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Supplemental Information

Fasting Imparts a Switch to Alternative Daily Pathways in Liver and Muscle

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Figure S1

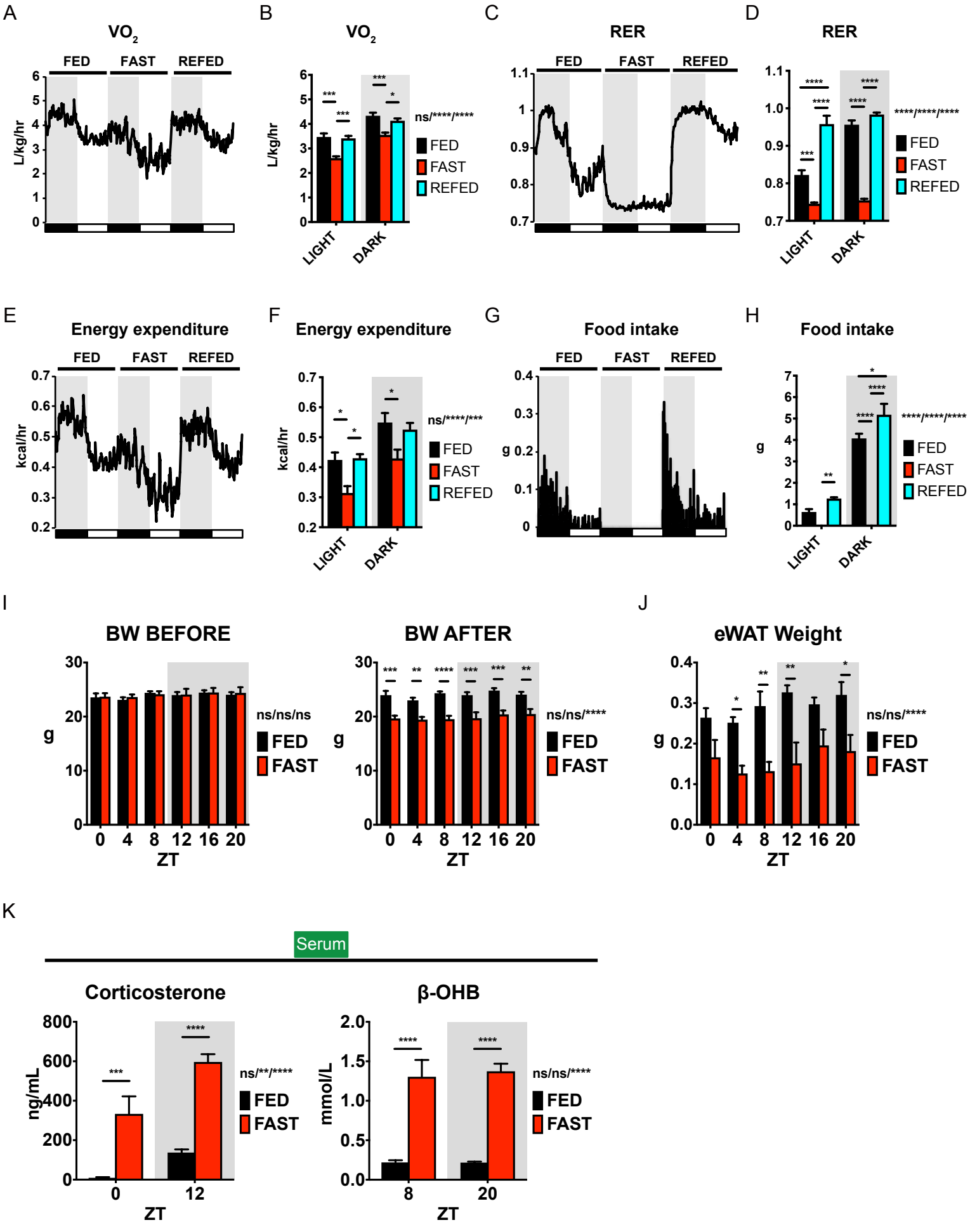

A
VO2
FED
FAST
REFED
L/kg/hr
B
VO2
L/kg/hr
ns/****/****
FED
FAST
REFED
LIGHT
DARK
C
RER
FED
FAST
REFED
D
RER
****/****/****
FED
FAST
REFED
LIGHT
DARK
E
Energy expenditure
FED
FAST
REFED
kcal/hr
F
Energy expenditure
kcal/hr
ns/****/***
FED
FAST
REFED
LIGHT
DARK
G
Food intake
FED
FAST
REFED
g
H
Food intake
g
****/****/****
FED
FAST
REFED
LIGHT
DARK
I
BW BEFORE
g
ns/ns/ns
FED
FAST
ZT
BW AFTER
g
ns/ns/****
FED
FAST
ZT
J
eWAT Weight
g
ns/ns/****
FED
FAST
ZT
K
Serum
Corticosterone
ng/mL
ns/**/****
FED
FAST
ZT
β-OHB
mmol/L
ns/ns/****
FED
FAST
ZT

Figure S1. Physical metabolic paradigms after 24-hr fasting and refeeding. Related to Figure 1.

