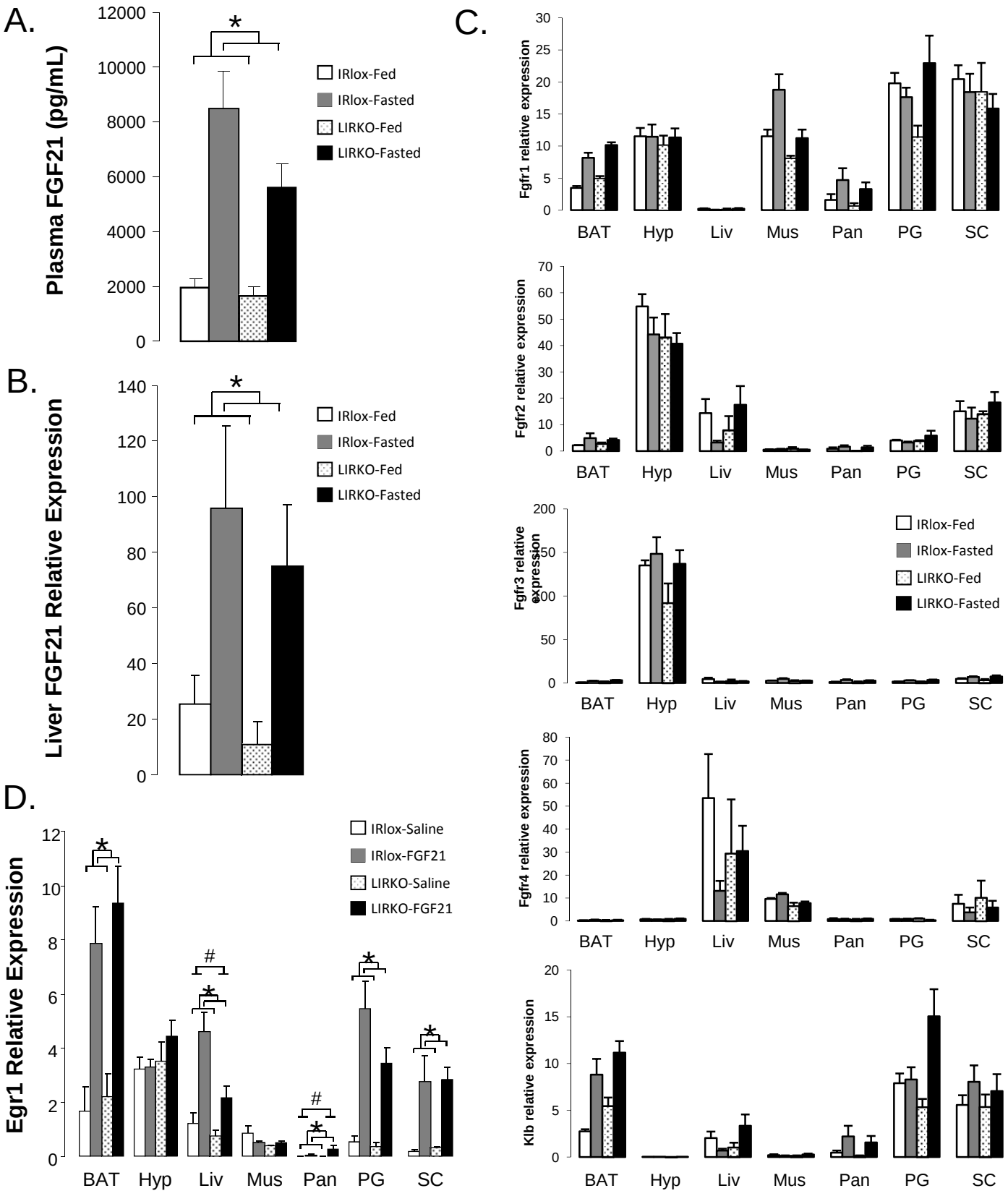
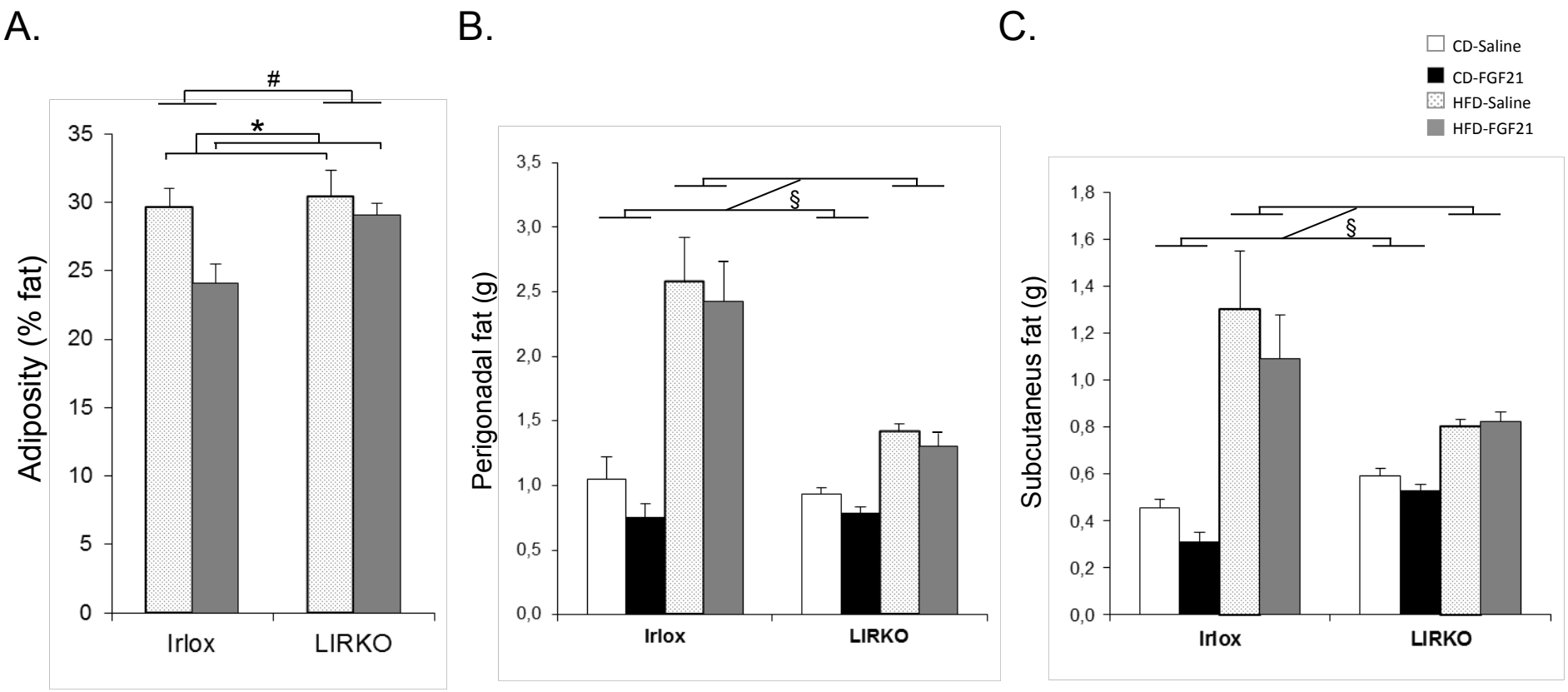
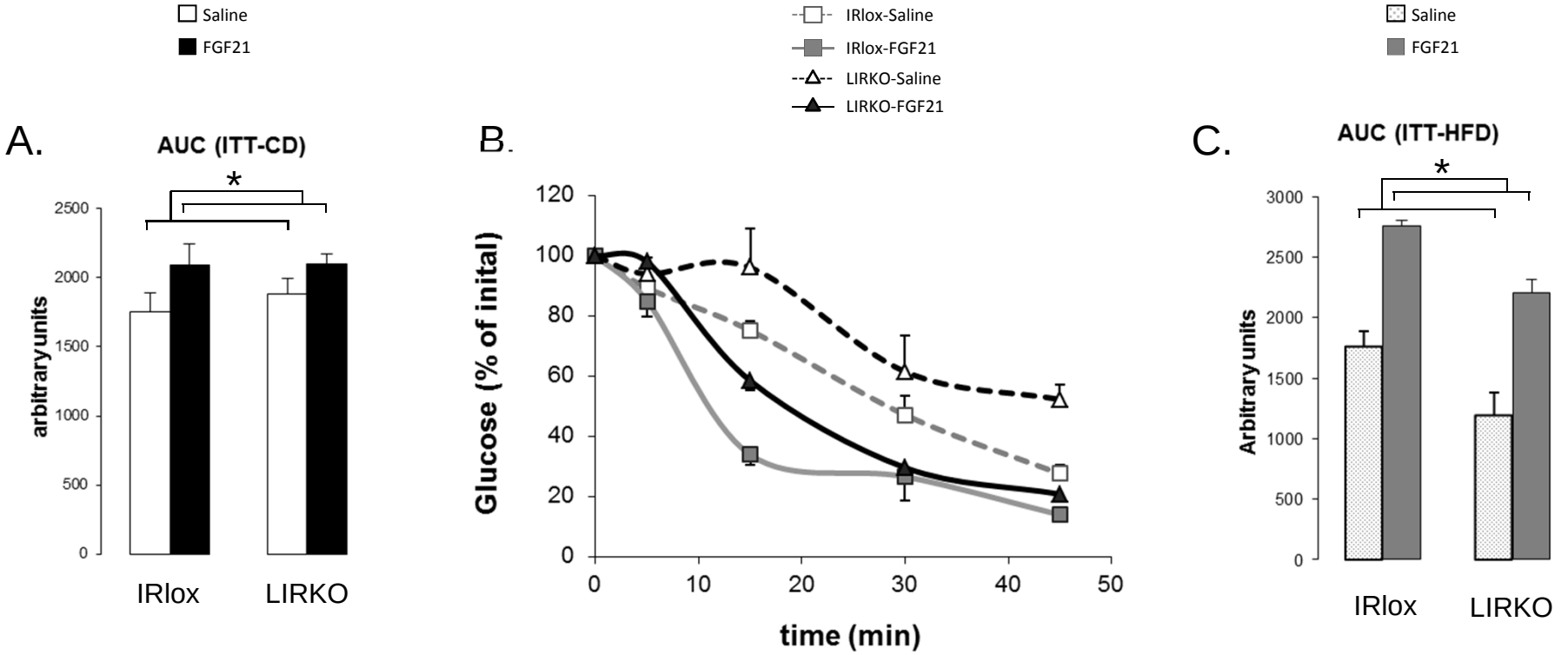


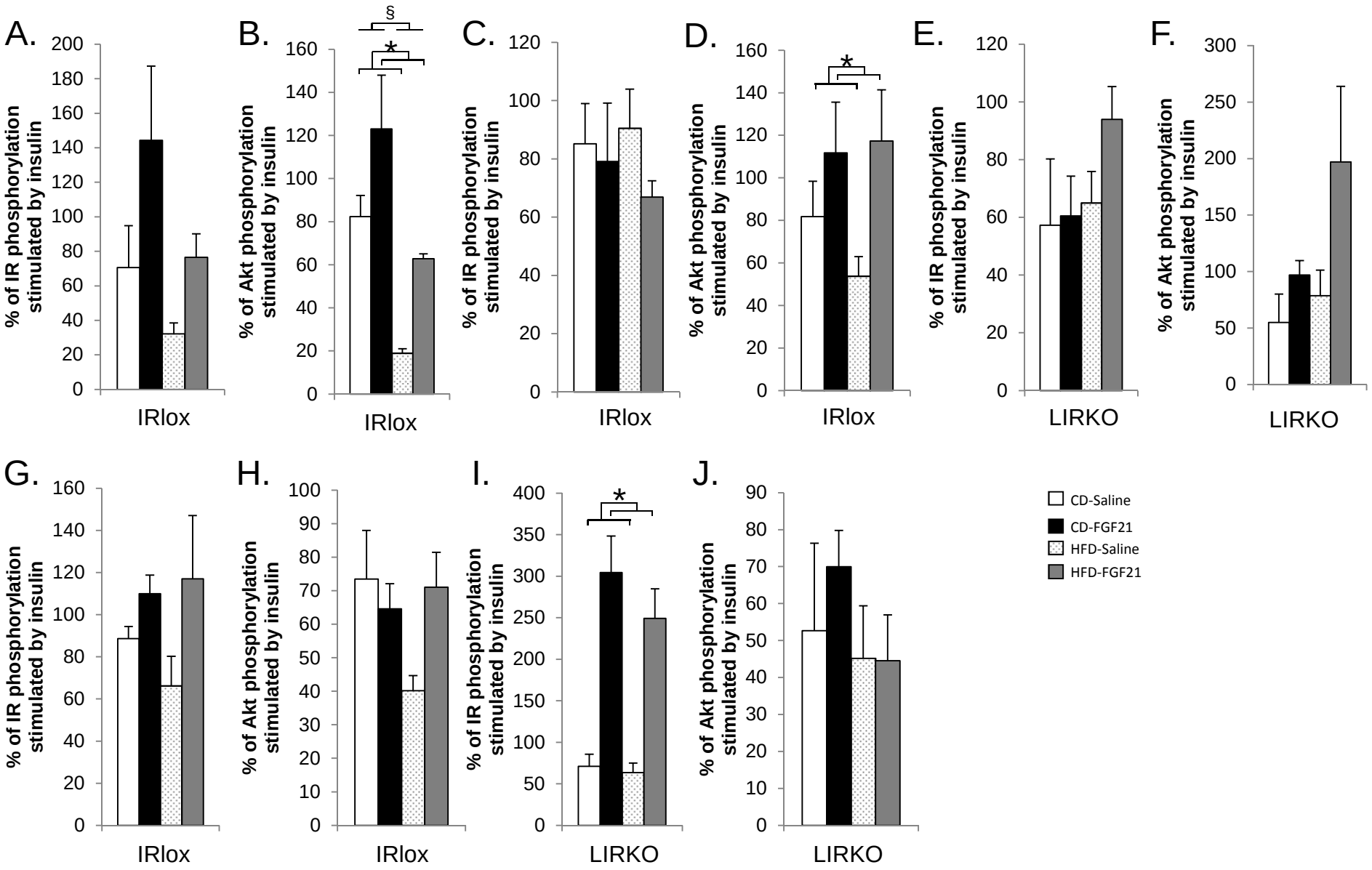
Supplementary Figure 1: FGF21 response to fasting, FGF21 receptor expression profile, and FGF21 sensitivity in LIRKO mice. Means ± SEM are represented. P-value was calculated by 2-way ANOVA. * p-value<0.05. A-C. Control and LIRKO mice were fed ad libitum, or fasted overnight. Open boxes represent fed state and plain gray boxes represent fasted state in control mice, black dotted boxes represent fed state and plain black boxes represent fasted state in LIRKO mice. A. Serum FGF21. B. Liver FGF21 gene expression. C. Fgfr 1, 2, 3, 4 and Klb gene expression in various tissues. D. Egr1 gene expression following intraperitoneal