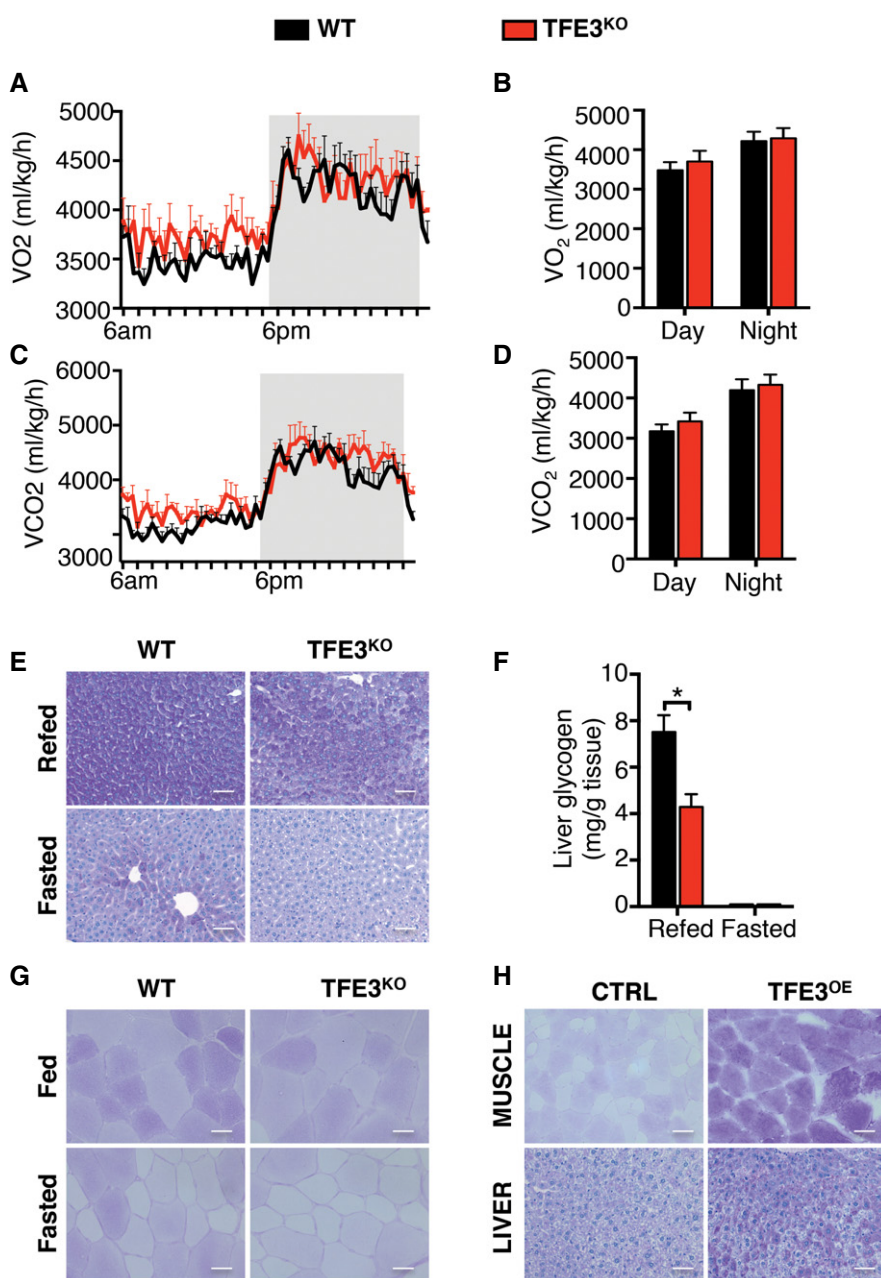


Expanded View Figures



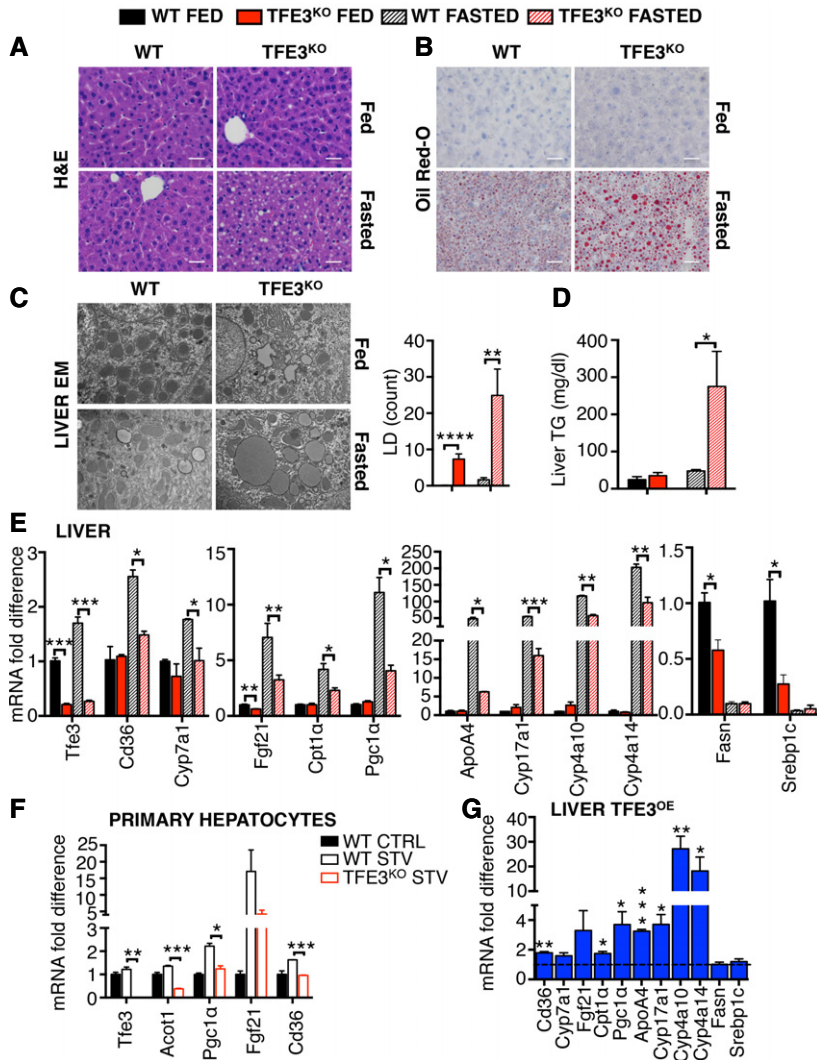


Figure EV2. TFE3 regulates β -oxidation during starvation.

A–D Haematoxylin and eosin (H&E) staining (A), Oil Red O (B) and electron microscopy images (C) with the relative quantification of the lipid droplets and liver triglyceride (TG) levels (D) of livers isolated from fed and 24-h-fasted *Tfe3* KO and control mice ($n = 5$ per group) (scale bars H&E: 20 μ m; scale bars Oil Red O: 50 μ m). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: **** $P < 0.0001$; ** $P = 0.0041$; * $P = 0.0288$.

E Quantification of mRNA levels of genes involved in lipid metabolism in livers from WT and *Tfe3* KO mice treated as indicated ($n = 3$ per group). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: *Tfe3* **** $P < 0.001$; *Cd36* * $P = 0.0162$; *Cyp7a1* * $P = 0.05$; *Fgf21* ** $P = 0.0011$; *Cpt1a* * $P = 0.0307$; *Pgc1a* * $P = 0.0147$; *ApoA4* * $P = 0.0147$; *Cyp17a1* **** $P = 0.0005$; *Cyp4a10* ** $P = 0.0056$; *Cyp4a14* ** $P = 0.0048$; *Fasn* ** $P = 0.0275$; *Srebp1c* * $P = 0.0255$.

