Expanded View Figures

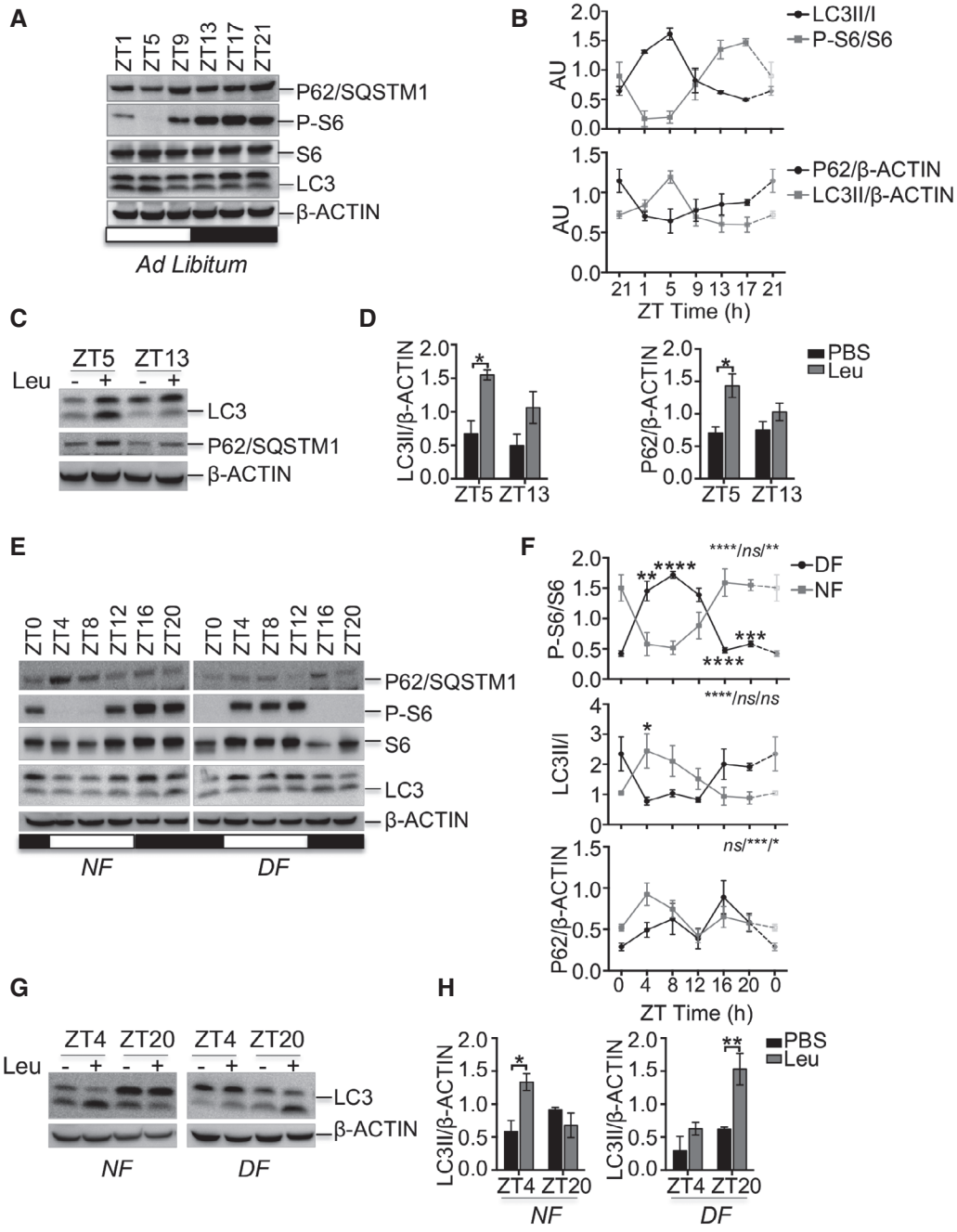

Figure EV1.

Figure EV1. Activation of autophagy and mTORC1 pathways depends on feeding time.

