Supplemental Figure Legends

Figure S1, related to Figure 1. β-klotho adipose-specific knockout mice have normal body weight and insulin sensitivity. (A) Western blot analysis of KLB protein expression in epididymal white adipose tissue (eWAT) and brown adipose tissue (BAT) of WT and β-klotho adipose-specific knockout mice (KLB AdipoKO) mice. β-actin serves as a loading control. (B-D) *Egr1* and *cFos* mRNA expression in (B) eWAT, (C) BAT, and (D) liver of WT and KLB AdipoKO mice (n = 6-7/group) administered vehicle or FGF21 (1 mg/kg) via i.p. injection for 1 hour. (E) Body weights of lean, 12-14 week old, male KLB AdipoKO mice and WT littermate controls (n = 6/group). (F) Insulin tolerance tests in the mice in (E) (n = 6/group). (G) Body weights of diet-induced obese (DIO), male KLB AdipoKO mice and WT littermate controls (n = 6/group). (H) Insulin tolerance tests in the mice in (G) (n = 6/group). (I) Fold change of adiponectin levels in media of primary white adipocytes treated with vehicle or FGF21 (1 ug/mL) for the indicated time. (J) Plasma glucose levels in 12-14 week old, male WT and Adiponectin knockout (Adipoq KO) littermates co-injected with insulin and either vehicle or FGF21 (1 mg/kg) (n = 6/group). Values are mean +/- SEM. (*, $P< 0.05$; **, $P<0.01$; ***, $P< 0.005$; and #, $P<0.001$ compared to WT).

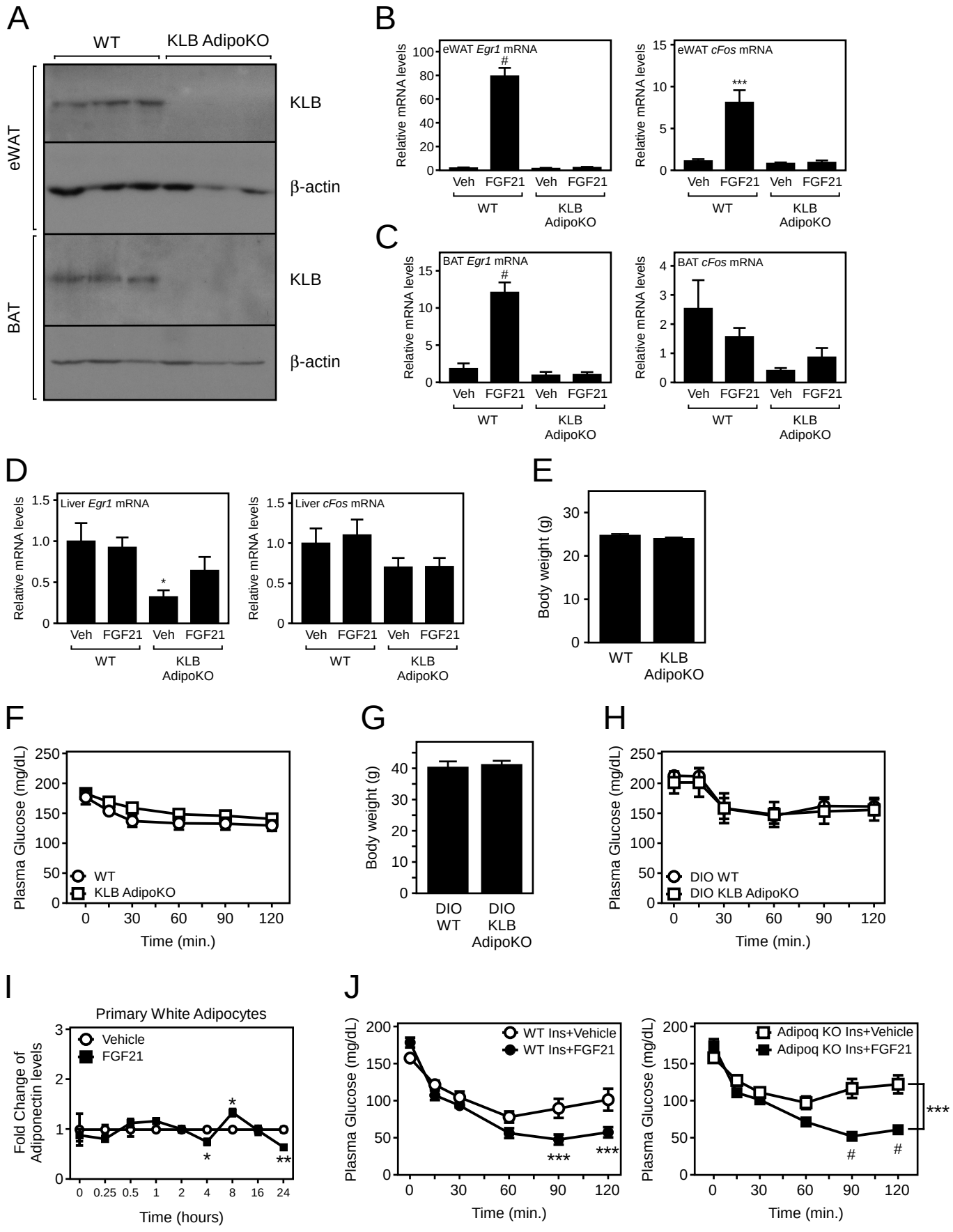
Figure S2, related to Figure 2. FGF21 induces brown adipose tissue metabolic gene expression. *Bmp8b* and *Zbtb16* mRNA expression in brown adipose tissue (BAT) of diet-induced obese (DIO) WT and Adiponectin (Adipoq) KO mice administered vehicle or FGF21 for 3 weeks via i.p. injection (n = 5-9/group). Values are mean +/- SEM. (*, $P< 0.05$; ***, $P< 0.005$ compared to WT).

