Ferroptosis and Oxidative Stress
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Supplementary information

GPX4 is not required for the thermogenesis function of brown adipose tissue in mice

Yifan Zhang, Cheng Li, Yu Liu, Wei Wang, Wanling You, Zhenyu Ju, Fudi Wang, Qian Hu

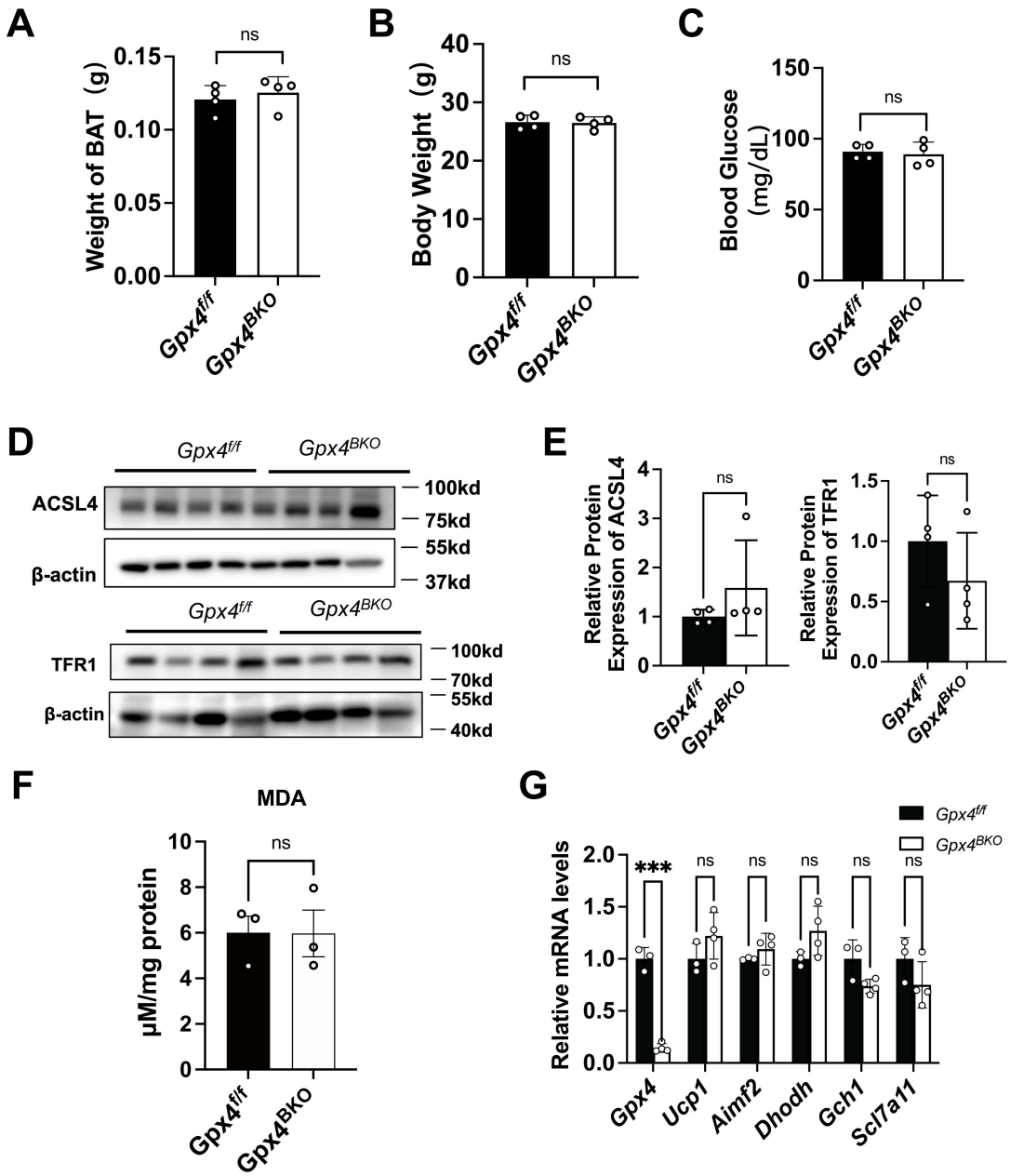

Figure S1. (A-B) Body weight and BAT mass of *Gpx4*^{BKO} mice compared with *Gpx4*$^{f/f}$ mice (n = 4); (C) Fasting blood glucose levels of *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice fed a chow diet (n = 4); (D) Immunoblot analysis in BAT from *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice; (E) Quantification of protein levels normalized to β-actin in (D); (F) MDA levels in BAT of *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice after 8 hours of cold exposure were determined by TBARS assay (n = 3); (G) q-pcr analysis in BAT from 8-week-old *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice exposed to 4 ℃ for 8 h, the mRNA levels were normalized to 18 s. The values were presented as mean ± SD; ns, no significance, ***p < 0.001. BAT: brown adipose tissue; TBARS: thiobarbituric acid reactive substances; SD: standard deviation; MDA: malondialdehyde.