A, B Autophagy markers (LC3 and P62/SQSTM1) and mTORC1 activity as indicated by the phosphorylation of S6 ribosomal protein were measured during the 24-h cycle in the liver from mice fed *ad libitum*. The plots represent average values of $n = 3$ for each time point expressed as ratio of LC3II/LC3I or LC3II/actin, p62/actin, and phosphorylated S6 versus pan-S6. ZT21 is double-plotted for visualization. Data are represented as mean ± SEM.

C, D Autophagy flux analysis in liver from WT mice fed *ad libitum* injected with leupeptin or PBS (C) and relative quantification (D) ($n = 3$ per group). Data are presented as mean ± SEM (two-way ANOVA test followed by the Bonferroni *post hoc* test: $^*P < 0.05$).

E, F Autophagy markers and mTORC1 activity as indicated by the phosphorylation of S6 ribosomal protein measured during the 24-h cycle in the liver from mice fed during the night (NF) or during the day (DF). The plots represent average values of $n = 3$ for each time point. The gray bars represent the dark cycle. Zeitgeber time ZT0: lights on; ZT12: light off. ZT0 is double-plotted for visualization. Data are presented as mean ± SEM (two-way ANOVA test (interaction/time/group) followed by the Bonferroni *post hoc* test: (*ns* non-significant; $^*P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$).

G, H Autophagy flux analysis in liver from WT mice fed during the night (NF) or during the day (DF) injected with leupeptin or PBS (G) and relative quantification (H) ($n = 3$ per group). Data are presented as mean ± SEM. (two-way ANOVA test followed by the Bonferroni *post hoc* test: $^*P < 0.05$; $^{**}P < 0.01$).

Source data are available online for this figure.

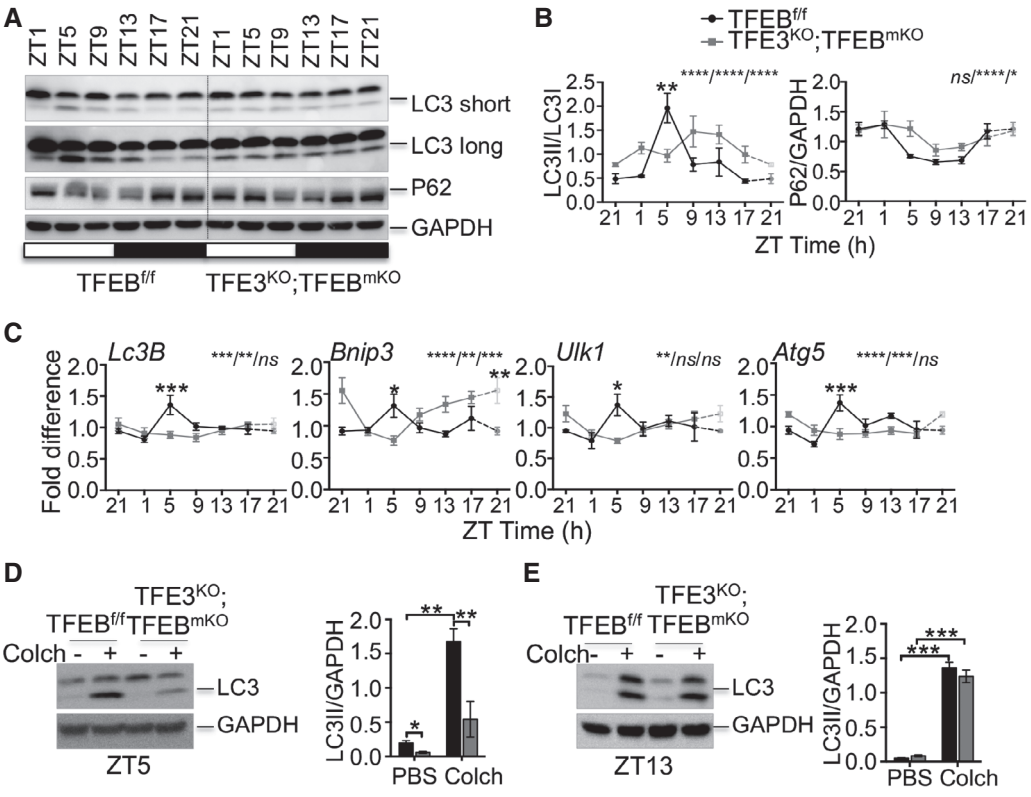

Figure EV2. Autophagy activation during the light phase is impaired in skeletal muscle from TFEB/TFE3-deficient mice.

