Supporting Information for

Original article

Activating Connexin43 gap junctions primes adipose tissue for therapeutic intervention

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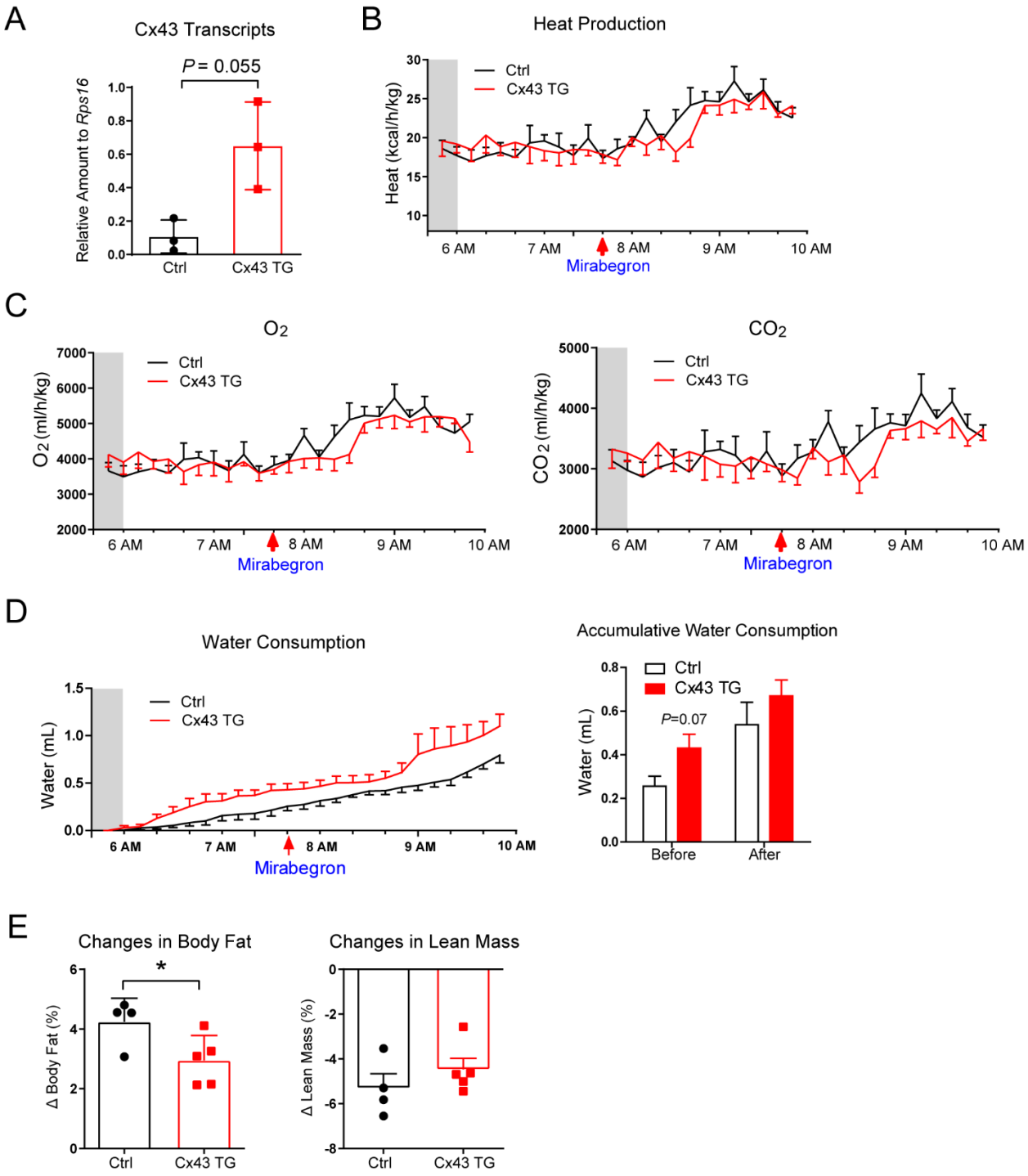