injection of FGF21 (1mg/kg body weight) in various tissues. Open boxes represent saline treated control mice, plain gray boxes represent FGF21 treated control mice, black dotted boxes represent saline treated LIRKO mice, and plain black boxes represent FGF21 treated LIRKO mice. # indicates a p-value<0.05 between genotypes. P-value was calculated using pooled pairwise non-parametric tests.



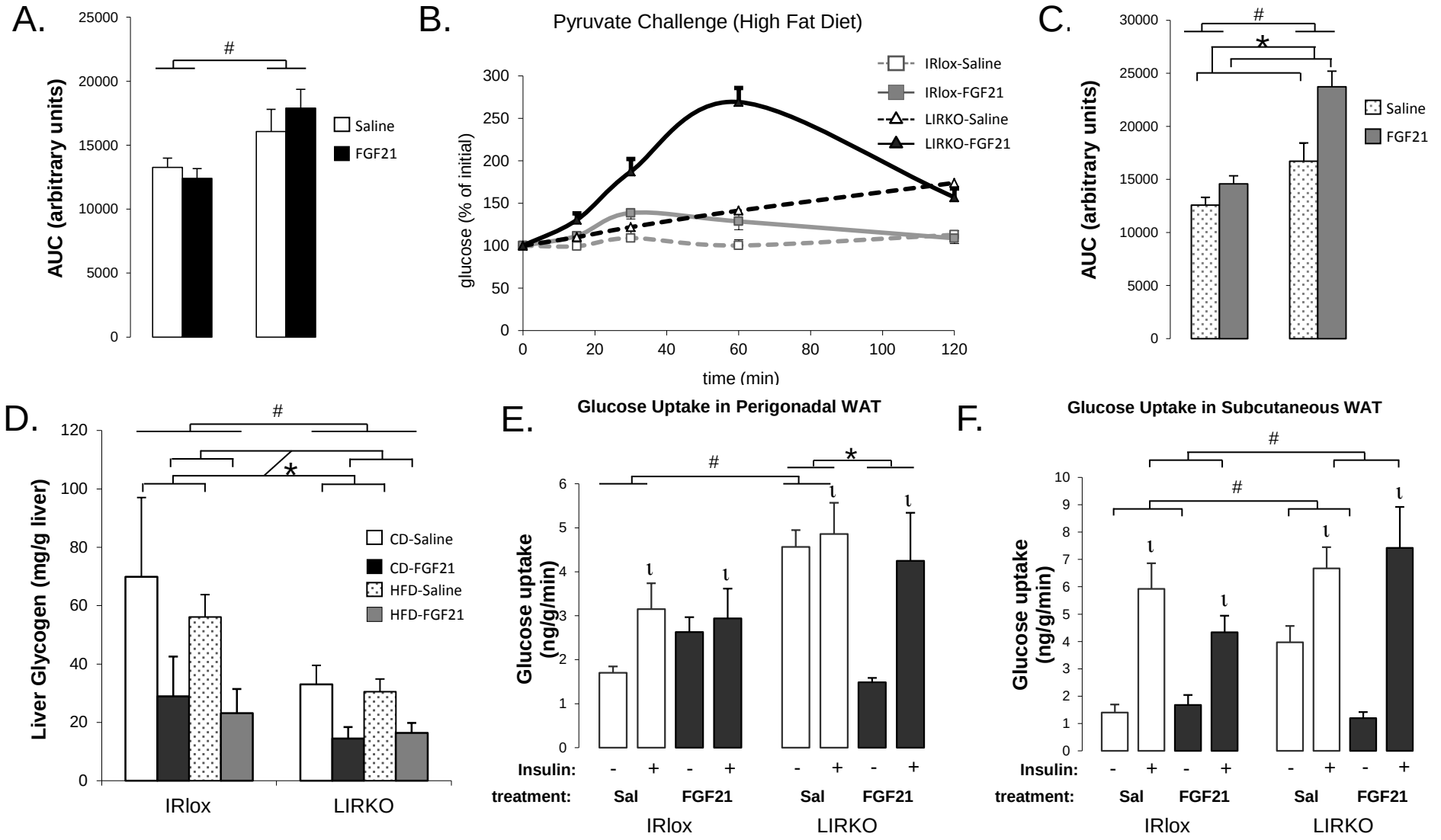
Supplementary Figure 2: Effect of chronic FGF21 treatment on Fat Tissue in control and LIRKO mice. Control and LIRKO mice either on CD or HFD for 7 weeks were treated with either saline or FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. Means ± SEM are represented. * indicates a p-value<0.05. # indicates a p-value<0.05 between genotypes. § indicates