(A and B) Metabolic cage analysis showing measured oxygen consumption (VO_2) for mice under FED, FAST, and REFED conditions and the average during the light and dark phases. (C and D) Metabolic cage analysis showing respiratory exchange ratio (RER) calculated by VCO_2/VO_2 for mice under FED, FAST, and REFED conditions and the average during the light and dark phases. (E and F) Metabolic cage analysis showing energy expenditure for mice under FED, FAST, and REFED conditions and the average during the light and dark phases. (G and H) Metabolic cage analysis showing food intake and the total amount consumed during the light and dark phases. Each parameter was recorded every 10 min over the circadian cycle in wild-type male mice subject to normal chow diet in *ad libitum* for the first 24-hr, subsequently to fasting for 24-hr, and lastly to refeeding for 24-hr. Data are shown as mean for the time series, and as mean + SEM for the average (n=5 biological replicates). (I and J) Body weight of mice before and after 24-hr fasting, and epididymal fat weight after 24-hr fasting at each time point. (K) Serum corticosterone and β-OHB levels from mice under FED and FAST conditions. Data are shown as mean + SEM (n=5 biological replicates per time point per group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ by two-way ANOVA (interaction/phase or time/group) with Bonferroni post-hoc tests.

Figure S2

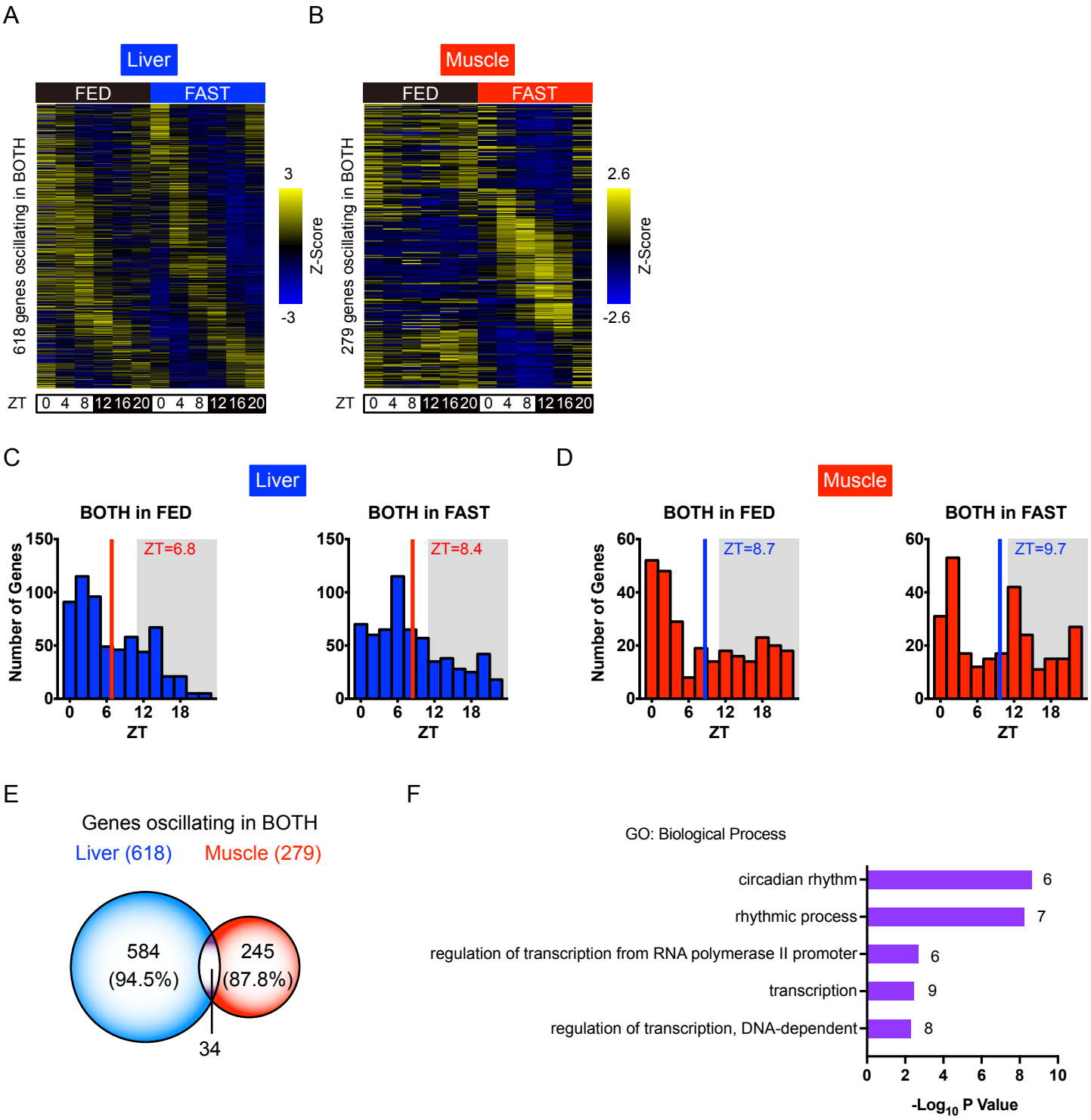

A
Liver
FED
FAST
618 genes oscillating in BOTH
3
Z-Score
-3
ZT 0 4 8 12 16 20 0 4 8 12 16 20
B
Muscle
FED
FAST
279 genes oscillating in BOTH
2.6
Z-Score
-2.6
ZT 0 4 8 12 16 20 0 4 8 12 16 20
C
Liver
BOTH in FED
ZT=6.8
Number of Genes
ZT
BOTH in FAST
ZT=8.4
ZT
D
Muscle
BOTH in FED
ZT=8.7
Number of Genes
ZT
BOTH in FAST
ZT=9.7
ZT
E
Genes oscillating in BOTH
Liver (618)
Muscle (279)
584
(94.5%)
245
(87.8%)
34
F
GO: Biological Process
circadian rhythm
rhythmic process
regulation of transcription from RNA polymerase II promoter
transcription
regulation of transcription, DNA-dependent
-Log10 P Value

Figure S2. Hepatic and muscle circadian transcriptomes. Related to Figure 1.