Figure S1 Other metabolic parameters in control and Cx43 TG mice treated with Mirabegron. (**A**) Cx43 expression in iWAT from 12-week-old control (Ctrl) and Cx43 TG mice after overnight Dox10 chow diet treatment. (**B**) Heat production (normalized to body weight) during the metabolic cage experiment shown in Fig. 1D and 1E (n = 4–5 mice). (**C**) O_2 consumption and CO_2 emission rate (normalized to body weight) during the metabolic cage experiment in Fig. 1D and 1E (n = 4–

5 mice). The shaded area indicates the dark cycle. (**D**) Left: Water consumption during the experiment in Fig. 1C and 1D. Right: accumulative water consumption before and after mirabegron treatment (n = 4–5 mice). (**E**). Changes in body fat (normalized to body weight, expressed as a percentage), and lean mass (normalized to body weight, expressed as a percentage) after metabolic cage experiment shown in Fig. 1D and 1E (n = 4–5 mice). All data are mean ± SEM. *$P < 0.05$.

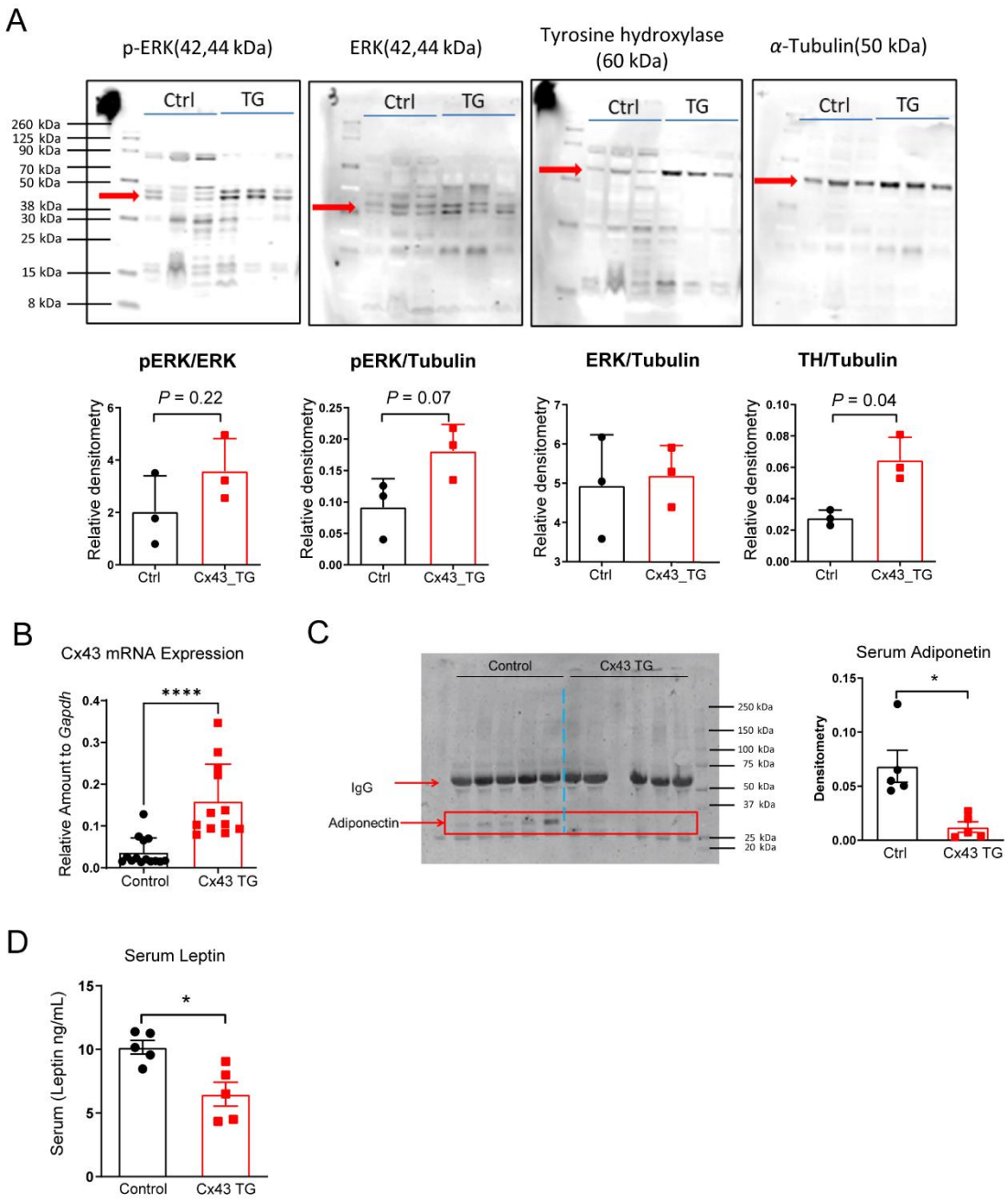