A, B Western blot analysis of autophagy protein throughout the day in muscle samples collected every 4 h for 24 h from control and $TFE3^{KO}$;$TFEB^{mKO}$ mice (A) and relative quantification (B) ($n = 3$ per group/time point). ZT21 is double-plotted for visualization. Data are presented as mean ± SEM. (two-way ANOVA test (interaction/time/group) followed by the Bonferroni *post hoc* test: *ns* non-significant; $^*P \leq 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$).

C mRNA expression analysis at different time points of genes involved in autophagy in WT and $TFE3^{KO}$;$TFEB^{mKO}$ muscle determined by qPCR. ($n = 3$ per group/time point). ZT21 is double-plotted for visualization. Data are presented as mean ± SEM (two-way ANOVA test (interaction/time/group) followed by the Bonferroni *post hoc* test: *ns* non-significant; $^*P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$).

D, E LC3 protein levels in muscle from mice kept under constant darkness and injected with PBS or colchicine at ZT5 (D) and ZT13 (E) ($n = 3$ per group). Data are presented as mean ± SEM. (two-way ANOVA test followed by the Bonferroni *post hoc* test: $^*P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Source data are available online for this figure.

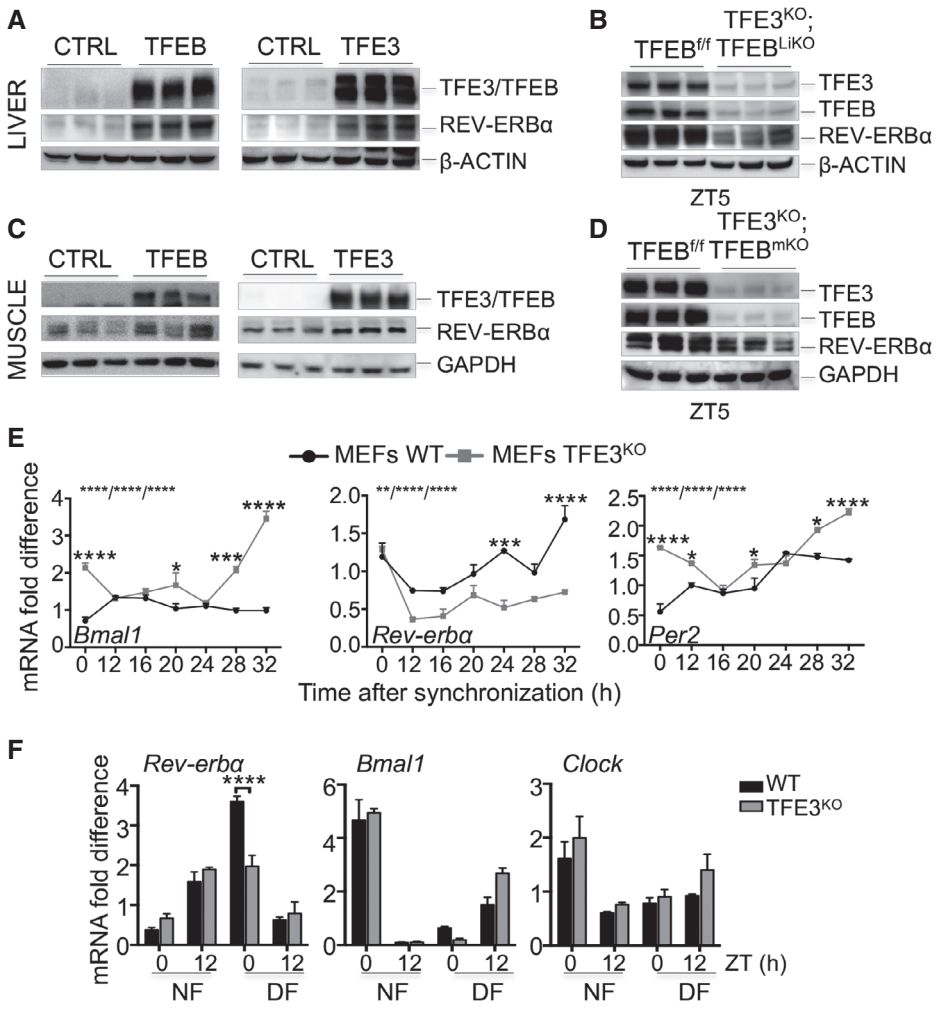

Figure EV3. TFEB and TFE3 regulate *Rev-erbα* expression.

A–D TFEB and TFE3 overexpression results in increased levels of REV-ERBα protein in liver (A) and muscle (C), while their depletion reduced REV-ERBα protein amount (B, D).

E Time course expression of clock genes in WT and TFE3^{KO} MEFs following dexamethasone synchronization (n = 3 per group/time point). Data are presented as mean ± SEM (two-way ANOVA test (interaction/time/group) followed by the Bonferroni *post hoc* test: (*$P \leq 0.05$; ***$P < 0.001$ ****$P < 0.0001$).

F qPCR analysis of total liver RNA isolated from control and TFE3^{KO} mice subjected to night (NF) or day (DF) feeding as indicated in the method section (n = 3 per group/time point). Data are presented as mean ± SEM. (two-way ANOVA test followed by the Bonferroni *post hoc* test: (***$P < 0.001$).

Source data are available online for this figure.