a p-value<0.05 between diets. A. Adiposity. B. Perigonadal fat depot weight. C. Subcutaneous fat depot weight. Open boxes represent saline treatment in mice on CD; black boxes represent FGF21 treatment in mice on CD. Gray dotted boxes represent saline treatment in mice on HFD, and plain gray boxes represent FGF21 treatment in mice on HFD. P-value was calculated by 2-way ANOVA (A.) or using pooled pairwise non-parametric tests (B. and C.).



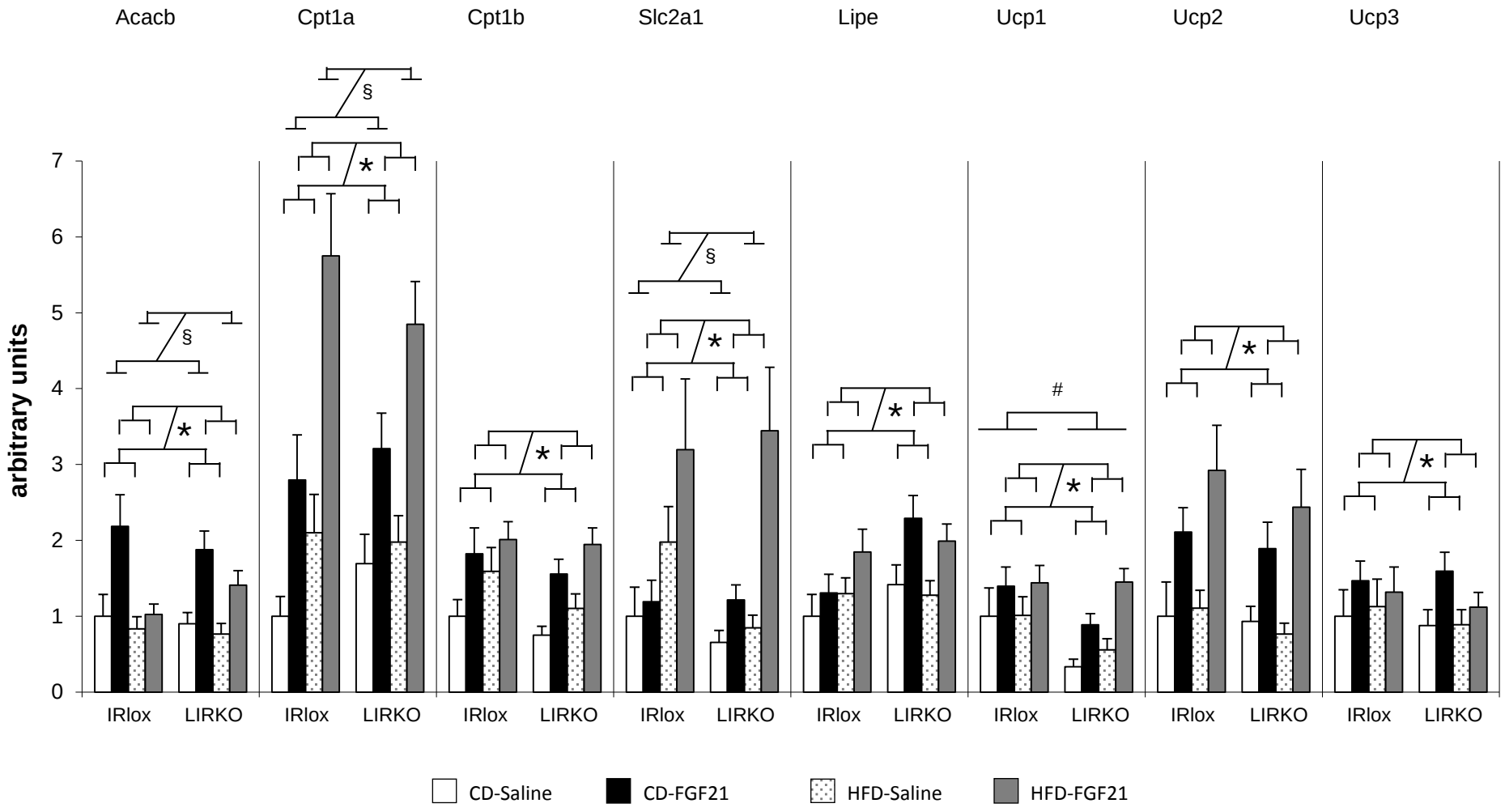
Supplementary Figure 3: Effect of FGF21 on Insulin Sensitivity. Control and LIRKO mice either on CD or HFD for 7 weeks were treated with either saline or FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. Means $\pm$ SEM are represented. P-value was calculated by 2-way ANOVA. * p-value<0.05. A. Area under the curve for Insulin Tolerance Test in mice on CD was estimated by calculating the area between the 100% initial glucose baseline and the line defined by the % of initial glucose over time after insulin injection, i.e., it is the area downward from the baseline. Open boxes represent saline treatment in mice on CD; black boxes represent FGF21 treatment in mice on CD. B. Insulin Tolerance Test in mice on HFD. Gray lines with squares represent controls; black lines with triangles represent LIRKO. Dashed lines represent saline treated mice; plain lines represent FGF21 treated mice. C. Area under the curve for Insulin Tolerance Test in mice on HFD. Gray dotted boxes represent saline treatment in mice on HFD, and plain gray boxes represent FGF21 treatment in mice on HFD.