Figure S2 Biochemical analysis of control and Cx43 TG mice. (**A**) Western blotting for p-ERK, ERK, tyrosine hydroxylase (TH), and *α*-tubulin in brown adipose tissue lysates generated from control and Cx43 TG mice fed with HFD supplemented with 200 mg/kg doxycycline for 4 weeks. The protein ladder (LI-COR Chameleon Duo 928-60000) shows two different patterns in respective channels. Control samples are labeled as "Ctrl", Cx43 TG samples are labeled as "TG". Analysis of densitometry is shown below blots (n = 3 mice). (**B**) Cx43 mRNA levels from iWAT harvested from Cx43 TG mice used in Fig. 2B, which were kept on 50 mg/kg doxycycline HFD

for 10 days (n = 12–14 mice). (**C**) Western blotting for serum adiponectin levels from control and Cx43 TG mice fed with HFD supplemented with 200 mg/kg doxycycline for 4 weeks. Quantification of serum adiponectin abundance (normalized to mouse serum IgG) is shown on the right. Protein ladder (LI-COR Odyssey Protein Molecular Weight Marker 928–60000). (n = 5 mice). (**D**). Serum leptin levels from control and Cx43 TG mice fed with HFD supplemented with 200 mg/kg doxycycline for 4 weeks (n = 6 mice). All data are mean ± SEM; *P < 0.05, ****P < 0.0001.

