Expanded View Figures

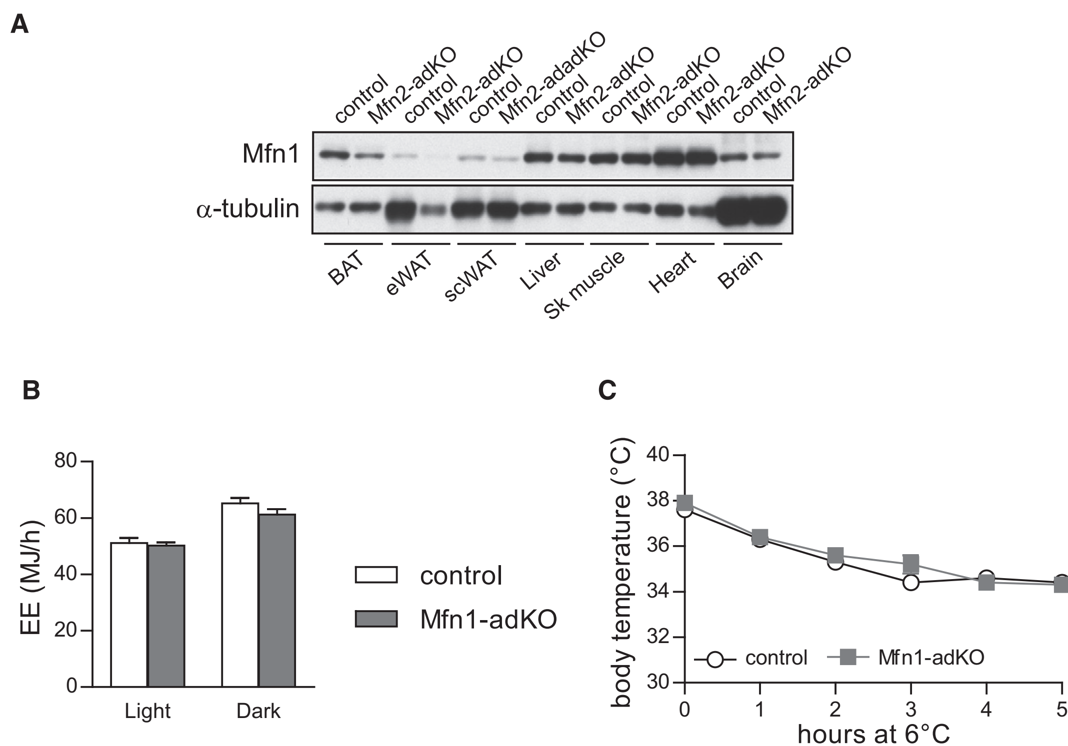

Figure EV1. Brown adipose tissue function is not affected in Mfn1-adKO mice.

A Mfn1 protein levels were measured in tissues from 20-week-old male control and Mfn2-adKO mice.

B Three-month-old control and Mfn1-adKO mice were fed *ad libitum* with a low-fat diet, and energy expenditure (EE) was measured during indirect calorimetry tests using a comprehensive laboratory animal monitoring system (CLAMS).

C A cold resistance test was used to evaluate thermogenic activity in control and Mfn1-adKO mice.

Data information: All values are presented as mean ± SEM of $n = 9$ mice for each genotype.