F Quantification of mRNA levels of genes involved in lipid metabolism in primary hepatocytes from WT and *Tfe3* KO mice treated as indicated. Data are presented as mean \pm SEM. Student's two-tailed *t*-test: *Tfe3* ** $P < 0.003$; *Acot1* *** $P = 0.0007$; *Pgc1a* * $P = 0.0159$; *Cd36* *** $P = 0.0002$.

G Expression of genes involved in lipid metabolism in livers from HDAd-PEPCK-*TFE3* injected mice ($n = 3$ per group). Values were normalized to control livers (dashed line). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: *Cd36* ** $P = 0.0018$; *Cpt1a* * $P = 0.0449$; *Pgc1a* * $P = 0.0207$; *ApoA4* *** $P = 0.0004$; *Cyp17a1* * $P = 0.0162$; *Cyp4a10* ** $P = 0.0067$; *Cyp4a14* * $P = 0.0480$.

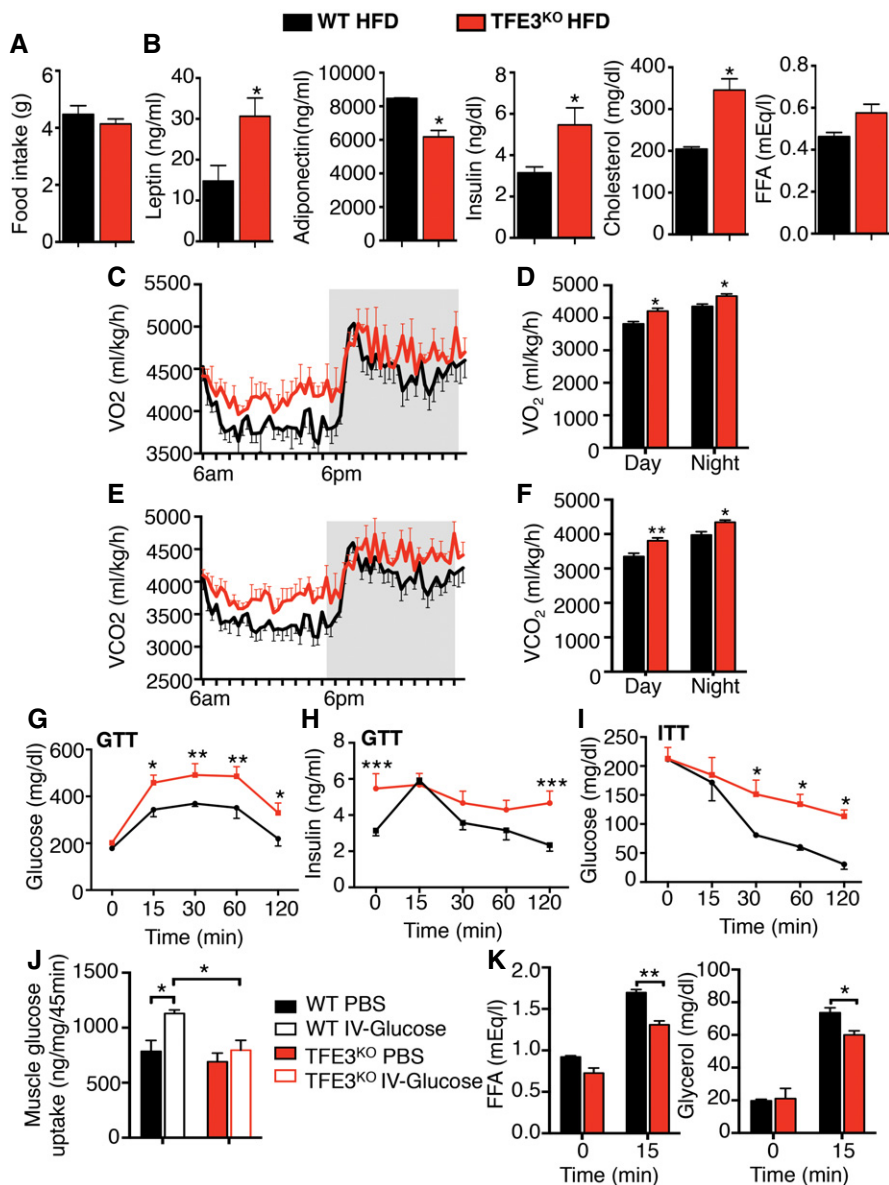


Figure EV3. Metabolic profile of *Tfe3* KO mice fed a HFD.

- A, B** Food intake (A) ($n = 5$) and serum panel (B) from WT and *Tfe3* KO mice after 1 month of HFD. Data are presented as mean \pm SEM. Student's two-tailed *t*-test: leptin ($n = 3$) $*P = 0.0451$; adiponectin ($n = 3$) $*P = 0.0192$; insulin ($n = 5$) $*P = 0.0287$; cholesterol ($n = 3$) $*P = 0.0277$.
- C** Oxygen consumption (V_{O_2}) in WT (black line) ($n = 5$) and *Tfe3* KO mice (red line) ($n = 5$) after 1 month of HFD. Grey areas indicate dark periods (6 PM to 6 AM). Data are presented as mean \pm SEM.
- D** Bar graph represents average V_{O_2} values during day and night ($n = 5$ per group). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: day $*P = 0.0122$; night $*P = 0.0174$.