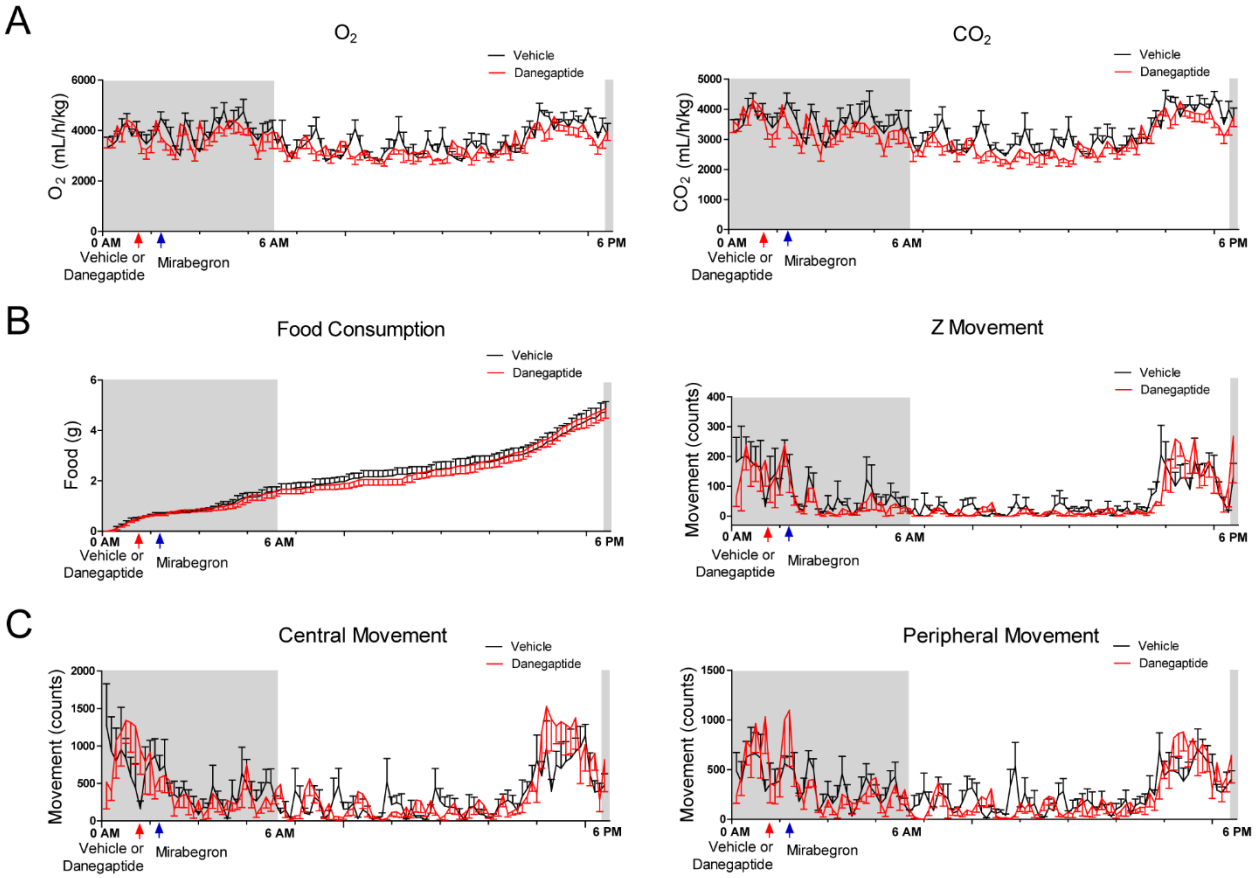

Figure S3 Metabolic characterization of mice treated with danegaptide and mirabegron. (**A**) O_2 consumption and CO_2 emission rate (normalized to body weight) were measured in the metabolic cage experiment shown in Fig. 3B (n = 6 mice). (**B**) Accumulative food consumption and food-seeking activity (Z movement) were measured in the metabolic cage experiment shown in Fig. 3B (n = 6 mice). (**C**) Central and peripheral activities were measured in the metabolic cage experiment shown in Fig. 3B (n = 6 mice). The shaded area indicates the dark cycle. All data are mean ± SEM.