Figure S3, related to Figure 3. Generation of inducible, adipose-specific, constitutively active FGFR1 transgenic mice. (A) Plasma insulin levels during glucose tolerance tests in DIO WT and KLB AdipoKO mice after 2 weeks daily intraperitoneal (i.p.) injection of vehicle or FGF21 (1 mg/kg) (n = 5-8/group). (B) Area under the curve (AUC) for mice in (A). (C) Body weights and (D) percent fat mass of wild-type (WT), TRE-caFGFR1 transgenic, and Adipo-rtTa/TRE-caFGFR1 double transgenic mice were induced to obesity by 12 weeks of high fat diet (HFD) feeding (no doxycycline (DOX); n = 7-10/group). (E) *caFGFR1* mRNA expression in epididymal white adipose tissue (eWAT), subcutaneous white adipose tissue (scWAT), and brown adipose tissue (BAT) of DIO WT, TRE-caFGFR1 transgenic, and Adipo-rtTa/TRE-caFGFR1 double transgenic mice on HFD + DOX for 2 weeks (n = 7-10/group). (F) Western blot analysis of Myc and phospho-ERK1/2 expression in skeletal muscle (SKM) from mice with the indicated genotypes. Total ERK served as a loading control. (G) Plasma triglyceride levels in DIO WT, TRE-caFGFR1 transgenic, and Adipo-rtTa/TRE-caFGFR1 double transgenic mice on HFD + Dox for 2 weeks. Values are mean +/- SEM. (*, $P< 0.05$; #, $P< 0.001$ compared to WT).

Figure S4, related to Figure 4. Generation and characterization of KLB BatKO mice.

(A) Western blot analysis of KLB protein expression in brown adipose tissue (BAT) and epididymal white adipose tissue (eWAT) of WT and β-klotho brown adipose tissue-specific knockout mice (KLB BatKO) mice. β-actin serves as a loading control. (B-D) *Egr1* and *cFos* mRNA expression in (B) eWAT, (C) BAT, and (D) liver of WT and KLB BatKO mice (n = 5-7/group) administered vehicle or FGF21 (1 mg/kg) via i.p. injection for 1 hour. (E) Plasma glucose levels plasma in 12-14 week old, male KLB BatKO mice and WT littermates co-injected with insulin and either vehicle or FGF21 (1 mg/kg) (n = 6/group). (F) *Insulin receptor (IR)*