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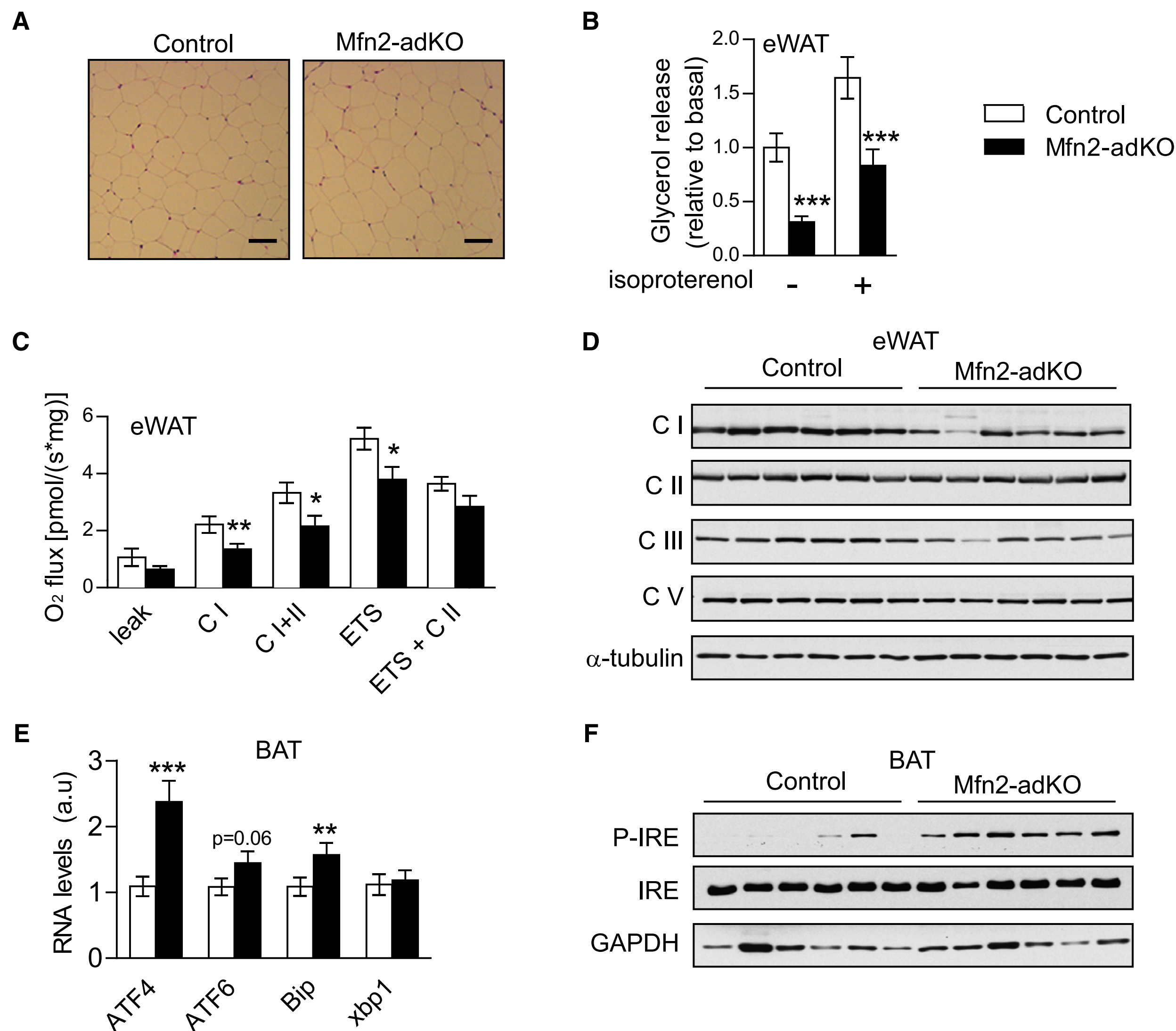

Figure EV2. eWAT function is not affected in Mfn2-adKO mice and ER stress is increased in Mfn2-adKO BAT on low-fat diet.

A H&E staining of eWAT (scale bar, 100 μm).

B Lipolysis was evaluated by measuring glycerol release in isolated eWAT stimulated or not with 1 μM isoproterenol for 5 h.

C Oxidative phosphorylation and electron transfer capacity in eWAT.

D Mitochondrial proteins levels in eWAT.

E, F mRNA (E) and proteins levels (F) for ER stress markers in BAT.