(A and B) Heat maps show hepatic and muscle transcripts cycling both in FED and FAST (JTK_CYCLE $P < 0.01$). (C and D) Phase distributions showing the peak phase of hepatic and muscle genes oscillating both in FED and FAST mice. The peak phase in FED is shown on the left panels, and FAST on the right panels. Mean phases are indicated in the graphs. (E) Venn diagrams showing the number of hepatic and muscle cycling transcripts both in FED and FAST. (F) Gene ontology analysis shows top biological processes enriched in rhythmic genes in both FED and FAST in both liver and muscle, with the number of genes indicated on the graph.

Figure S3

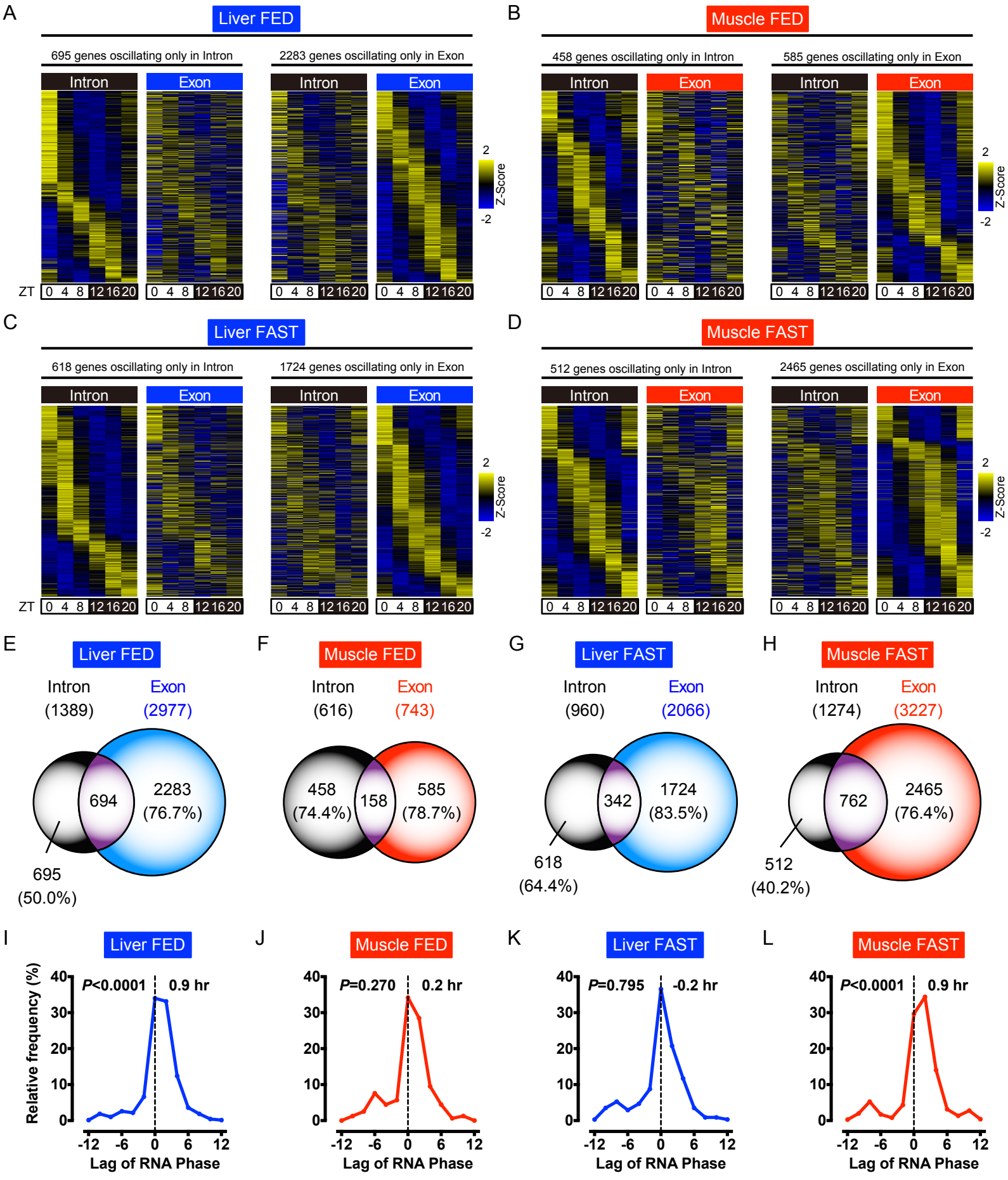

A
Liver FED
695 genes oscillating only in Intron
2283 genes oscillating only in Exon
Intron
Exon
ZT 0 4 8 12 16 20
2
Z-Score
-2
B
Muscle FED
458 genes oscillating only in Intron
585 genes oscillating only in Exon
C
Liver FAST
618 genes oscillating only in Intron
1724 genes oscillating only in Exon
D
Muscle FAST
512 genes oscillating only in Intron
2465 genes oscillating only in Exon
E
Liver FED
Intron (1389)
Exon (2977)
694
2283 (76.7%)
695 (50.0%)
F
Muscle FED
Intron (616)
Exon (743)
458 (74.4%)
158
585 (78.7%)
G
Liver FAST
Intron (960)
Exon (2066)
342
1724 (83.5%)
618 (64.4%)
H
Muscle FAST
Intron (1274)
Exon (3227)
762
2465 (76.4%)
512 (40.2%)
I
Liver FED
P<0.0001
0.9 hr
J
Muscle FED
P=0.270
0.2 hr
K
Liver FAST
P=0.795
-0.2 hr
L
Muscle FAST
P<0.0001
0.9 hr
Relative frequency (%)
0 10 20 30 40
-12 -6 0 6 12
Lag of RNA Phase

Figure S3. Post-transcriptional control of temporal response to fasting. Related to Figures 1 and 2.

(A-D) Exon Intron Split Analysis was implemented using RNA-seq data from liver and skeletal muscle in FED and FAST conditions. Heat maps show cycling transcripts only in intronic reads (left panels) or in exonic reads (right panels) (JTK_CYCLE $P < 0.01$). (E-H) Venn diagrams indicate the number of oscillating intronic and exonic transcripts in the liver and skeletal muscle in FED and FAST conditions. (I-L) Graphs show the distribution of phase lags of exonic reads compared to intronic reads in the liver and skeletal muscle in FED and FAST conditions. Wilcoxon Signed Rank Test was performed to determine if there is a significant lag compared to theoretical median value (zero).