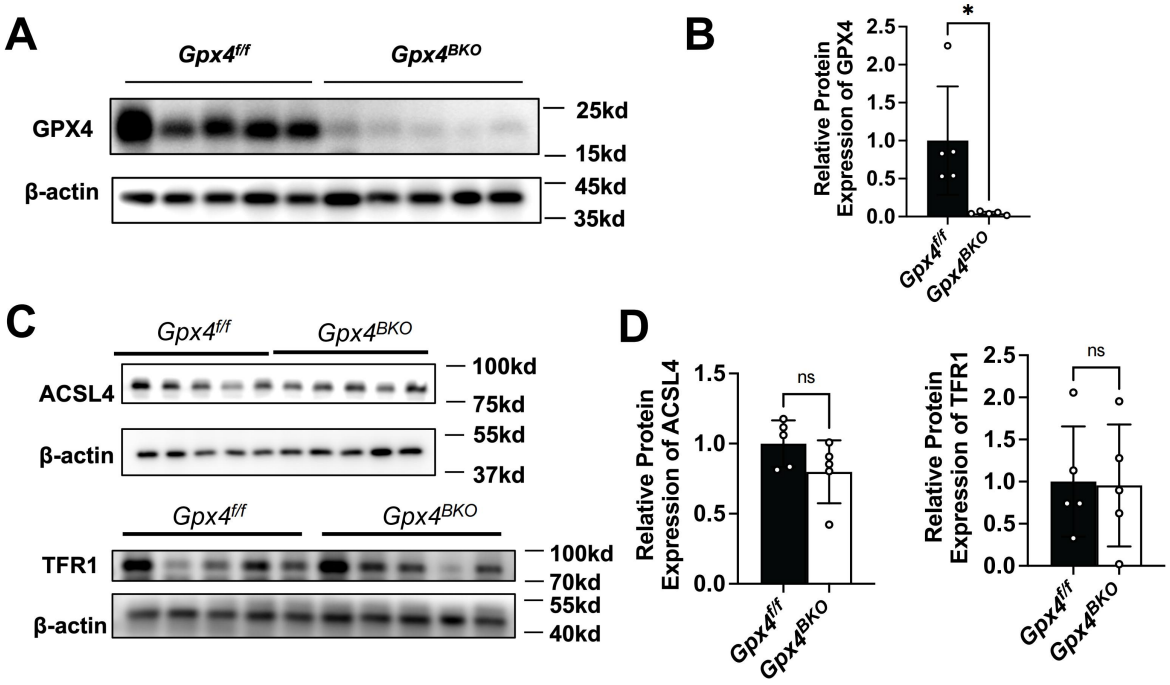

Figure S2. (A-B) *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice (aged 8 weeks) were treated with an HFD for 16 weeks; (A) GPX4 deletion in the BAT of *Gpx4*^{BKO} was verified by Western blot, and (B) the relative GPX4 levels were normalized to β-actin; (C-D) (C) Immunoblots analysis of ACSL4 and TFR1 in BAT from *Gpx4*$^{f/f}$ mice and *Gpx4*^{BKO} feeding with *n* HFD, and (D) the relative ACSL4 and TFR1 levels were normalized to β-actin. The values were presented as mean ± SD; ns, no significance, **p* < 0.05. HFD: high-fat diet; BAT: brown adipose tissue; GPX4: glutathione peroxidase 4; SD: standard deviation.