Data information: All values are presented as mean ± SEM of n = 9–10 mice for each genotype. *, **, and *** indicate statistically significant difference between control (white bars) and Mfn2-adKO mice (black bars) at $P < 0.05$, $P < 0.03$, and $P < 0.01$, respectively (two-tailed Student's t-test).

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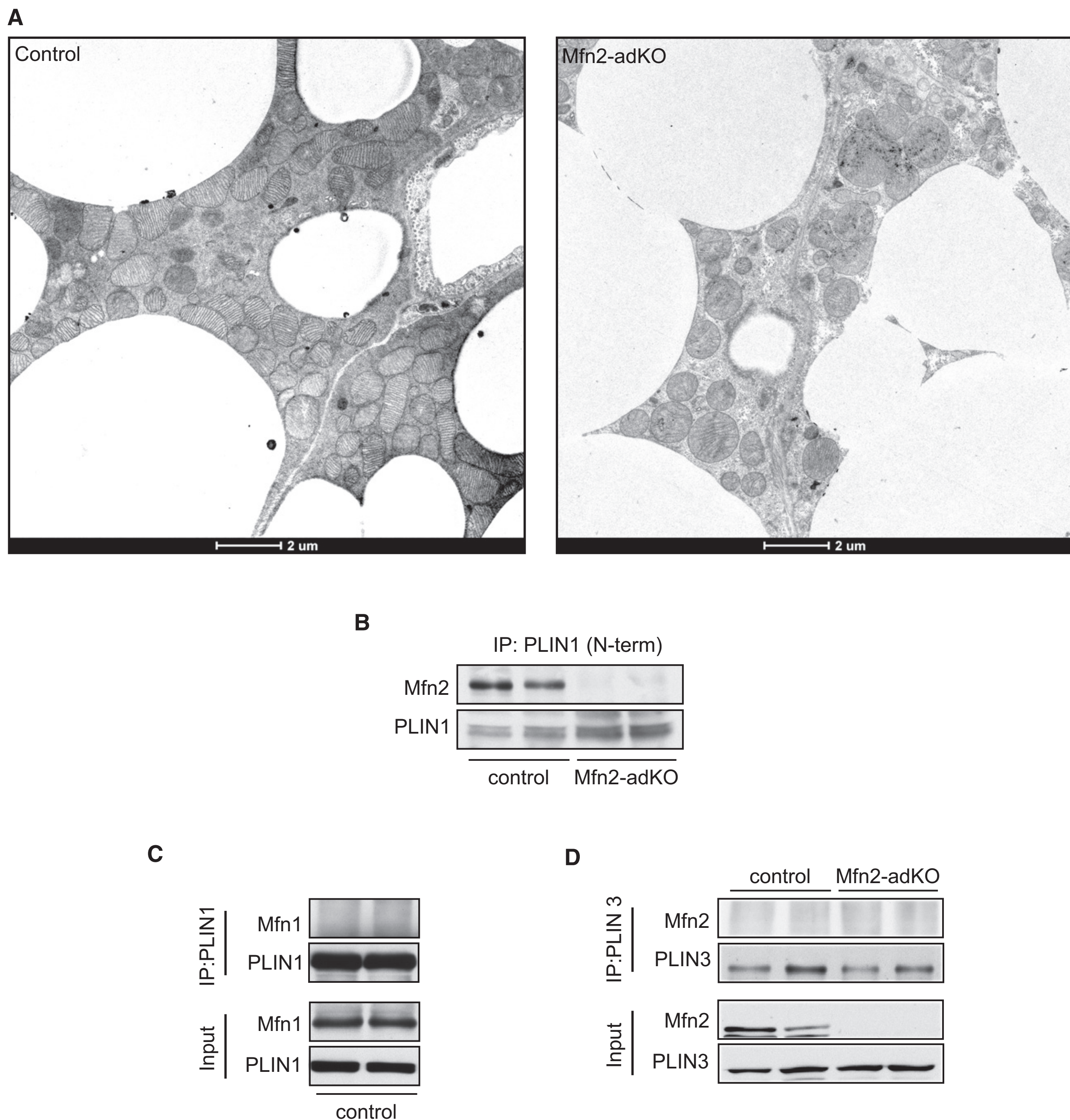

Figure EV3. Mfn2 influences mitochondria–lipid droplet connectivity and specifically interacts with PLIN1.