Figure S4

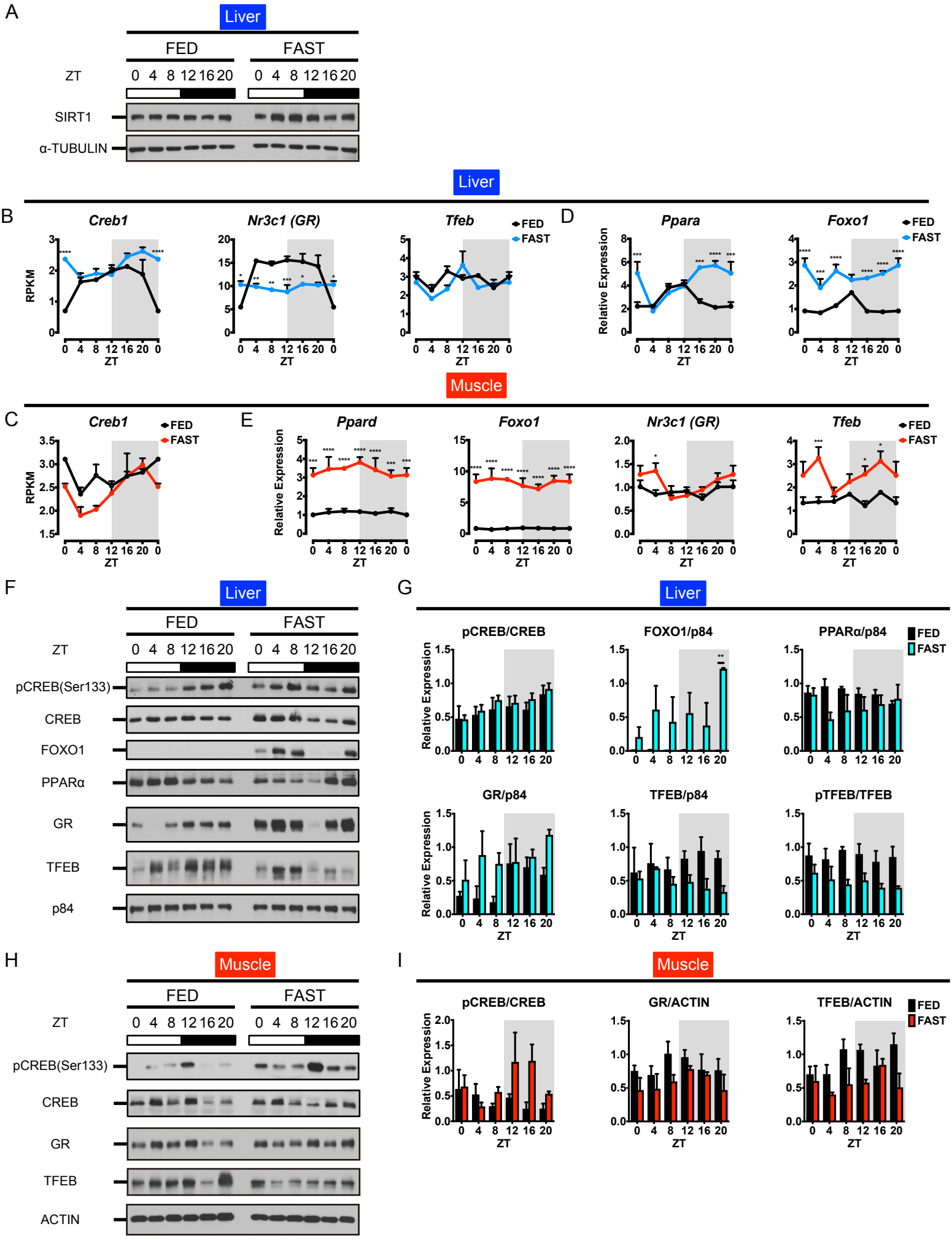

A
Liver
FED
FAST
ZT
0 4 8 12 16 20 0 4 8 12 16 20
SIRT1
α-TUBULIN
Liver
B
Creb1
Nr3c1 (GR)
Tfeb
FED
FAST
RPKM
ZT
D
Ppara
Foxo1
Relative Expression
Muscle
C
Creb1
E
Ppard
Foxo1
Nr3c1 (GR)
Tfeb
F
Liver
pCREB(Ser133)
CREB
FOXO1
PPARα
GR
TFEB
p84
G
Liver
pCREB/CREB
FOXO1/p84
PPARα/p84
GR/p84
TFEB/p84
pTFEB/TFEB
H
Muscle
ACTIN
I
Muscle
GR/ACTIN
TFEB/ACTIN

Figure S4. Differential gene regulation by the clock and fasting sensing pathways in the liver and skeletal muscle. Related to Figures 3 and 4.

(A) Representative immunoblots of hepatic SIRT1 and α-TUBULIN in whole cell lysates from FED and FAST mice over the circadian cycle. (B and C) Gene expression profiles of hepatic *Creb1*, *Nr3c1* (*GR*), *Tfeb*, and muscle *Creb1* measured by RNA-seq. Transcript levels were presented as mean + SEM (n=3 biological replicates per time point per group). ZT0 is double plotted for visualization. (D and E) Gene expression profiles of hepatic *Ppara* and *Foxo1,* and muscle *Ppard*, *Foxo1, Nr3c1* (*GR*), and *Tfeb* measured by RT-qPCR. Transcript levels were normalized to 18S ribosomal RNA and presented as mean + SEM (n=5 biological replicates per time point per group). ZT0 is double plotted for visualization. (F) Representative immunoblots and of hepatic phospho-CREB (pCREB), CREB, FOXO1, PPARα, GR, TFEB, and p84 in nuclear extracts from FED and FAST mice. (G) Quantitation of the blot band density of the hepatic proteins presented as mean + SEM (n=3 biological replicates per time point per group). $^{**}p < 0.01$ by two-way ANOVA with Bonferroni post-hoc tests. The upper band density of hepatic TFEB was quantitated as phospho-TFEB (pTFEB). (H) Representative immunoblots of muscle pCREB, CREB, GR, TFEB, and ACTIN in whole cell lysates from FED and FAST mice. (I) Quantitation of the blot band density of the muscle proteins presented as mean + SEM (n=3 biological replicates per time point per group).