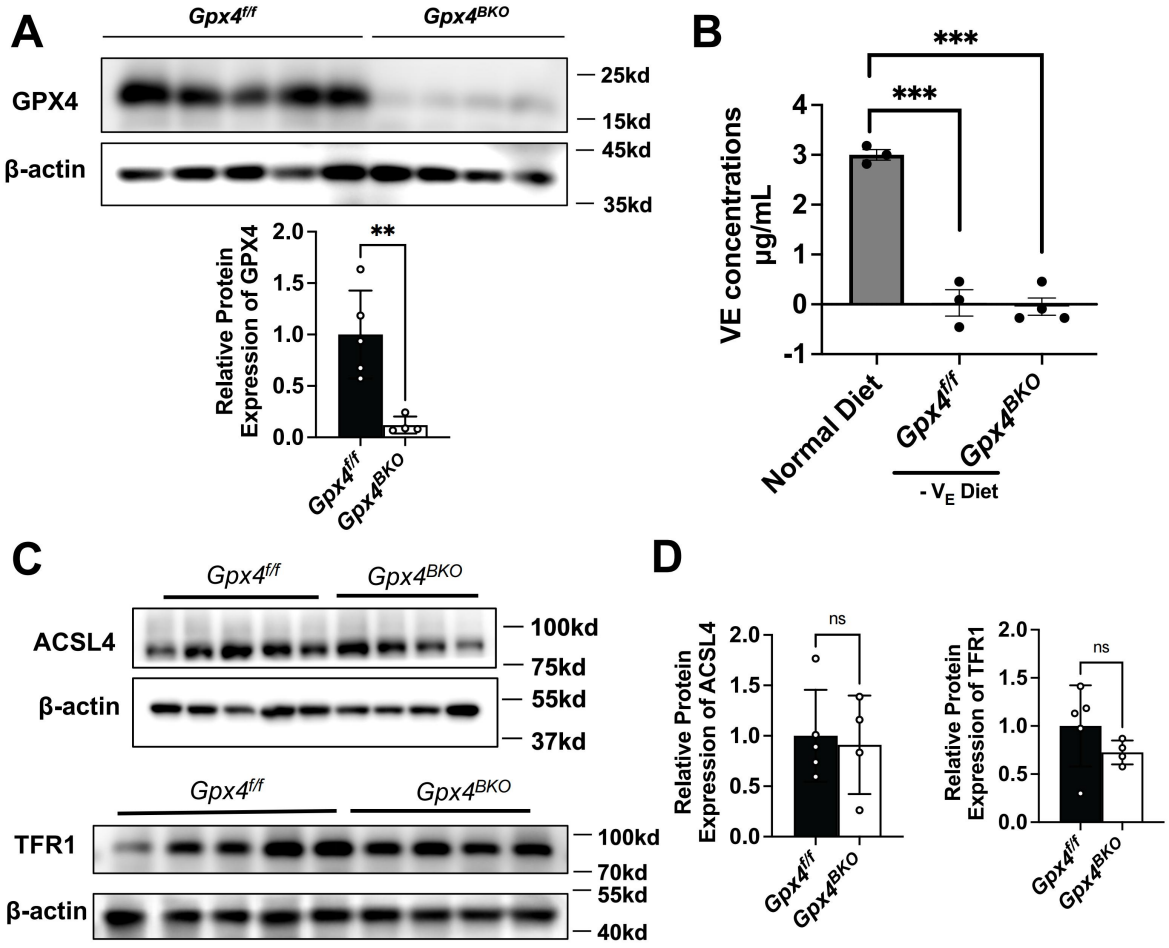

Figure S3. (A) *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice (aged 8 weeks) were treated with a vitamin E depletion diet for 4 weeks. GPX4 deletion in the BAT of *Gpx4*^{BKO} was verified by Western blot, and the relative GPX4 levels were normalized to β-actin; (B) Serum vitamin E levels in mice fed a vitamin E-deprived diet versus a normal control diet; (C-D) (C) Immunoblots analysis of ACSL4 and TFR1 in BAT from *Gpx4*$^{f/f}$ mice and *Gpx4*^{BKO} feeding with V_E depletion diets, and (D) the relative ACSL4 and TFR1 levels were normalized to β-actin. The values were presented as mean ± SD; ns, no significance, ***$p < 0.001$. GPX4: glutathione peroxidase 4; SD: standard deviation.