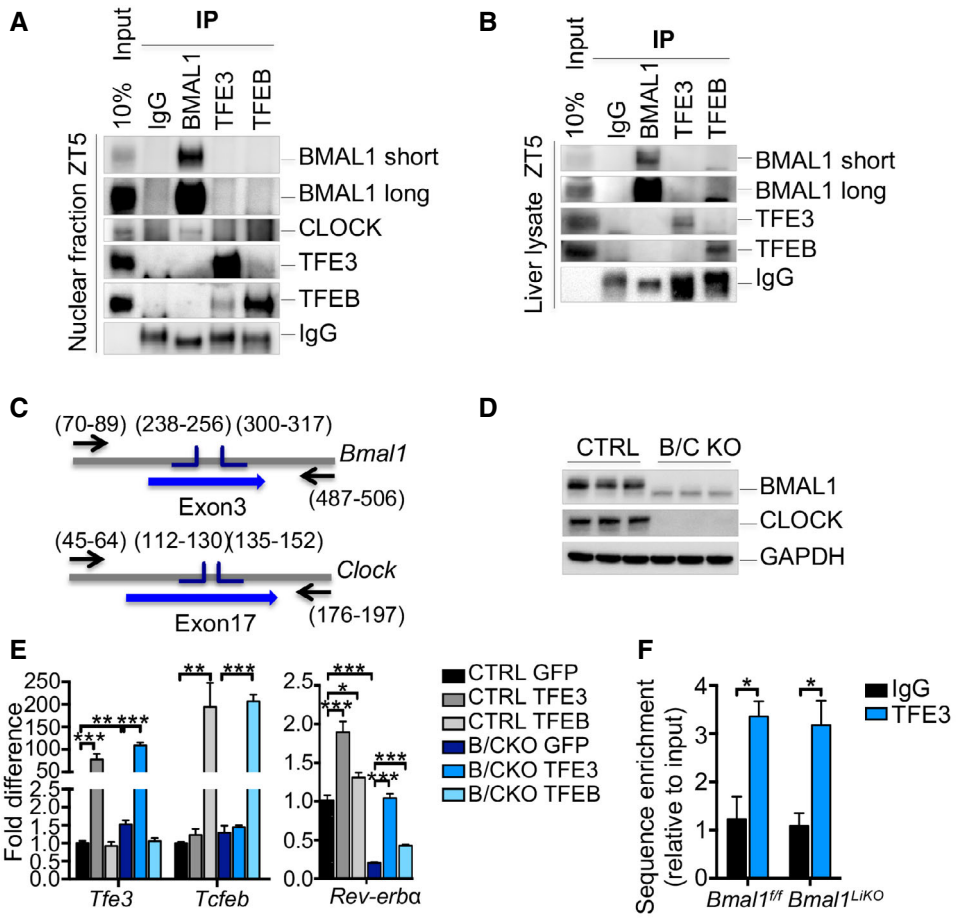

Figure EV4. TFEB and TFE3 act in parallel and not in combination with BMAL1/CLOCK.

A, B Co-immunoprecipitation analysis using the indicated antibodies in nuclear fraction (A) or whole lysate (B) from liver of WT mice fed *ad libitum* at ZT5.

C Representation of the strategy used for the generation of the BMAL1/CLOCK KO (B/C KO) Hepa1-6 cells using CRISPR/Cas9 technology.

D Validation of the B/C KO cells by Western blot analysis.

E qPCR analysis of WT and B/C KO cells overexpressing TFEB and TFE3 (n = 3 per group). Data are represented as mean ± SEM (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ by Student's t-test).

F ChIP analysis for *Rev-erbα* in liver from mice of the indicated genotypes (n = 3 per group). Bar graph represents the amount of immunoprecipitated DNA as detected by qPCR assay. Values were normalized to the input and plotted as relative enrichment over the IgG control. Data are represented as mean ± SEM (*$P \leq 0.05$ by Student's t-test).