mRNA levels in epididymal white adipose tissue (eWAT), subcutaneous WAT (scWAT), brown adipose tissue (BAT), and liver of WT and insulin receptor adipose-specific knockout mice (IR AdipoKO) mice (n = 5-7/group). (G) Plasma glucose levels plasma in 12-14 week old, male IR AdipoKO mice and WT littermates co-injected with insulin and either vehicle or FGF21 (1 mg/kg) (n = 5-7/group). Values are mean +/- SEM. (*, $P< 0.05$; **, $P<0.01$; ***, $P< 0.005$; and #, $P<0.001$ compared to WT).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WT
KLB AdipoKO
eWAT
BAT
KLB
β-actin
KLB
β-actin
B
eWAT Egr1 mRNA
eWAT cFos mRNA
Relative mRNA levels
Veh FGF21 Veh FGF21
WT
KLB AdipoKO
C
BAT Egr1 mRNA
BAT cFos mRNA
D
Liver Egr1 mRNA
Liver cFos mRNA
E
Body weight (g)
F
Plasma Glucose (mg/dL)
WT
KLB AdipoKO
Time (min.)
G
Body weight (g)
DIO WT
DIO KLB AdipoKO
H
Plasma Glucose (mg/dL)
DIO WT
DIO KLB AdipoKO
Time (min.)
I
Primary White Adipocytes
Fold Change of Adiponectin levels
Vehicle
FGF21
Time (hours)
J
Plasma Glucose (mg/dL)
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WT Ins+FGF21
Adipoq KO Ins+Vehicle
Adipoq KO Ins+FGF21
Time (min.)

Figure S1

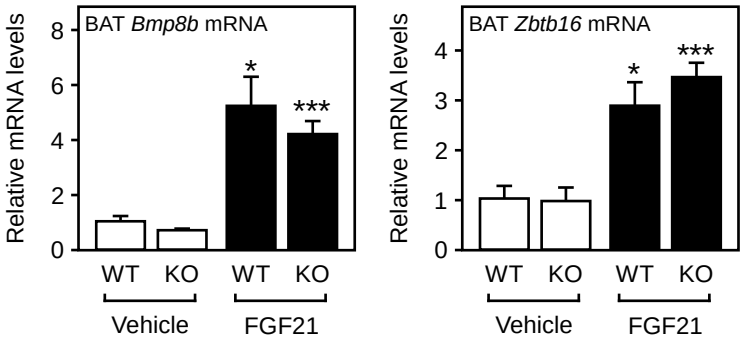
BAT *Bmp8b* mRNA
Relative mRNA levels
8
6
4
2
0
*
***
WT
KO
WT
KO
Vehicle
FGF21
BAT *Zbtb16* mRNA
Relative mRNA levels
4
3
2
1
0
*
***
WT
KO
WT
KO
Vehicle
FGF21

