Figure EV1. Metabolic profile of Tfe3 KO mice. 
A Oxygen consumption (\( V_{\text{O}_2} \)) in WT (black line) \((n = 5)\) and Tfe3 KO mice (red line) \((n = 4)\) fed a chow diet. Grey areas indicate dark periods \((6\text{ PM to 6 AM})\). Data are presented as mean ± SEM. 
B Bar graph represents average \( V_{\text{O}_2} \) values during day and night \((n = 5\text{ WT and } n = 4\text{ Tfe3 KO})\). Data are presented as mean ± SEM. 
C CO\(_2\) production (\( V_{\text{CO}_2} \)) in WT (black line) \((n = 5)\) and Tfe3 KO mice (red line) \((n = 4)\) fed a chow diet. Grey areas indicate dark periods \((6\text{ PM to 6 AM})\). Data are presented as mean ± SEM. 
D Bar graph represents average \( V_{\text{CO}_2} \) values during day and night \((n = 5\text{ WT and } n = 4\text{ Tfe3 KO})\). Data are presented as mean ± SEM. 
E, F Periodic acid–Schiff (PAS) staining of liver (E) and relative glycogen content measurement (F) in 24-h-fasted or 24-h-fasted plus 3 h of refeeding WT and Tfe3 KO mice \((n = 4\text{ per group})\) (scale bars: 50 \(\mu\text{m}\)). Data are presented as mean ± SEM. Student’s two-tailed t-test: *\(P = 0.0120\). 
G PAS staining in muscle sections from fed and 24-h-fasted WT and Tfe3 KO mice (scale bars: 20 \(\mu\text{m}\)). 
H Representative PAS images from TFE3-overexpressing muscle and liver (scale bars muscle: 20 \(\mu\text{m}\); scale bars liver: 50 \(\mu\text{m}\)).
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 μm; scale bars Oil Red O: 50 μm). Data are presented as mean ± SEM. Student’s two-tailed t-test: ****P < 0.0001; ***P = 0.0042, *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 ± 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, Aco1 **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 ± SEM. Student’s two-tailed t-test: Tfe3 ***P < 0.003, Aco1 ***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 ± SEM. Student’s two-tailed t-test: Cd36 ***P = 0.0018, Cpt1a *P = 0.0449, Pgc1a *P = 0.0207, Aco1 **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 ± 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 O2) 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 ± SEM.

D Bar graph represents average V O2 values during day and night (n = 5 per group). Data are presented as mean ± SEM. Student’s two-tailed t-test: day *P = 0.0122; night *P = 0.0174.

E CO2 production (VCO2) 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 ± SEM.

F Bar graph represents average VCO2 values during day and night (n = 5 per group). Data are presented as mean ± 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 ± 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 ± 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 ± 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 ± 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 ± 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 ± 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 μm).