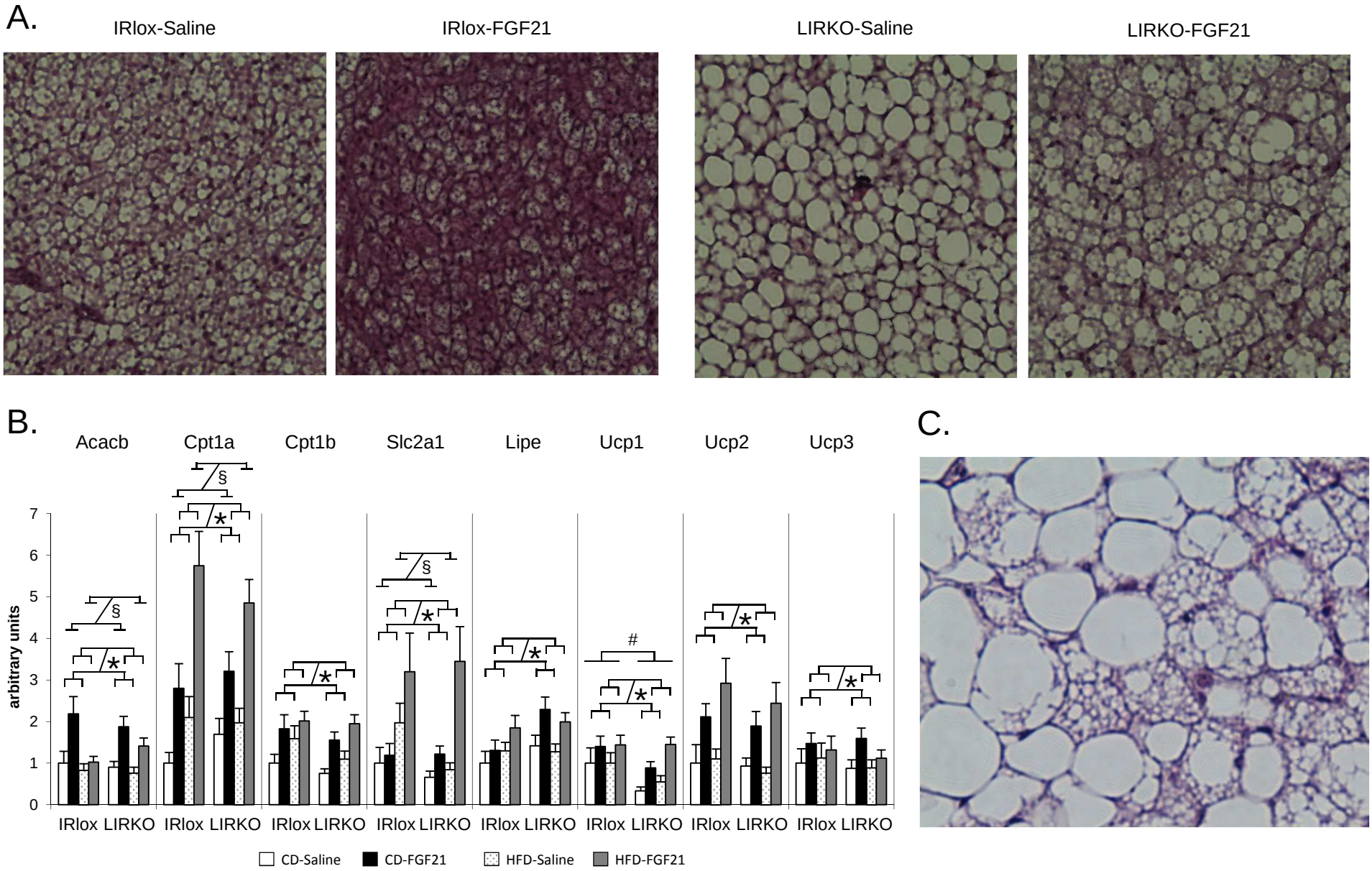
Supplementary Figure 4: Quantification of Insulin Signaling in mice with FGF21. Control and LIRKO mice either on CD or HFD for 7 weeks were treated with either saline or FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. Quantification of pIR in liver (A), skeletal muscle (C, E), subcutaneous fat (G; I), and of pAkt in liver (B), skeletal muscle (D, F), and subcutaneous fat (H, J) was determined by densitometry using the ImageJ software, all related to Akt expression. Means $\pm$ SEM are represented. P-value was calculated by 2-way ANOVA. § indicates a p-value<0.05 between diets. * indicates a p-value<0.05 upon FGF21 treatment. Open boxes represent saline treatment in mice on CD; black boxes represent FGF21 treatment in mice on CD. Light gray dotted boxes represent saline treatment in mice on HFD, and plain gray boxes represent FGF21 treatment in mice on HFD.