A Low-magnification EM images of BAT from control and Mfn2-adKO mice.

B Mfn2–PLIN1 interaction was evaluated by immunoprecipitating PLIN1 from BAT of control and Mfn2-adKO mice using an antibody recognizing the N-terminal region of PLIN1.

C Mfn1–PLIN1 interaction was evaluated by immunoprecipitating PLIN1 from BAT of control mice. The results illustrate the lack of co-IP between these two proteins.

D The interaction between Mfn2 and PLIN3 was evaluated by immunoprecipitating PLIN3 from BAT of control and Mfn2-adKO mice. The results illustrate the lack of co-IP between these two proteins.

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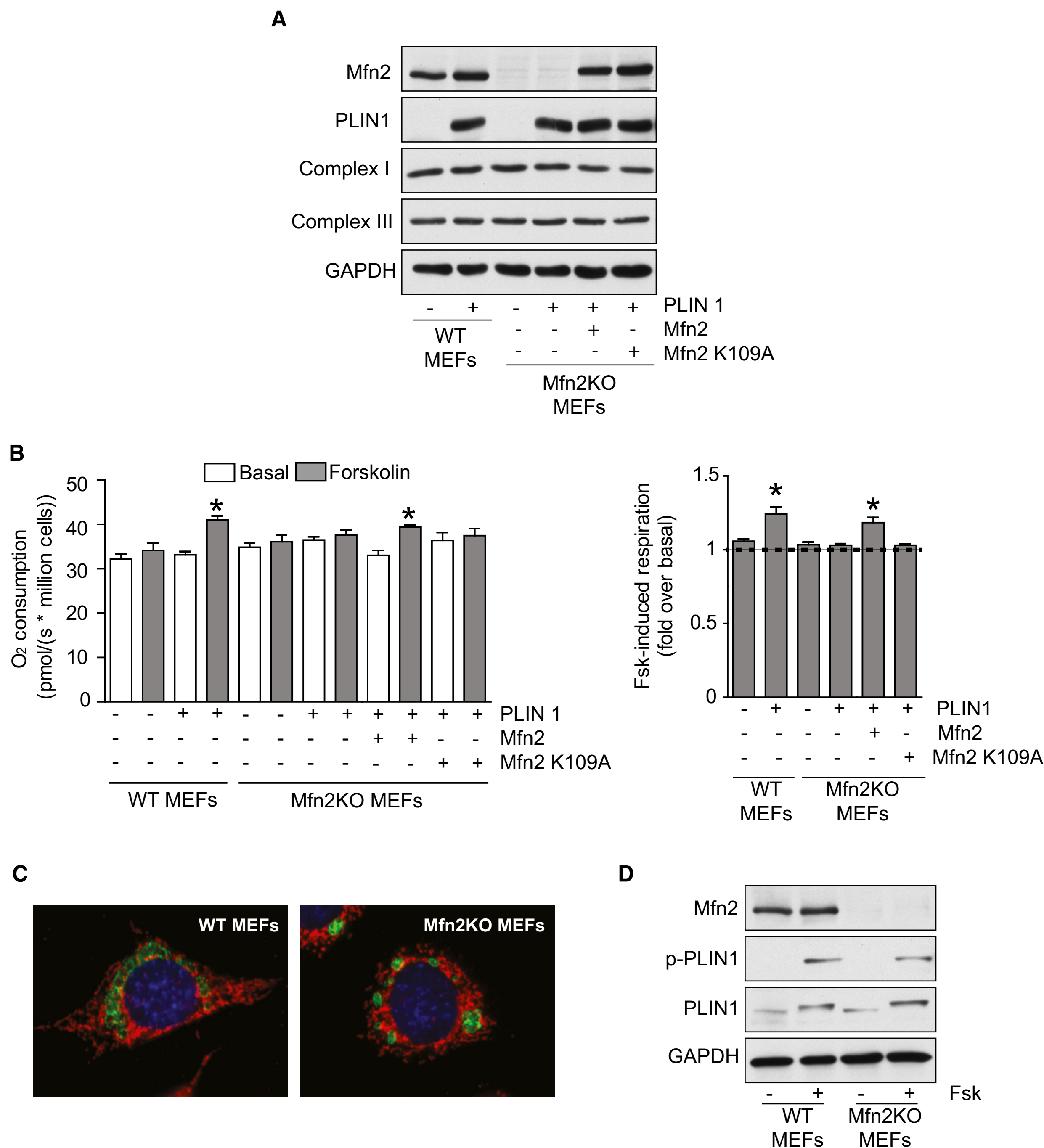

