZT16

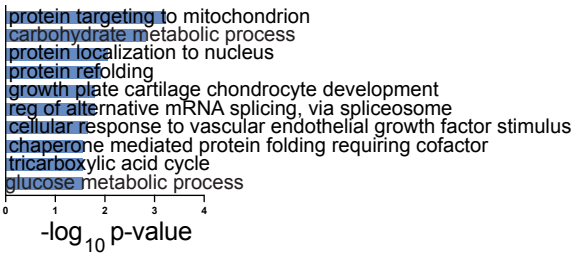


	WT	KO	LRE	LMRE	WT	KO	LRE	LMRE
significant within genotype	169	43	108	108				
significant ZT4 vs WT NF 4		39	20	23				
significant ZT16 vs WT NF 16		61	1	1				

KO rescued under NF



Liver-RE rescued under NF



Liver+Muscle-RE rescued under NF

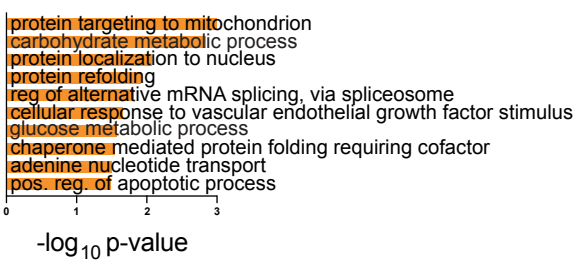


Figure S12. Transcriptional effects of night feeding on circadian and non-circadian genes in liver in RE mice using JTK_CYCLE. Related to Figure 6.

(A and B) Effect of night feeding on genes classified using JTK Venn diagram overlap approach (JTK $p < 0.01$ for classifications within each genotype) as only oscillating in WT liver (WT-*Alfp*-Cre and WT-*Alfp*+*Hsa*-Cre) under ad libitum feeding conditions and peaking between ZT2-6 (A) or ZT14-18 (B). To display on heatmap, single RE and KO mice under AL are normalized to single Cre WT control, double-RE mice under AL are normalized to double Cre WT control. For clarity, only double Cre WT is shown in AL panel. For both AL and NF, data is displayed as relative to WT controls within that feeding condition. Only genes detected as significantly different by DESeq2 (FDR<0.05) were included. DAVID gene ontology for biological process was performed on the subset of genes which were not significantly different from WT at each timepoint.

SUPPLEMENTARY FIGURE 13

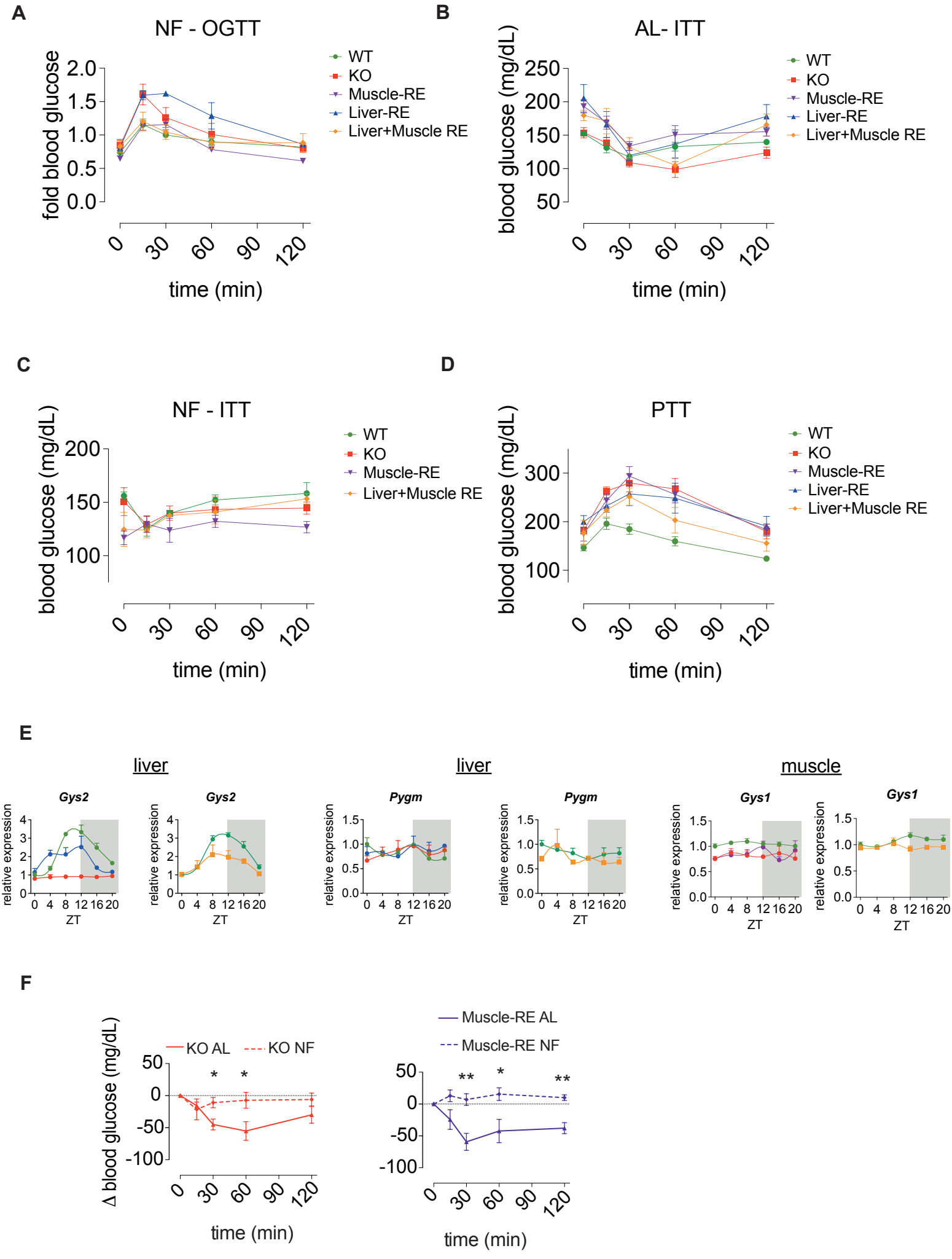


Figure S13. Metabolic tests in RE mice. Related to Figure 7.

(A) Raw data for night feeding oral glucose tolerance tests.

(B) Raw data for *ad libitum* insulin glucose tolerance tests.

(C) Raw data for night feeding insulin tolerance tests.

(D) Raw data for *ad libitum* pyruvate/lactate tolerance tests.

(E) Expression of gene related to glycogen metabolism. Data are normalized to WT ZT0.