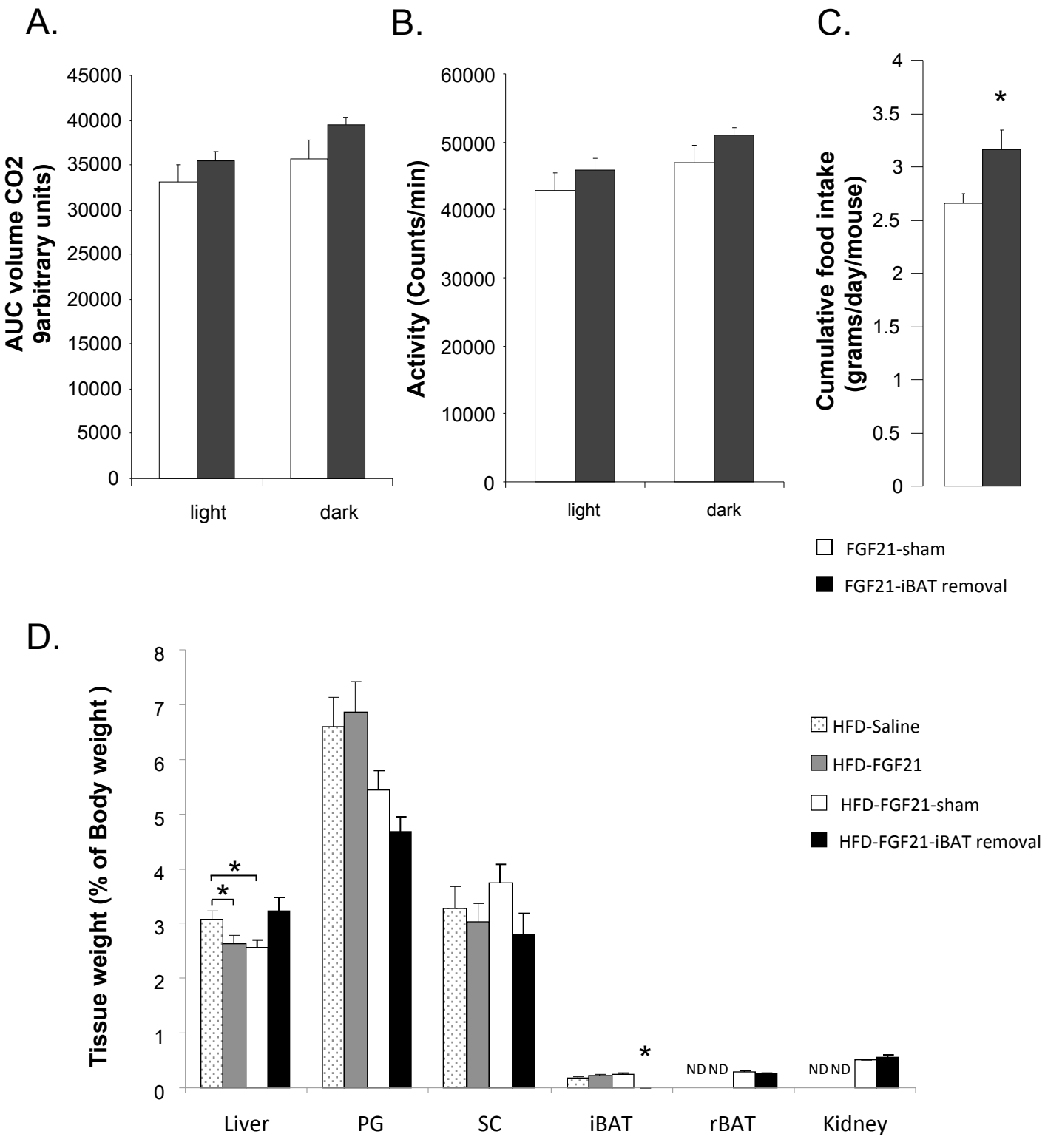
Supplementary Figure 5: Hepatic glucose homeostasis and glucose uptake in WAT in mice following FGF21 treatment. Control and LIRKO mice on CD or HFD for 7 weeks were treated with saline or FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. Means ± SEM are represented. P-value was calculated by 2-way or 3-way ANOVA (A, C, E, F) or using pooled pairwise non-parametric tests (D). # p-value<0.05 between genotypes. * p-value<0.05 upon FGF21 treatment. ι p-value<0.05 upon insulin stimulation. A. Area under the curve for Pyruvate challenge in mice on CD. Open boxes represent mice on CD receiving saline; black boxes represent mice on CD receiving FGF21. B. Pyruvate challenge in mice on HFD. Gray lines with squares represent controls; black lines with triangles represent LIRKO. Dashed lines represent mice receiving saline; plain lines represent FGF21 treated mice. C. Area under the curve for Pyruvate challenge in mice on HFD. Gray dotted boxes represent mice on HFD receiving saline, and plain gray boxes represent FGF21 treated mice on HFD. D. Glycogen content in Liver. Open boxes represent mice on CD receiving saline; black boxes represent mice on CD receiving FGF21. Gray dotted boxes represent mice on HFD receiving saline, and plain gray boxes represent FGF21 treated mice on HFD. E & F. In vivo glucose uptake was assessed by measuring [C14]DOG uptake in tissues (E. perigonadal white adipose tissue; F. subcutaneous white adipose tissue) of control and LIRKO mice fed with a CD after insulin stimulation or not. Open boxes represent saline treatment, plain boxes represent FGF21 treatment.