Figure S5

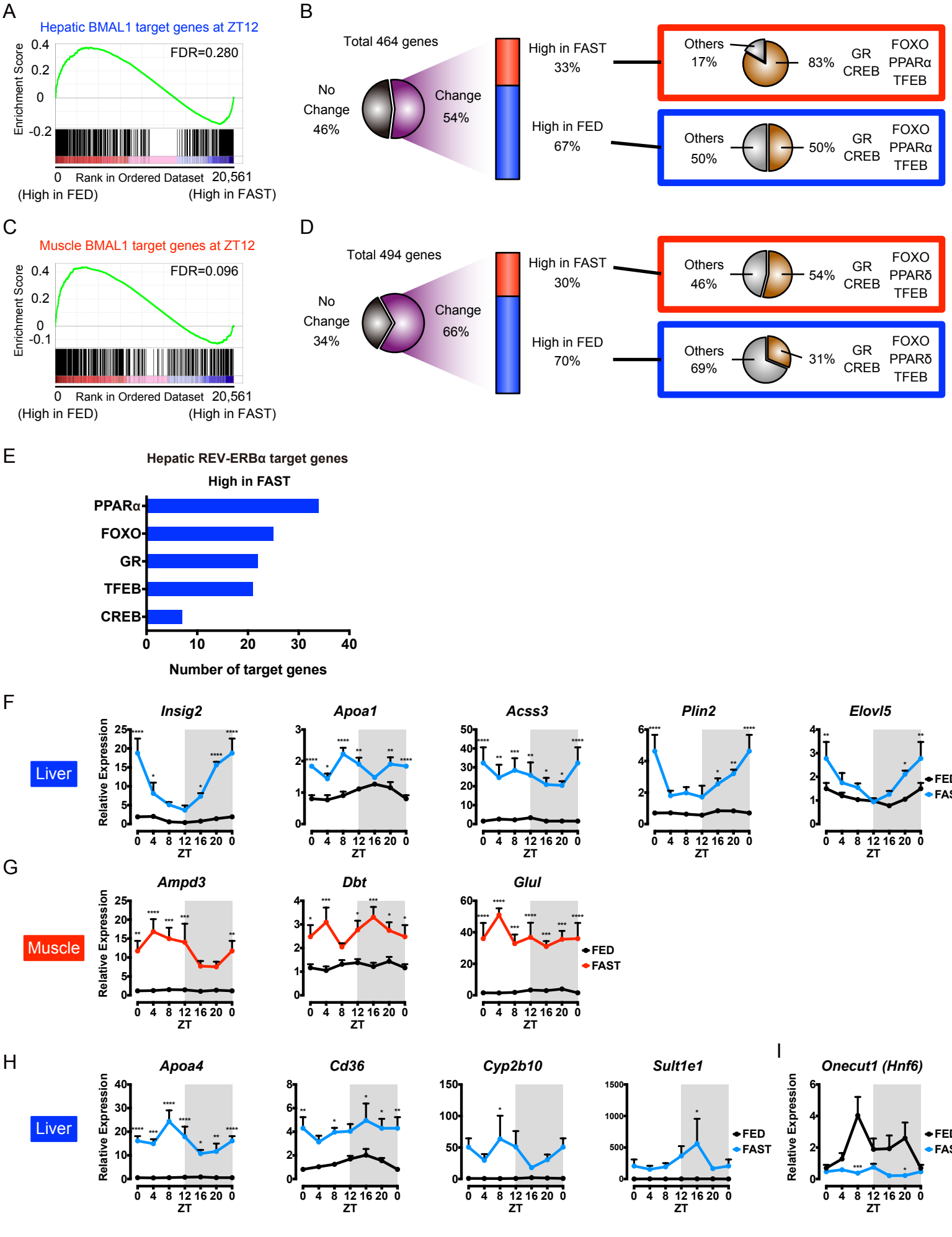
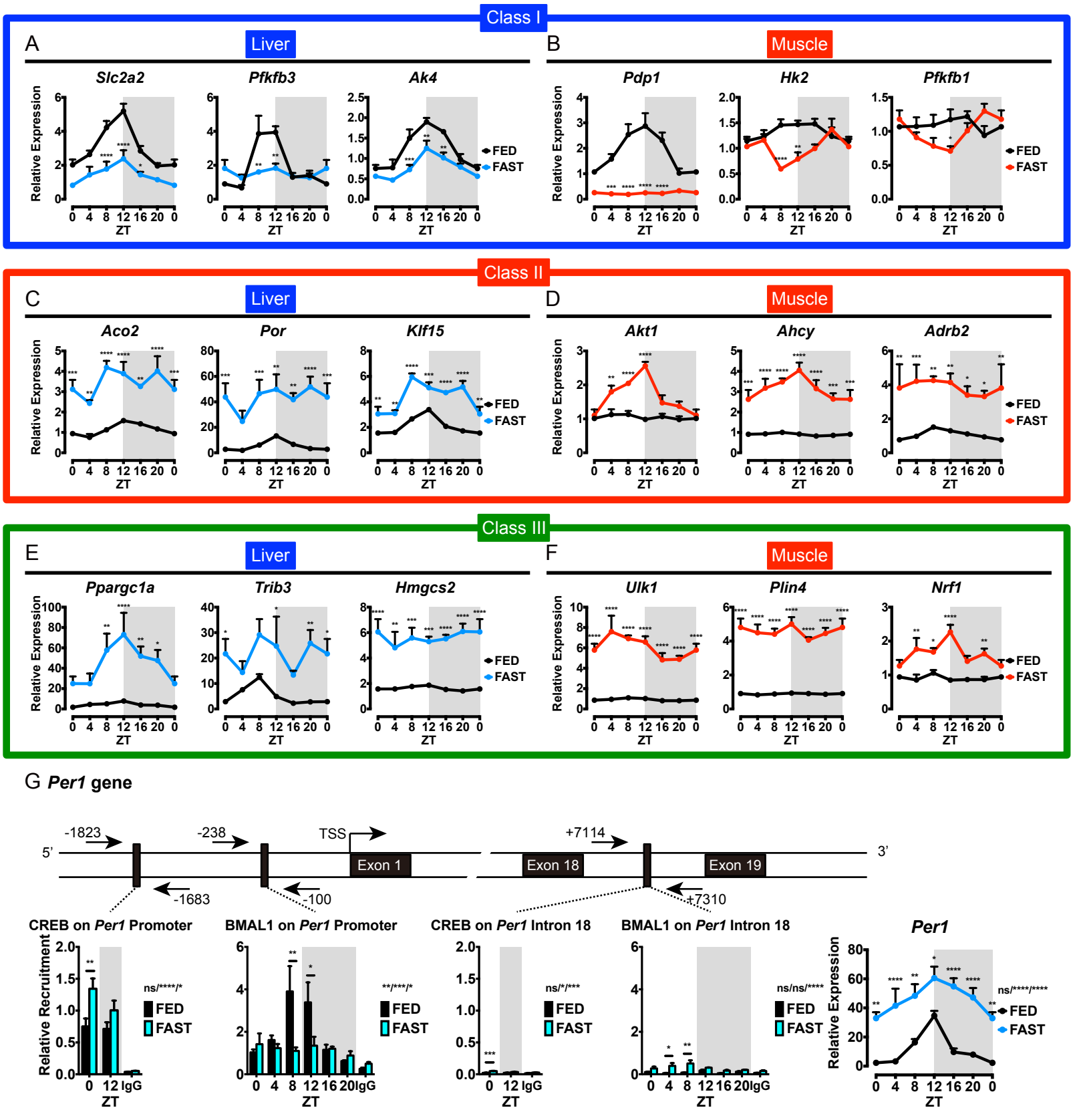

A
Hepatic BMAL1 target genes at ZT12
Enrichment Score
FDR=0.280
Rank in Ordered Dataset 20,561
(High in FED)
(High in FAST)
B
Total 464 genes
No Change 46%
Change 54%
High in FAST 33%
High in FED 67%
Others 17%
83%
GR CREB
FOXO PPARα TFEB
Others 50%
50%
GR CREB
FOXO PPARα TFEB
C
Muscle BMAL1 target genes at ZT12
FDR=0.096
D
Total 494 genes
No Change 34%
Change 66%
High in FAST 30%
High in FED 70%
Others 46%
54%
Others 69%
31%
FOXO PPARδ TFEB
E
Hepatic REV-ERBα target genes
High in FAST
PPARα
FOXO
GR
TFEB
CREB
Number of target genes
F
Liver
Insig2
Apoa1
Acss3
Plin2
Elovl5
Relative Expression
ZT
FED
FAST
G
Muscle
Ampd3
Dbt
Glul
H
Liver
Apoa4
Cd36
Cyp2b10
Sult1e1
I
Onecut1 (Hnf6)

Figure S5. Temporal pattern of differential gene regulation by BMAL1 and REV-ERBα under fasting. Related to Figure 4.