Figure EV4. PLIN1 and Mfn2 are both required for forskolin-induced fat oxidation in MEF cells.

A MEF cells were transfected with PLIN1, wild-type Mfn2, or Mfn2K109A as indicated. Then, 36 h later, cells were loaded with oleic acid (0.2 mM). After 12 h, protein extracts were obtained to test the markers indicated by Western blot.

B MEFs were treated as in (A). After 12 h of oleic acid loading, cells were trypsinized and counted. Then, two million cells were placed in respirometry chambers with air-equilibrated serum-free minimum essential medium. After evaluating basal respiration (basal; white bars), cells were treated with forskolin (Fsk; 1 μM; gray bars) and respiration rates were further evaluated. In the left graph, absolute respiration values are shown. In the right graph, the relative increase in respiration induced by Fsk is depicted. Values are presented as mean ± SEM of $n = 6$ independent experiments. * indicates statistically significant difference vs. the respective basal state at $P < 0.05$ (two-tailed Student's t-test). In the right panel, the ANOVA with Bonferroni *post-hoc* test was used to evaluate the differences in Fsk-induced respiration.

C MEFs were transfected with MitoDsRed to visualize mitochondria (in red) and with PLIN1 (in green) to visualize lipid droplets. Then, 36 h later, cells were loaded with oleic acid. After 12 h, cells were fixed with 4% paraformaldehyde and PLIN1 and mitochondria were visualized using fluorescence microscopy.

D Cells were treated as in (B). At the end of the treatment protein extracts were obtained and used for Western blot analysis of the indicated markers.