- E** CO_2 production (V_{CO_2}) in WT (black line) ($n = 5$) and *Tfe3* KO mice (red line) ($n = 5$) after 1 month of HFD. Grey areas indicate dark periods (6 PM to 6 AM). Data are presented as mean \pm SEM.
- F** Bar graph represents average V_{CO_2} values during day and night ($n = 5$ per group). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: day $**P = 0.0076$; night $*P = 0.0113$.
- G, H** Glucose (G) and insulin (H) levels at the indicated time point after glucose challenge ($n = 5$ per group). Data are presented as mean \pm SEM. ANOVA test followed by *post hoc* Bonferroni test: GTT $*P = 0.0106$ (15 min), $**P = 0.0066$ (30 min), $**P = 0.0029$ (60 min), $*P = 0.0144$ (120 min); insulin during GTT $***P = 0.0002$.
- I** Glucose levels at the indicated time point after insulin challenge ($n = 5$ per group). Data are presented as mean \pm SEM. ANOVA test followed by *post hoc* Bonferroni test: $*P = 0.04$ (30 min); $*P = 0.03$ (60 min); $*P = 0.014$ (120 min).
- J** Muscle glucose uptake in control and *Tfe3* KO mice after 1 month of HFD ($n = 3$ per group). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: WT PBS versus WT IV glucose $*P = 0.0294$; WT IV glucose versus *Tfe3* KO IV glucose $*P = 0.0245$.
- K** *In vivo* lipolysis measured in WT and *Tfe3* KO mice fed a HFD for one month as indicated in the Materials and Methods section ($n = 3$ per group). Data are presented as mean \pm SEM. Student's two-tailed *t*-test: FFA $**P = 0.040$; glycerol $*P = 0.0417$.