(A) Gene set enrichment analysis (GSEA) for hepatic BMAL1 target genes at ZT12 enriched in FED. (B) Differentially expressed BMAL1 target genes in the liver at ZT12 illustrating fasting-sensitive TF coordinated regulation. (C) GSEA for muscle BMAL1 target genes at ZT12 enriched in FED. (D) Differentially expressed BMAL1 target genes in the muscle at ZT12 illustrating fasting-sensitive TF coordinated regulation. Gene sets with false discovery rate (FDR) < 0.25 are considered significantly enriched. (E) The numbers of hepatic GR, CREB, FOXO, TFEB, and PPARα target genes among the hepatic REV-ERBα target genes expressed significantly higher in FAST mice. (F) Gene expression profiles of hepatic REV-ERBα targets by RT-qPCR of FED and FAST mice. (G) Gene expression profiles of muscle REV-ERBα/HDAC3 targets by RT-qPCR of FED and FAST mice. (H) Gene expression profiles of hepatic HNF6/REV-ERBα targets by RT-qPCR of FED and FAST mice. (I) Gene expression profile of hepatic *Onecut1* (*Hnf6*) by RT-qPCR of FED and FAST mice. Transcript levels were measured by RT-qPCR, normalized to 18S ribosomal RNA, and presented as mean + SEM (n=5 biological replicates per time point per group). ZT0 is double plotted for visualization. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ by two-way ANOVA with Bonferroni post-hoc tests.

Figure S6



G *Per1* gene

Figure S6. Differential gene regulation by clock and fasting responsive TFs. Related to Figure 5.

(A and B) Representative gene expression profiles of hepatic and muscle BMAL1 target genes repressed by fasting (Class I). (C and D) Representative gene expression profiles of hepatic and muscle BMAL1 target genes induced by fasting (Class II). (E and F) Representative gene expression profiles of hepatic and muscle fasting-induced genes of non-BMAL1 targets (Class III). Transcript levels were normalized to 18S ribosomal RNA and presented as mean + SEM (n=5 biological replicates per time point per group). ZT0 is double plotted for visualization. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ by two-way ANOVA with Bonferroni post-hoc tests. (G) Chromatin immunoprecipitation in liver for BMAL1, CREB, or IgG followed by RT-qPCR specific for *Per1* gene. Data are presented as mean + SEM (n=3~4 biological replicates per time point per group). Diagram above shows schematic representation of *Per1* gene illustrating regions of amplification by primers designed for analysis (arrows). TSS denotes transcription start site. *Per1* gene expression profile is also shown next to ChIP-qPCR data for visualization. Transcript levels were normalized to 18S ribosomal RNA and presented as mean + SEM (n=5 biological replicates per time point per group). ZT0 is double plotted for visualization. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ by two-way ANOVA (interaction/time/group) with Bonferroni post-hoc tests.