Figure S2

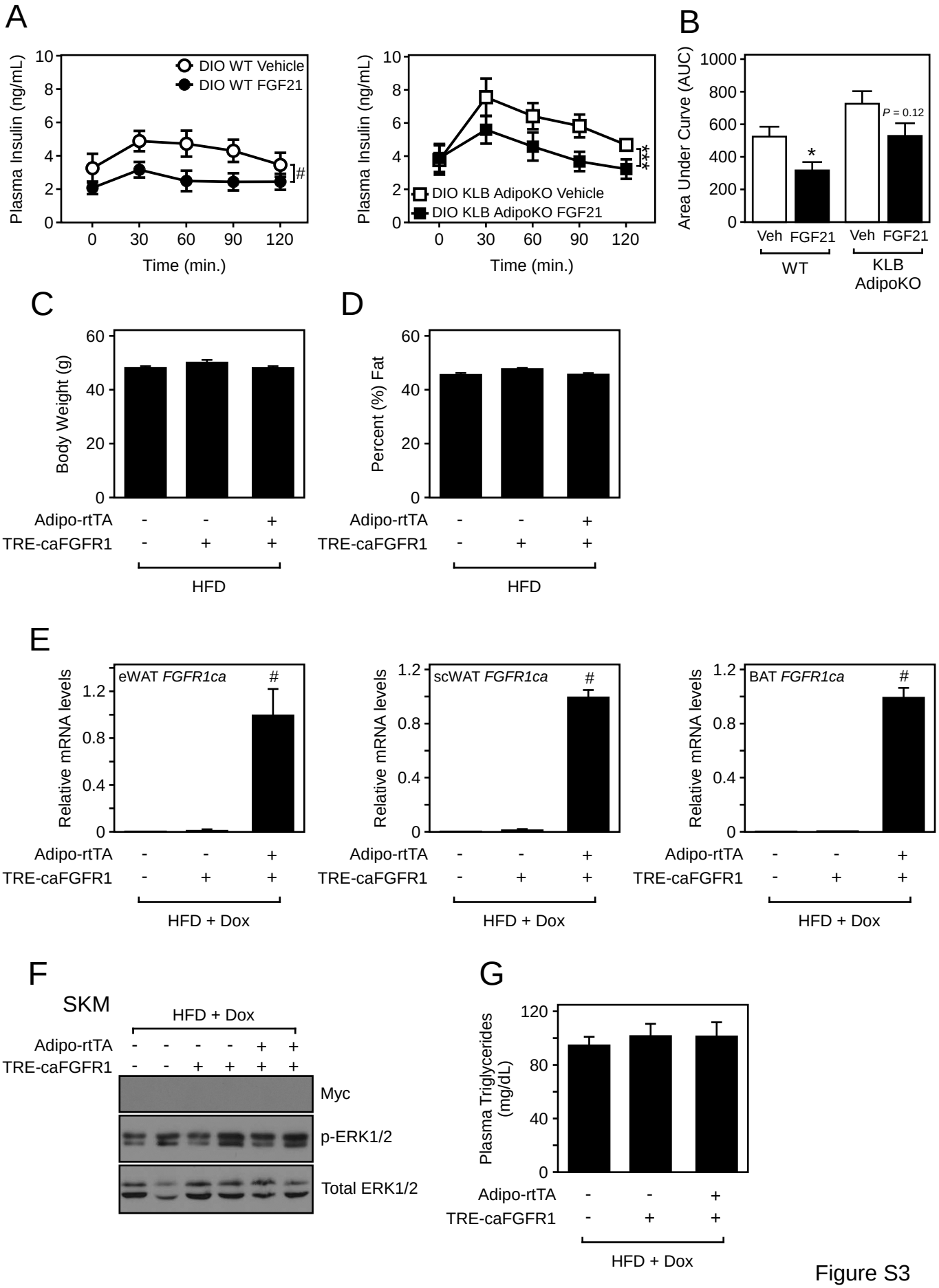
A
Plasma Insulin (ng/mL)
DIO WT Vehicle
DIO WT FGF21
Time (min.)
#
Plasma Insulin (ng/mL)
DIO KLB AdipoKO Vehicle
DIO KLB AdipoKO FGF21
Time (min.)
***
B
Area Under Curve (AUC)
P = 0.12
*
Veh FGF21 Veh FGF21
WT
KLB AdipoKO
C
Body Weight (g)
Adipo-rtTA
TRE-caFGFR1
HFD
D
Percent (%) Fat
Adipo-rtTA
TRE-caFGFR1
HFD
E
eWAT FGFR1ca
Relative mRNA levels
#
Adipo-rtTA
TRE-caFGFR1
HFD + Dox
scWAT FGFR1ca
Relative mRNA levels
#
Adipo-rtTA
TRE-caFGFR1
HFD + Dox
BAT FGFR1ca
Relative mRNA levels
#
Adipo-rtTA
TRE-caFGFR1
HFD + Dox
F
SKM
HFD + Dox
Adipo-rtTA
TRE-caFGFR1
Myc
p-ERK1/2
Total ERK1/2
G
Plasma Triglycerides (mg/dL)
Adipo-rtTA
TRE-caFGFR1
HFD + Dox