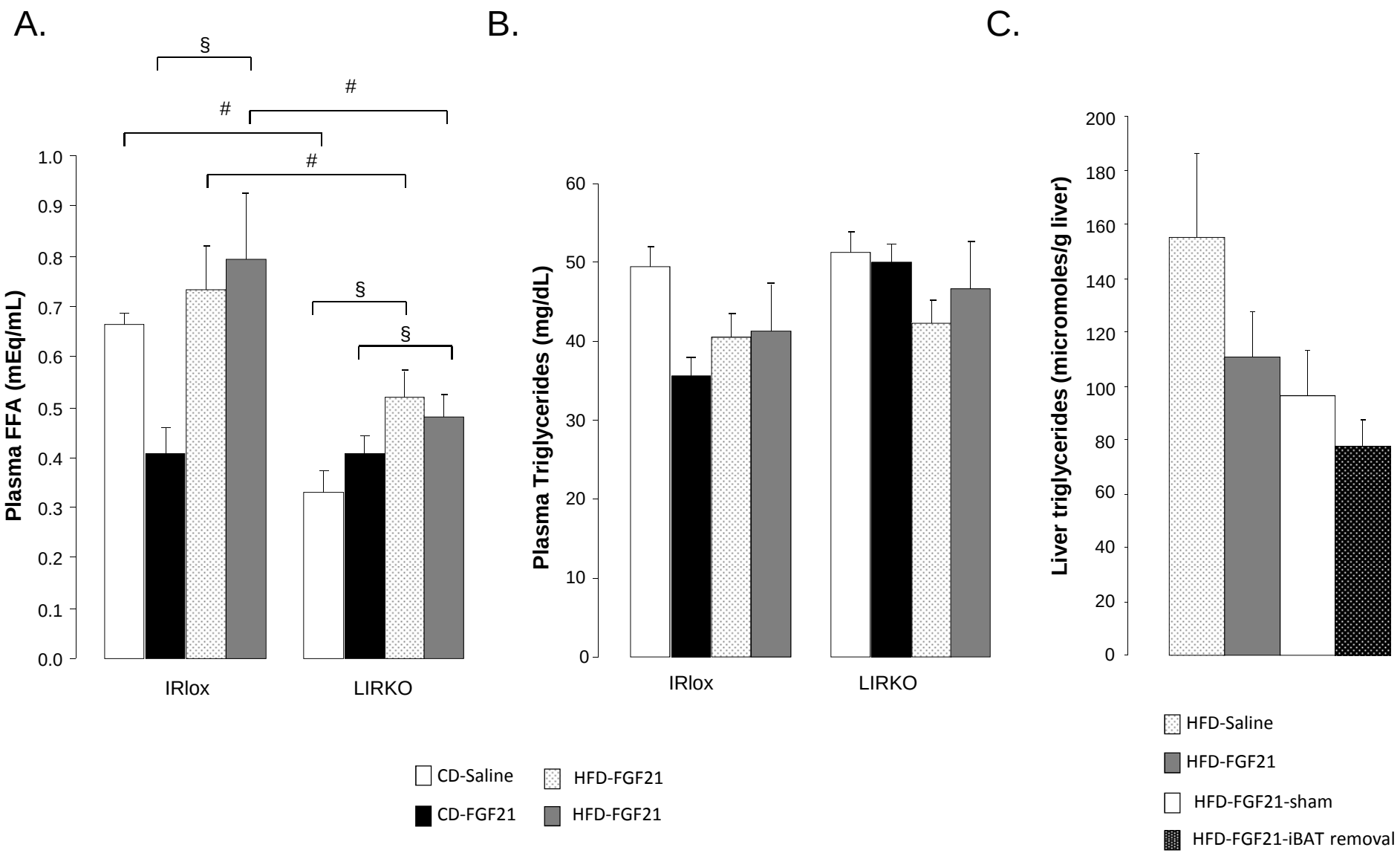
Supplementary Figure 6: Gene expression in BAT following FGF21 treatment. Control and LIRKO mice on CD or HFD for 7 weeks were treated with either saline or FGF21 (1mg/kg/day) delivered subcutaneously during 2 weeks using osmotic pumps. Means ± SEM are represented. P-value was determined by pooled pairwise non-parametric tests. #: p-value<0.05 between genotypes. §: p-value<0.05 between diets. *: p-value<0.05 upon FGF21 treatment. Open boxes represent saline treated mice on CD, and plain black boxes represent FGF21 treated mice on CD. Light gray dotted boxes represent saline treated mice on HFD, and plain gray boxes represent FGF21 treated mice on HFD. Acacb: Acetyl-CoA carboxylase beta; Cpt1: Carnitine palmitoyltransferase; Slc2a1: Glut1, Lipe: Hormone-sensitive lipase; Ucp: Uncoupling protein.