Figure S7

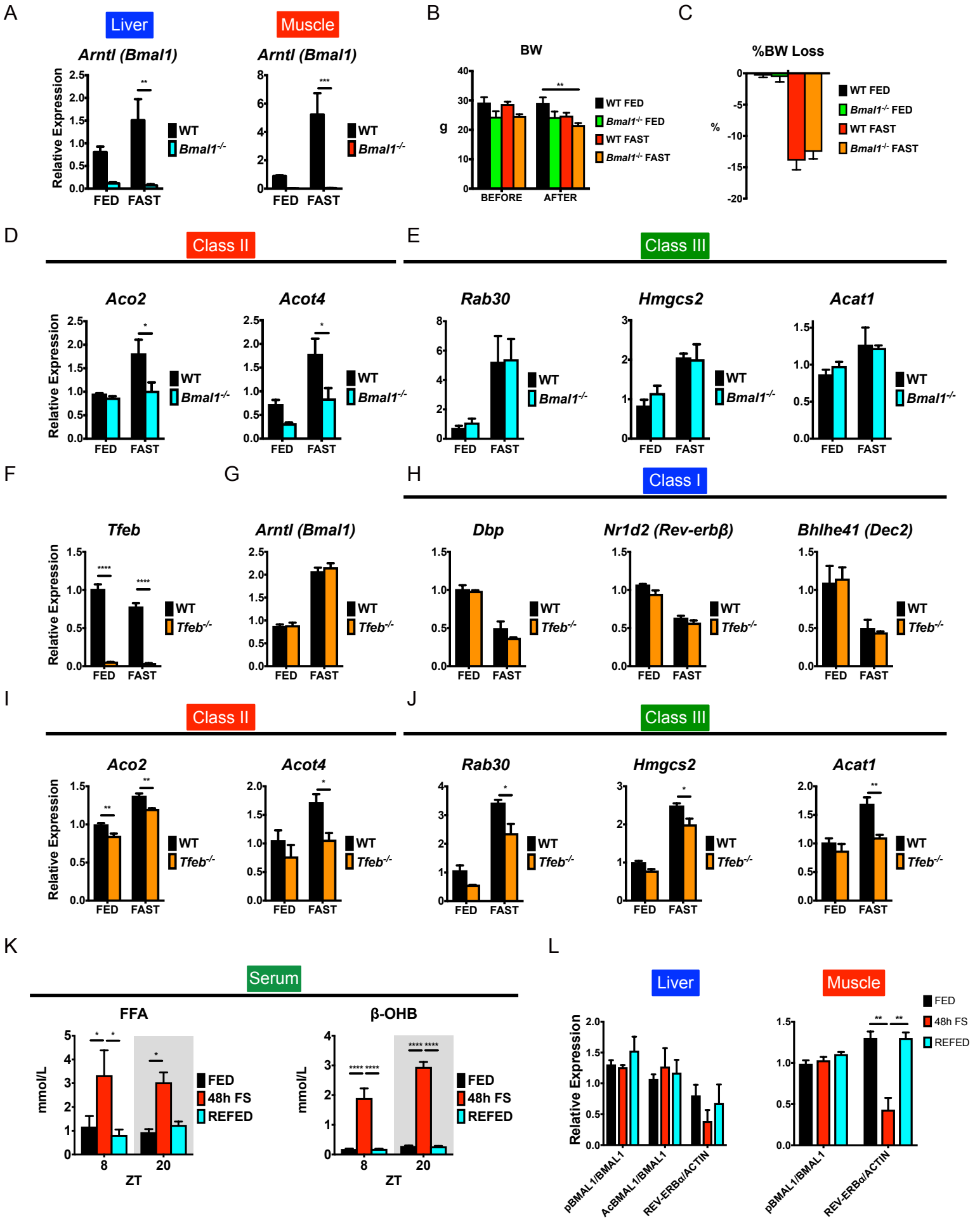

A
Liver
Arntl (Bmal1)
Relative Expression
WT
Bmal1-/-
FED FAST
Muscle
Arntl (Bmal1)
B
BW
g
WT FED
Bmal1-/- FED
WT FAST
Bmal1-/- FAST
BEFORE AFTER
C
%BW Loss
%
D
Class II
Aco2
Acot4
E
Class III
Rab30
Hmgcs2
Acat1
F
Tfeb
Tfeb-/-
G
Arntl (Bmal1)
H
Class I
Dbp
Nr1d2 (Rev-erbβ)
Bhlhe41 (Dec2)
I
Class II
Aco2
Acot4
J
Class III
Rab30
Hmgcs2
Acat1
K
Serum
FFA
β-OHB
mmol/L
FED
48h FS
REFED
ZT
8
20
L
Liver
Muscle
Relative Expression
pBMAL1/BMAL1
AcBMAL1/BMAL1
REV-ERBα/ACTIN
pBMAL1/BMAL1
REV-ERBα/ACTIN

Figure S7. Differential gene regulation by clock and fasting responsive TFs, and temporal response to fasting. Related to Figures 6 and 7.

(A) Hepatic and muscle gene expression of *Arntl* (*Bmal1*) in WT and *Bmal1*$^{-/-}$ mice at ZT8. Transcript levels were normalized to 18S ribosomal RNA and presented as mean + SEM (n=4~5 biological replicates per genotype per group). (B) Body weight of WT and *Bmal1*$^{-/-}$ mice before and after 24-hr fasting. Data are shown as mean + SEM (n=4~5 biological replicates per genotype per group). (C) % body weight loss after 24-hr fasting at ZT8. Data are shown as mean - SEM (n=4~5 biological replicates per genotype per group). (D) Representative gene expression profiles of hepatic BMAL1 target genes induced by fasting (Class II) in WT and *Bmal1*$^{-/-}$ mice at ZT8. (E) Representative gene expression profiles of hepatic fasting-induced genes of non-BMAL1 targets (Class III) in WT and *Bmal1*$^{-/-}$ mice at ZT8. Data are shown as mean + SEM (n=4~5 biological replicates per genotype per group). (F and G) Hepatic gene expression of *Tfeb* and *Arntl* (*Bmal1*) in WT and *Tfeb* LiKO (*Tfeb*$^{-/-}$) mice at ZT8. (H) Representative gene expression profiles of hepatic BMAL1 target genes repressed by fasting (Class I) in WT and *Tfeb*$^{-/-}$ mice at ZT8. (I) Representative gene expression profiles of hepatic BMAL1 and TFEB target genes induced by fasting (Class II) in WT and *Tfeb*$^{-/-}$ mice at ZT8. (J) Representative gene expression profiles of hepatic TFEB-target, non-BMAL1 target genes induced by fasting (Class III) in WT and *Tfeb*$^{-/-}$ mice at ZT8. Data are shown as mean + SEM (n=3~5 biological replicates per genotype per group). (K) Serum free fatty acid (FFA) and β-OHB levels from mice under *ad libitum* fed (FED), 48-hr fasting (48h FS), and 24-hr fasting with 24-hr refeeding (REFED) conditions. Data are shown as mean + SEM (n=3 biological replicates per time point per group). (L) Quantitation of the blot band density presented as mean + SEM (n=3 biological replicates per group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ by two-way ANOVA with Bonferroni post-hoc tests.