Source data are available online for this figure.

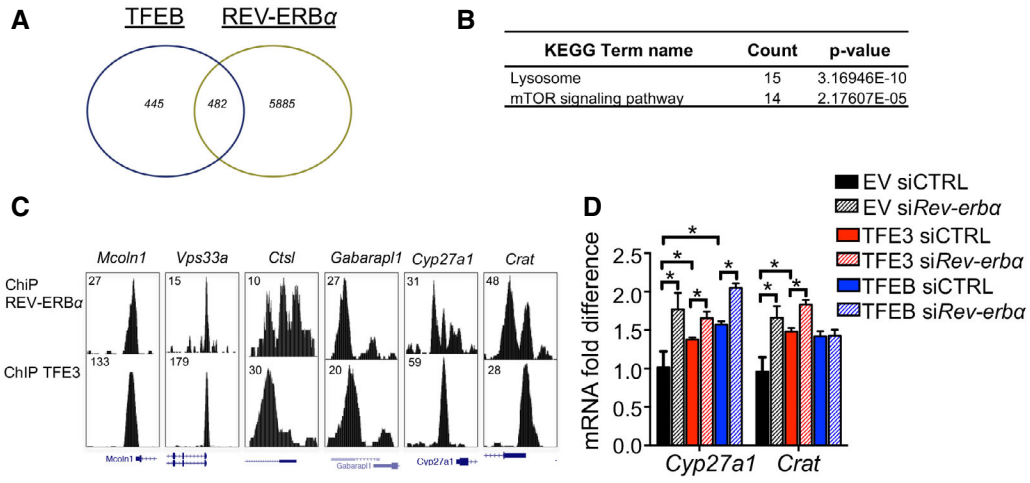

Figure EV5. REV-ERBα binds the promoter regions of several TFEB/TFE3 target genes.

A Venn diagram showing the unique and common TFEB and REV-ERBα bindings. Fisher's exact test *P*-value < 2.2e-16.

B KEGG analysis of the shared TFEB and REV-ERBα peaks.

C TFE3 and REV-ERBα cistromes in the promoter region of genes involved in autophagy and lysosome. Tag counts are shown in the corner.

D Expression analysis of circadian- and metabolism-related genes in Hepa1-6 overexpressing TFEB or TFE3 and depleted for *Rev-erbα* (n = 3 per group). mRNA levels were normalized using *Rps16* and expressed as relative to cells transfected with scramble siRNA and empty vector. Data are represented as mean ± SEM (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ by Student's t-test).