SUPPLEMENTAL FIGURE LEGENDS

Figure S1. *Acot7* expression remains unchanged in hypothalamus and liver of Acot7 Tg mice and the expression of other members of the family is identical in Acot7 transgenic islets than control. A,B) RT-qPCR expression data of *Acot7* mRNA in hypothalamus, liver and isolated islets from Acot7 Tg (line F26) (green) and littermate controls (black). Data (mean ± S.E.M) is presented as fold change *versus* control (A) or relative to actin (B). n=4-5 mice/genotype (each dot represents an individual mouse). **C)** RT-qPCR was used to evaluate the expression of the indicated Acot family members. n=4 mice/genotype. Data (mean ± S.E.M) presented as fold change *versus* control.

Figure S2. Acot7 over-expression does not affect body weight or randomly-fed glycemia. Body weight (left hand panels) and randomly-fed glycaemia (right hand panels) were periodically monitored in Acot7 Tg mice (Line F26; green lines) and littermate controls (black lines) following the beginning of the Doxycycline treatment (5 weeks old). n=8-10. ns: non-significant. Student t-test corrected for multiple comparisons by the Sidak-Bonferroni method.

Figure S3. β-cell-selective over-expression of mitochondrial *Acot7* impairs glucose tolerance (Lines F15 and F9). A) Glucose tolerance was measured in 25 week old female mice from Acot7 Tg lines F15 (left) or F9 (right) fed a regular chow diet. **B)** Glucose tolerance was measured in 9 week old male mice from Acot7 Tg lines F15 (left) or F9 (right) fed a high fat diet during 4 weeks. Acot7 Tg are shown in green and littermate controls in black. The area under the curve (AUC) is shown underneath each graph. n=5-8 mice/genotype. ns: non-significant, *p<0.05, **p<0.01; Two-way ANOVA Fisher least significance different test (Glucose tolerance test); Student's t test (AUC).

Figure S4. Over-expression of Acot7 does not affect insulin resistance and β-cell mass. A) Insulin tolerance was measured in 9 week-old females Acot7 Tg (green) or littermate control (black) mice (line F26) fed a high-fat diet (left panel) and 25 week-old females Acot7 Tg (green) or control (black) (line F15) fed a chow diet (right hand-side graph). n=5-6 mice/genotype (left), 3-4 (right).**B, C)** Pancreata from Acot7 Tg (F26) and littermate control mice fed a normal diet ((B), 25 week-old) or a high-fat diet ((C), 9 week-old), as indicated were fixed and subjected to immunocytochemical analysis for insulin and glucagon, as indicated. Representative islets are shown on the left. Scale bar: 50µm. ImageJ software was used to quantify β-cell mass which is presented as a percentage of the pancreatic surface and corresponds to quantification of the insulin-positive area per pancreas area quantified in whole pancreas sections. n=6 mice/genotype (3 male,3 female

each); 2 pancreas sections per animal were used, separated by 50µm. ns: non-significant; Unpaired Student's t test.

Figure S5. The expression of genes essential for GSIS remained unchanged upon ACOT7 over-expression in islets. RT-qPCR was used to evaluate the expression of the indicated genes in isolated islets from Acot7 Tg (F26) (green) and littermate controls (black). Data (mean ± S.E.M) presented as fold change *versus* control. n=4 mice/genotype.

Figure S6. A) The expression of *Ldha* and *Slc16a1* remained unchanged upon ACOT7 over-expression in islets. RT-qPCR was used to evaluate the expression of *Ldha* and *Slc16a1* in isolated islets from Acot7 Tg (F15 and F26, as indicated) (green) and littermate controls (black). Data (mean ± S.E.M) presented as fold change *versus* control. n=8-10 mice/genotype. **B) Overexpression of *Acot7* in INS1 (832/13) cells impairs leucine plus glutamine-induced insulin secretion. Leucine plus glutamine**-stimulated insulin secretion was evaluated in INS1(832/13) cells over-expressing mitochondrial Acot7 (Acot7) or an empty vector (C). Insulin secretion was measured after a 30min. stimulation at 3mM glucose (G: Glucose) alone or in the presence of 10mM leucine (Leu) and 10mM glutamine (Gln). Data are from four independent experiments and eight technical replicates (means ± S.E.M). *p<0.05. Matched two-way ANOVA, Bonferroni test. The experiments were performed 24h after cell transfection and the cells were pre-incubated at 3mM glucose during 1h before starting the experiments.