Figure S3

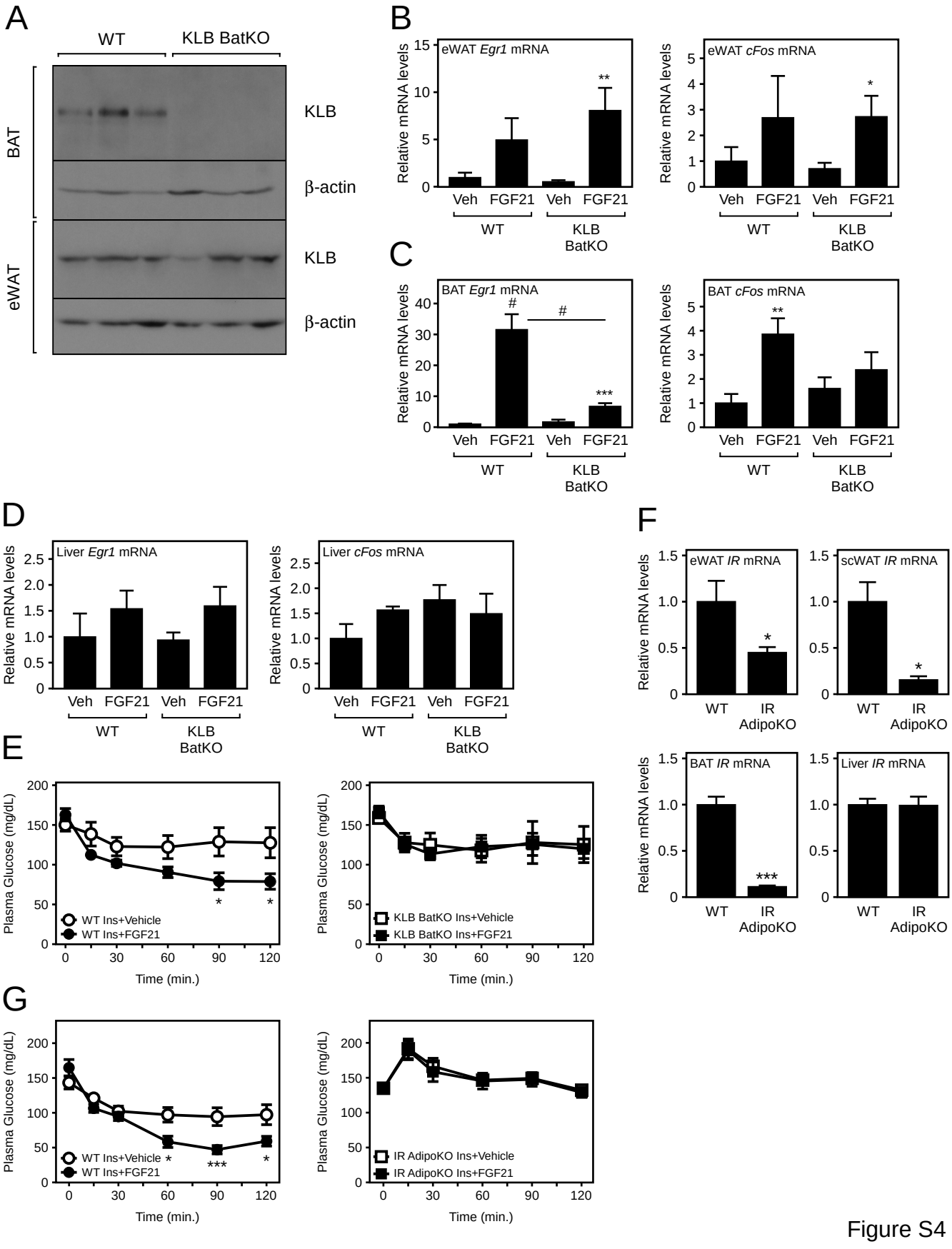
A
WT
KLB BatKO
BAT
eWAT
KLB
β-actin
KLB
β-actin
B
eWAT Egr1 mRNA
eWAT cFos mRNA
Relative mRNA levels
Veh FGF21 Veh FGF21
WT
KLB BatKO
C
BAT Egr1 mRNA
BAT cFos mRNA
D
Liver Egr1 mRNA
Liver cFos mRNA
E
Plasma Glucose (mg/dL)
Time (min.)
WT Ins+Vehicle
WT Ins+FGF21
KLB BatKO Ins+Vehicle
KLB BatKO Ins+FGF21
F
eWAT IR mRNA
scWAT IR mRNA
BAT IR mRNA
Liver IR mRNA
WT
IR AdipoKO
G
IR AdipoKO Ins+Vehicle
IR AdipoKO Ins+FGF21

Figure S4