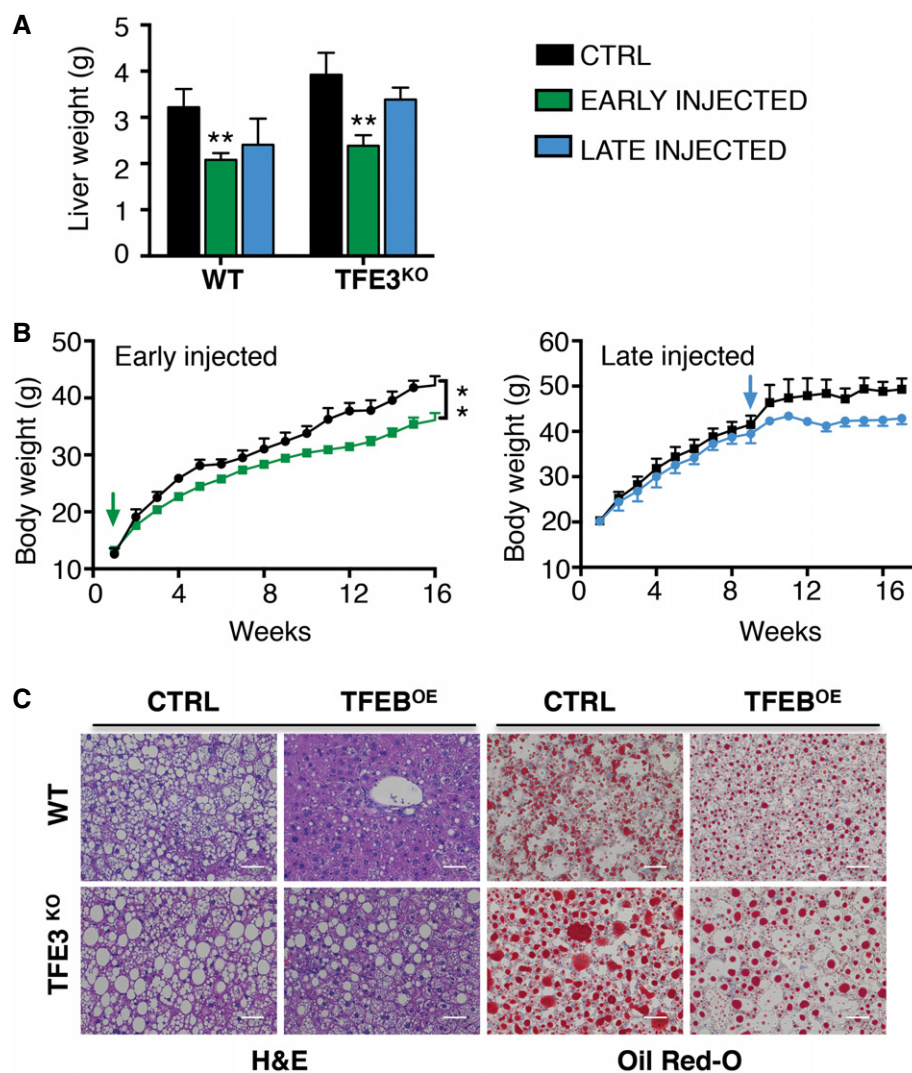


Figure EV4. TFEB overexpression rescues diet-induced obesity in *Tfe3* KO mice.

- A Liver weight from WT and *Tfe3* KO mice injected with the HDAd-PEPCK-hTFEB prior to HFD administration (early) ($n = 13$) or 8 weeks into HFD (late) ($n = 4$) and controls ($n = 6$) of the indicated genotypes. Data are presented as mean \pm SEM. ANOVA test followed by *post hoc* Bonferroni test: WT $**P = 0.0032$; *Tfe3* KO $**P = 0.0041$.
- B Body weight in *Tfe3* KO mice injected with an HDAd-PEPCK-TFEB. Arrows indicate the time of injection. Left panel: controls ($n = 4$) and early-injected mice ($n = 13$). Right panel: controls ($n = 4$) and late-injected mice ($n = 3$). Data are presented as mean \pm SEM. ANOVA test followed by *post hoc* Bonferroni test: early $**P < 0.01$.
- C H&E (left panel) and Oil Red O staining (right panel) of liver sections from late-injected mice of the indicated genotypes after 15 weeks of HFD (scale bars: 50 μ m).