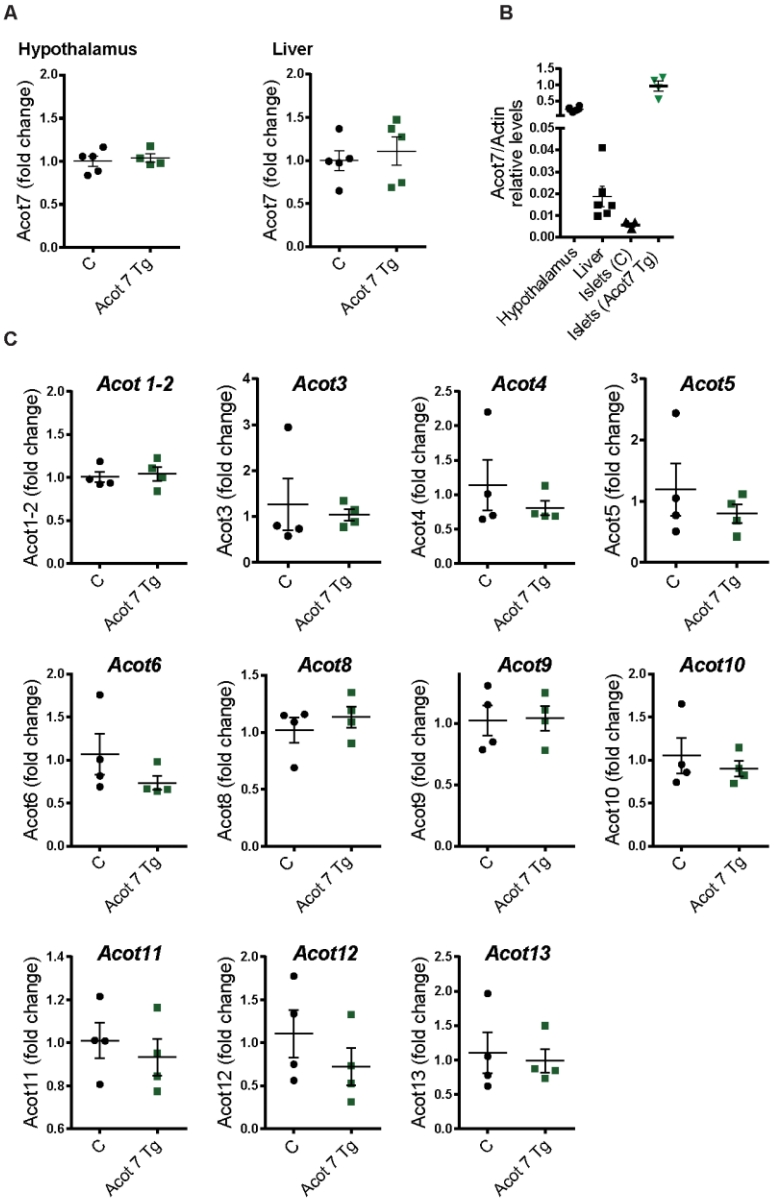
A
Hypothalamus
Acot7 (fold change)
C
Acot 7 Tg
Liver
Acot7 (fold change)
C
Acot 7 Tg
B
Acot7/Actin relative levels
Hypothalamus
Liver
Islets (C)
Islets (Acot7 Tg)
C
Acot 1-2
Acot1-2 (fold change)
Acot3
Acot3 (fold change)
Acot4
Acot4 (fold change)
Acot5
Acot5 (fold change)
Acot6
Acot6 (fold change)
Acot8
Acot8 (fold change)
Acot9
Acot9 (fold change)
Acot10
Acot10 (fold change)
Acot11
Acot11 (fold change)
Acot12
Acot12 (fold change)
Acot13
Acot13 (fold change)

Figure S1.

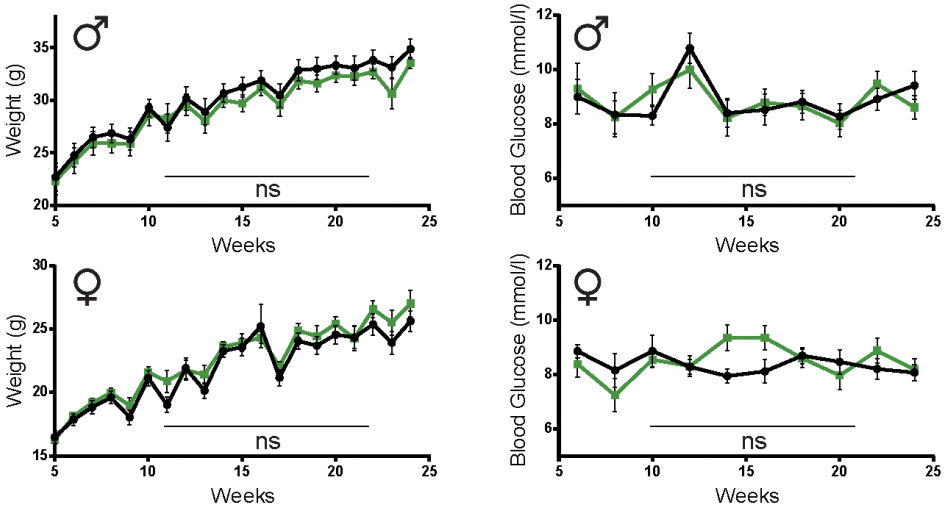
♂
Weight (g)
35
30
25
20
5
10
15
20
25
Weeks
ns
♂
Blood Glucose (mmol/l)
12
10
8
6
5
10
15
20
25
Weeks
ns
♀
Weight (g)
30
25
20
15
5
10
15
20
25
Weeks
ns
♀
Blood Glucose (mmol/l)
12
10
8
6
5
10
15
20
25
Weeks
ns

Figure S2.