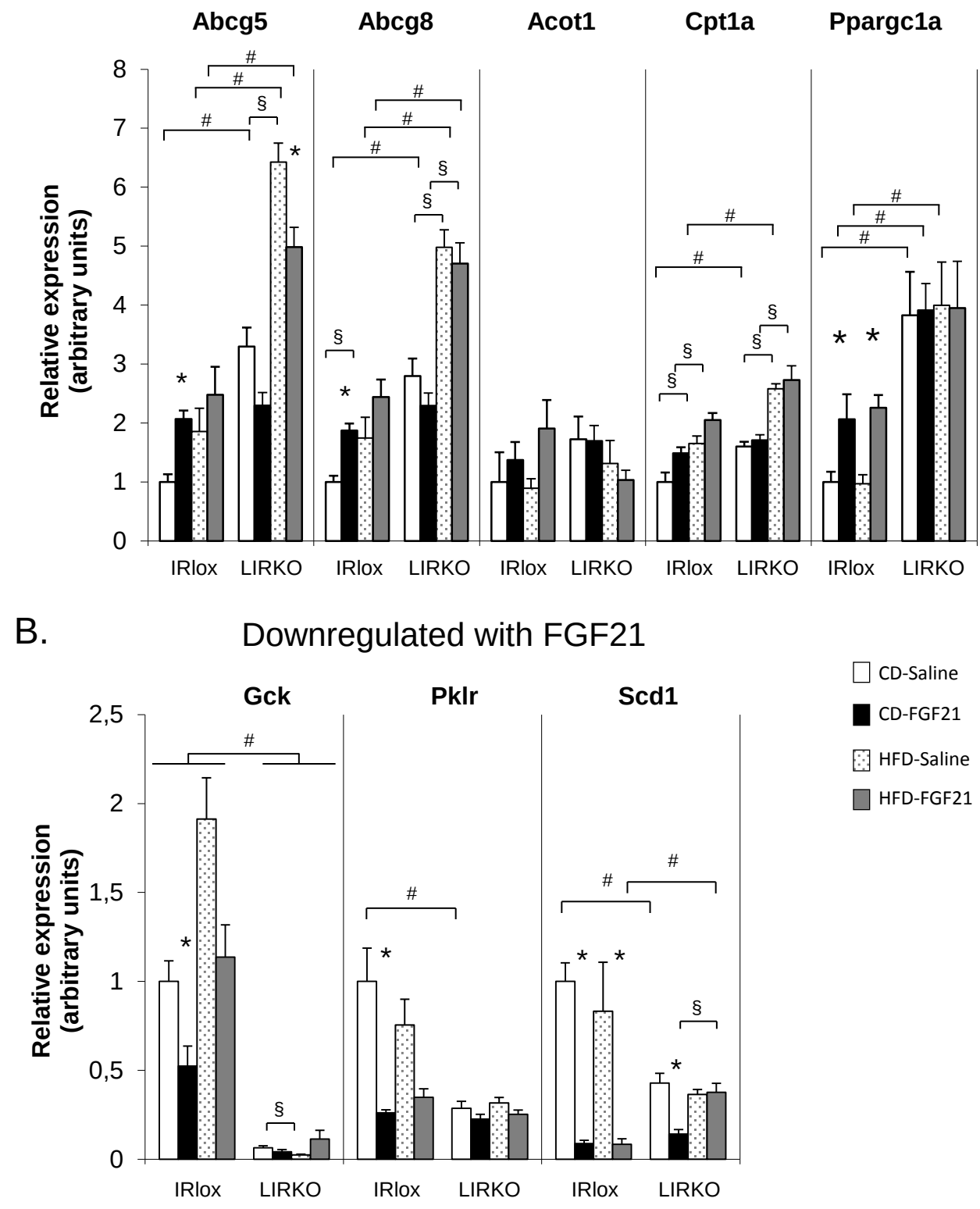
Supplementary Figure 7: Characteristics of Fat tissues following FGF21 treatment. Control and LIRKO mice on CD were treated with either saline or FGF21 (1mg/kg/day) delivered subcutaneously during 2 weeks using osmotic pumps. A. H&E staining of interscapular brown adipose tissue sections. Magnification 10X. B. Gene expression in BAT. Means ± SEM are represented. P-value was determined by pooled pairwise non-parametric tests. #: p-value<0.05 between genotypes. §: p-value<0.05 between diets. *: p-value<0.05 upon FGF21 treatment. Open boxes represent saline treated mice on CD, and plain black boxes represent FGF21 treated mice on CD. Light gray dotted boxes represent saline treated mice on HFD, and plain gray boxes represent FGF21 treated mice on HFD. Acacb: Acetyl-CoA carboxylase beta; Cpt1: Carnitine palmitoyltransferase; Slc2a1: Glut1, Lipe: Hormone-sensitive lipase; Ucp: Uncoupling protein. C. H&E staining of a subcutaneous adipose tissue section from control mice. Magnification 40X.



Supplementary Figure 8: Effect of iBAT removal on FGF21 response. A., B., C. Control mice on HFD for 12 weeks, with iBAT (open boxes) or without iBAT (black boxes) were treated with FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. A. CO2 production per animal. B. Activity. C. Food intake. D. Tissue weight. Control mice on HFD for 7 weeks were treated with either saline (grey dotted boxes) or FGF21 (1 mg/kg/day) delivered subcutaneously (plain grey boxes) during the last 2 weeks of the diet using osmotic pumps. Control mice on HFD for 12 weeks, with iBAT (open boxes) or without iBAT (black boxes) were treated with FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. PG: perigonadal fat pad, SC: subcutaneous fat pad, iBAT: interscapular brown adipose tissue. rBAT: remaining adipose tissue in the subscapular region. Mean $\pm$ SEM are represented. Statistical comparisons were done by T-test. * p-value<0.05.



Supplementary Figure 9: Plasma and liver lipids. A., B. Control mice or LIRKO mice either on CD or HFD for 7 weeks were treated with either saline or FGF21 (1 mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. Open boxes represent saline treated mice and plain dark boxes represent FGF21 treated mice, on CD. Dotted grey boxes represent saline treated mice and plain grey boxes represent FGF21 treated mice, on HFD. Plasma levels of triglycerides (A.) and free fatty acids (B.) in the fed state. C. Liver triglycerides content. Control mice on HFD for 7 weeks receiving saline (dotted grey boxes) or FGF21 (plain grey boxes), or for 12 weeks and receiving FGF21, with iBAT (open boxes) or without iBAT (black boxes with white dots). Means ± SEM are represented. P-value was calculated by 3-way ANOVA. #: p-value<0.05 between genotypes. §: p-value<0.05 between diets.



Supplementary Figure 10: Regulation of lipid metabolism gene expression in liver upon FGF21 chronic infusion. A., B. Control and LIRKO mice either on CD or HFD for 7 weeks were treated with either saline or FGF21 (1mg/kg/day) delivered subcutaneously during the last 2 weeks of the diet using osmotic pumps. Gene expression was assessed by quantitative real time PCR. Mean ± SEM are represented. Statistical comparisons were done by pooled pairwise non-parametric tests, followed by Kruskall-Wallis 3-way tests and pairwise Wilcoxon tests. * p value<0.05. Open boxes represent saline treatment in mice on CD, black boxes represent FGF21 treatment in mice on CD, gray dotted boxes represent saline treatment in mice on HFD, and plain gray boxes represent FGF21 treatment in mice on HFD. Abcg: ATP binding cassette, G; Acot1: Acyl-CoA thioesterase 1; Cpt1a: Carnitine palmitoyltransferase 1A; Ppargc1a: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; Gck: Glucokinase; Pklr: Pyruvate kinase, liver and RBC; Scd1: Stearoyl-CoA desaturase 1.