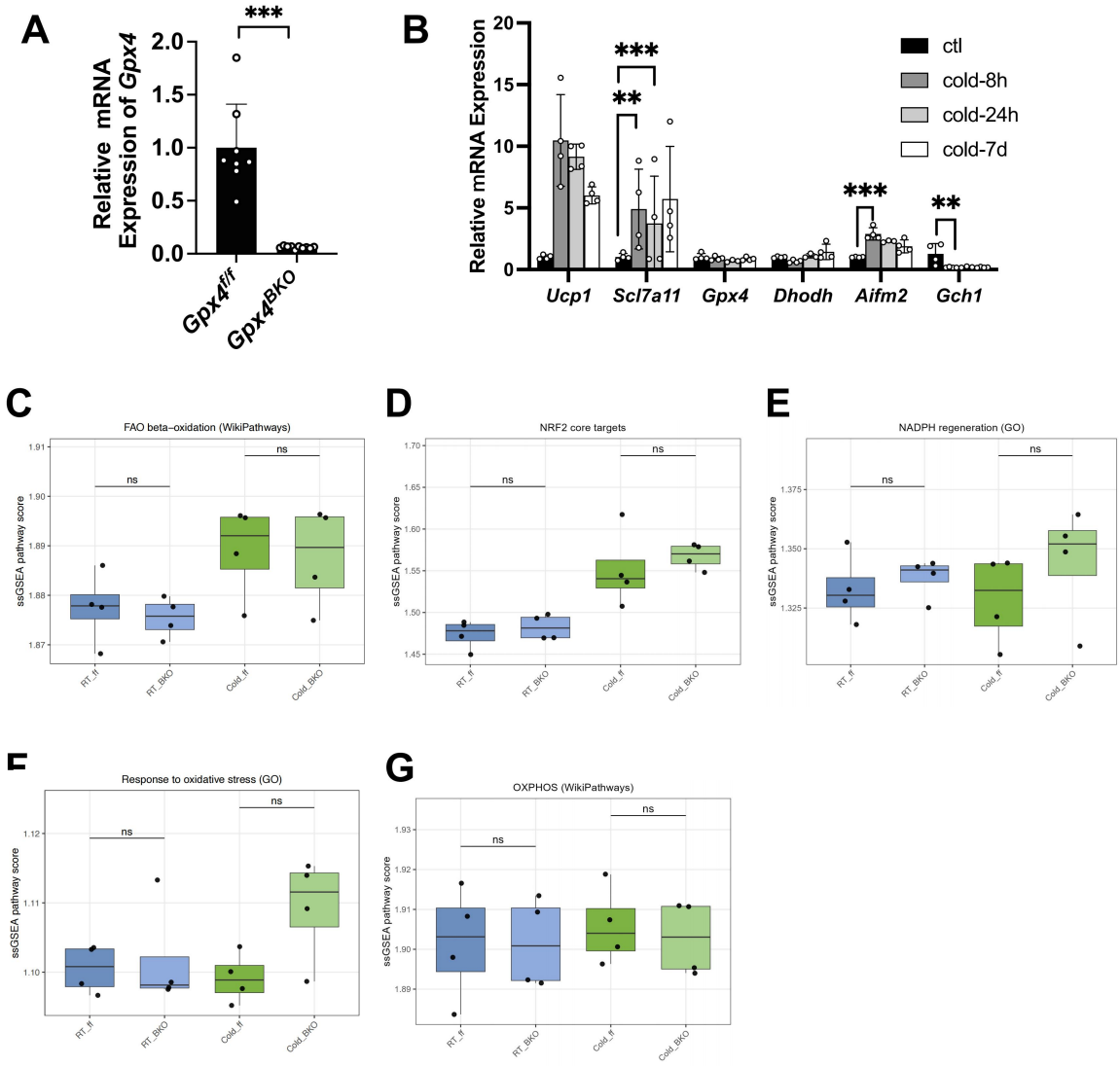

Figure S4. (A) The relative mRNA levels of *Gpx4* in the BAT of *Gpx4*$^{f/f}$ and *Gpx4*^{BKO} mice; (B) QPCR analysis in BAT from 8-week-old wild-type mice exposed to 4 ℃ for indicated durations, the mRNA levels were normalized to 18 s; (C-G) Pathway activity scores were evaluated using ssGSEA across experimental conditions. Plots depict the distribution of enrichment scores for (C) fatty acid beta-oxidation, (D) NRF2 core targets, (E) NADPH regeneration, (F) oxidative stress and redox homeostasis, and (G) OXPHOS. Data are presented as box plots (box, interquartile range; center line, median; whiskers, 1.5× IQR), with individual biological replicates shown as black dots (n = 4). The values were presented as mean ± SD; ns, no significance, *p < 0.05, **p < 0.01, ***p < 0.001. QPCR: quantitative polymerase chain reaction; BAT: brown adipose tissue; GPX4: glutathione peroxidase 4; OXPHOS: oxidative phosphorylation; SD: standard deviation.