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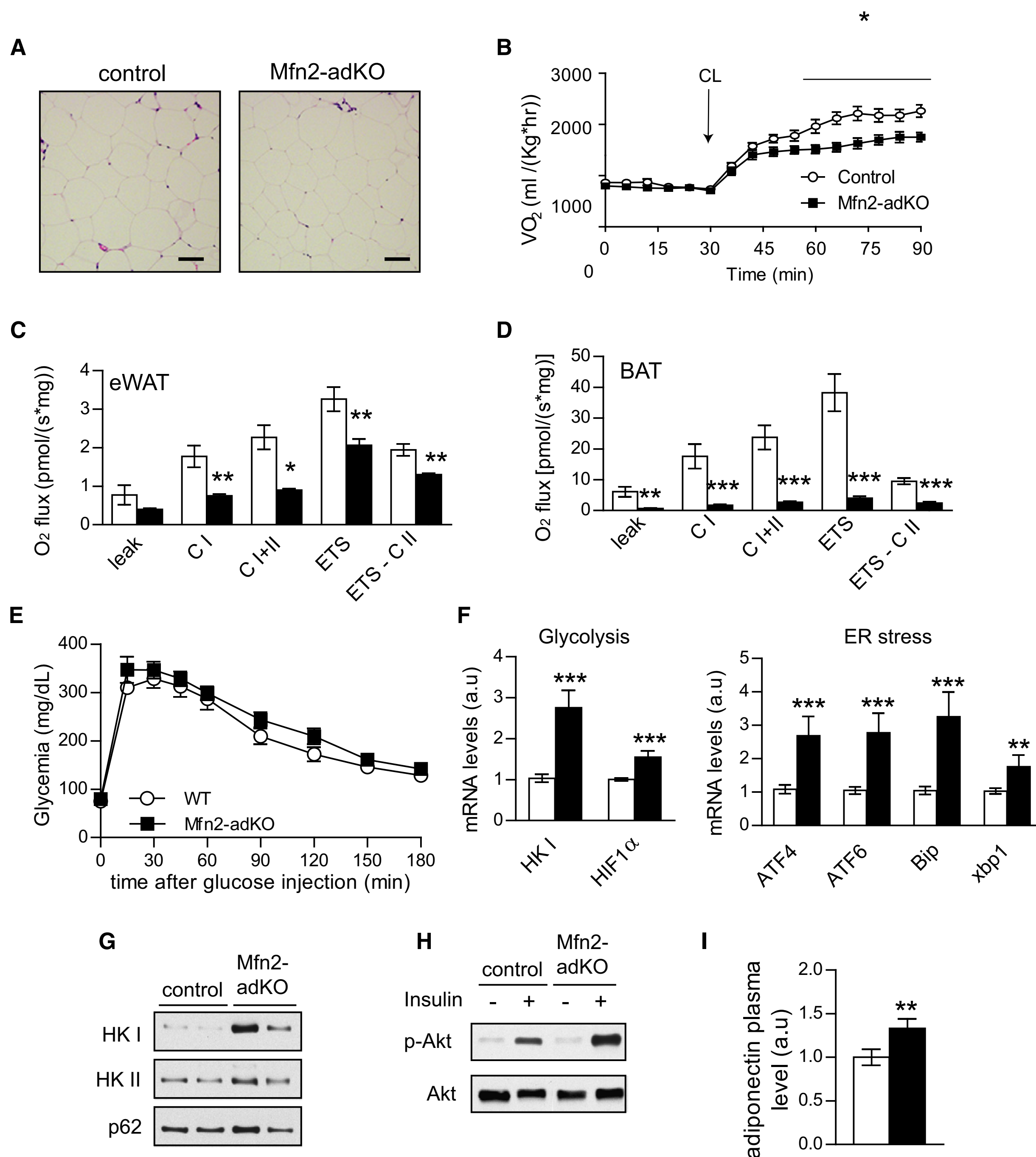

Figure EV5. Mfn2-adKO mice display brown adipose tissue dysfunction on high-fat diet.

- A H&E staining of eWAT on HFD (scale bar, 100 μm).
- B Non-shivering thermogenesis was evaluated by measuring baseline and CL (1 mg/kg)-induced O_2 consumption in anesthetized mice at 30°C (n = 8 mice per genotype).
- C, D Oxidative phosphorylation and electron transfer system (ETS) capacity in eWAT (C) and BAT (D) from HFD-fed control and Mfn2-adKO mice.
- E An intraperitoneal glucose tolerance test was performed on low-fat diet-fed control and Mfn2-adKO mice.
- F Glycolytic and ER stress markers gene expression in BAT of HFD-fed control and Mfn2-adKO mice.
- G Protein levels of glycolytic markers in mitochondrial fractions from control and Mfn2-adKO BAT.
- H Insulin signaling in *ex vivo* incubated BAT with or without insulin (50 nM) from control and Mfn2-adKO mice fed on HFD.
- I Adiponectin plasma level from HFD-fed control and Mfn2-adKO mice.

Data information: All values are presented as mean ± SEM of, unless otherwise stated, n = 9 mice per genotype. *, **, and *** indicate statistically significant difference between control (white bars and circles) and Mfn2-adKO mice (black bars and circles) at $P < 0.05$, $P < 0.03$, and $P < 0.01$, respectively (two-tailed Student's t-test).

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