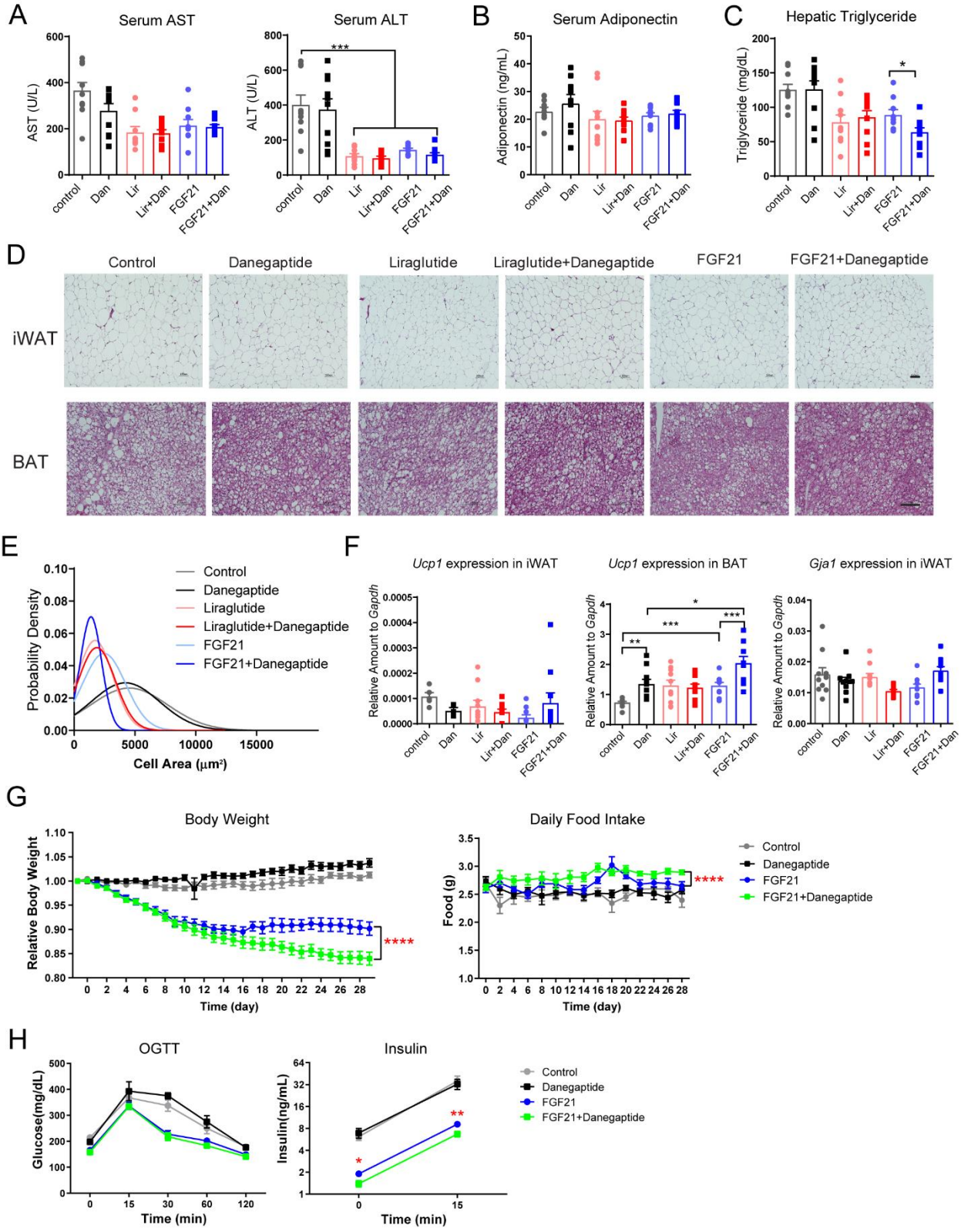

Figure S4 Further improvement of metabolic function when combing FGF21 with danegaptide. The treatment condition for Panels A–E is illustrated in Fig. 4A. The treatment condition for Panels

G and H was similar except that mice were treated with drugs for 29 days. (**A**) Serum AST and ALT levels after treatment (*n* = 9–10 mice). (**B**) Serum adiponectin levels after treatment (*n* = 9–10 mice). (**C**) Hepatic triglyceride content after the treatment (*n* = 9–10 mice). (**D**) Representative histology of the iWAT and BAT from at least three mice. Scale bar = 100 μm. (**E**) Summary of iWAT adipocytes area distribution in each treatment condition. (**F**) *Ucp1* expression in iWAT and BAT. Cx43 expression in iWAT (*n* = 10 mice). (**G**) Weight-loss and food intake during 4 weeks of FGF21 (1 mg/kg body weight) and danegaptide (10 mg/kg body weight) combinatory treatment (*n* = 6–10 mice). (**H**) Oral glucose tolerance (OGTT) (left) and insulin levels (right) after 4 weeks FGF21 (1 mg/kg body weight) and danegaptide (10 mg/kg body weight) treatment (*n* = 6–9 mice). All data are mean ± SEM; **P* < 0.05, ***P* < 0.01, ***<*P*<0.001, ****<*P*<0.0001.