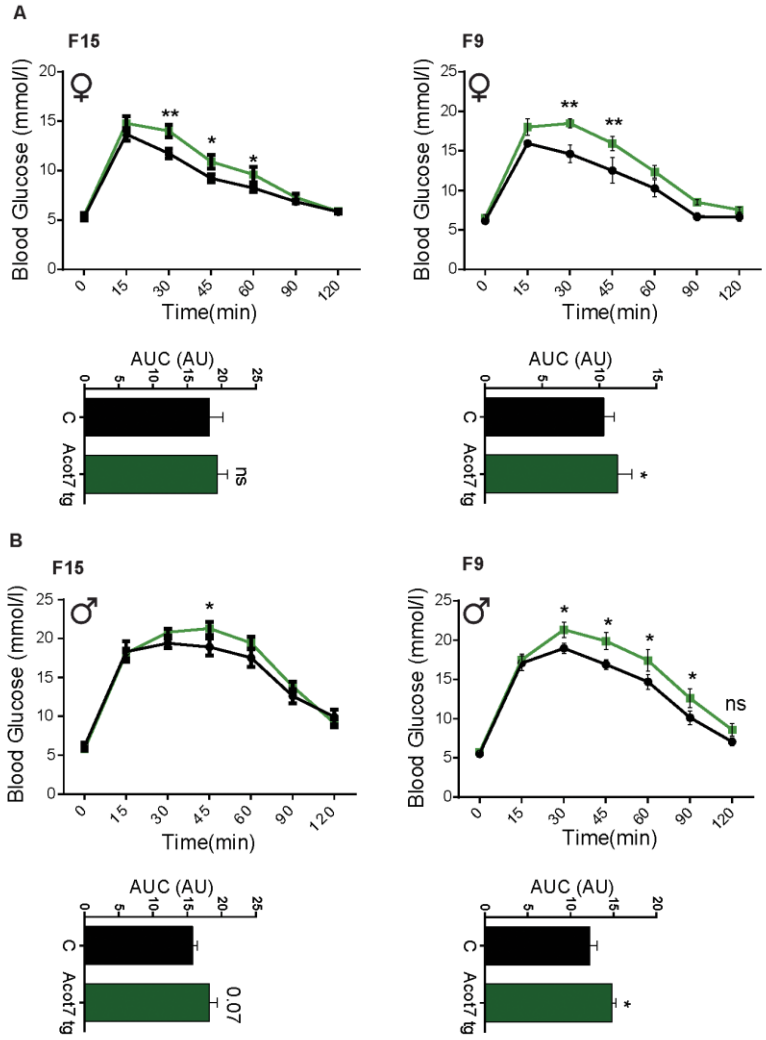

Figure S3.

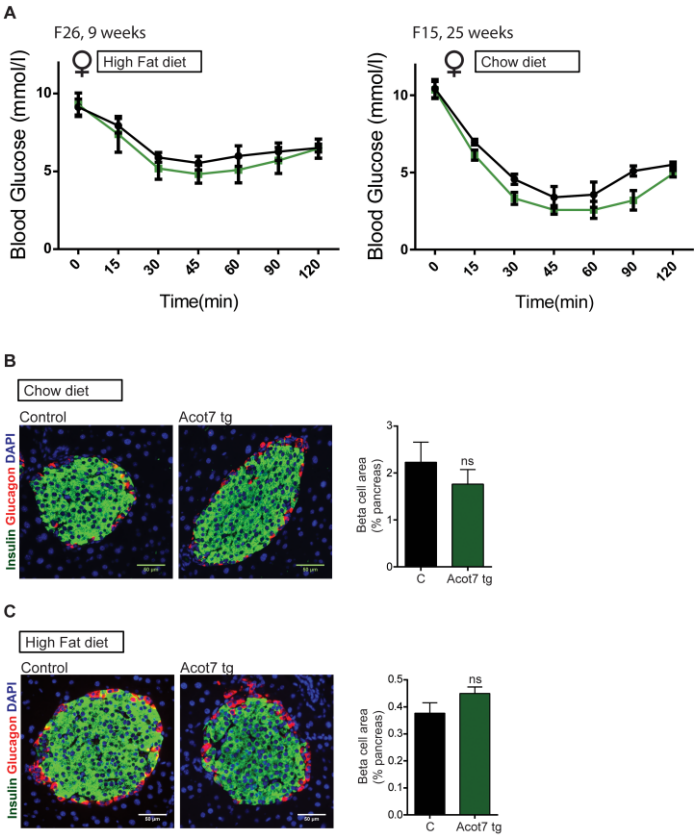
A
F26, 9 weeks
High Fat diet
Blood Glucose (mmol/l)
Time(min)
F15, 25 weeks
Chow diet
Blood Glucose (mmol/l)
Time(min)
B
Chow diet
Control
Acot7 tg
Insulin Glucagon DAPI
Beta cell area (% pancreas)
ns
C
Acot7 tg
C
High Fat diet
Control
Acot7 tg
Insulin Glucagon DAPI
Beta cell area (% pancreas)
ns
C
Acot7 tg

Figure S4.

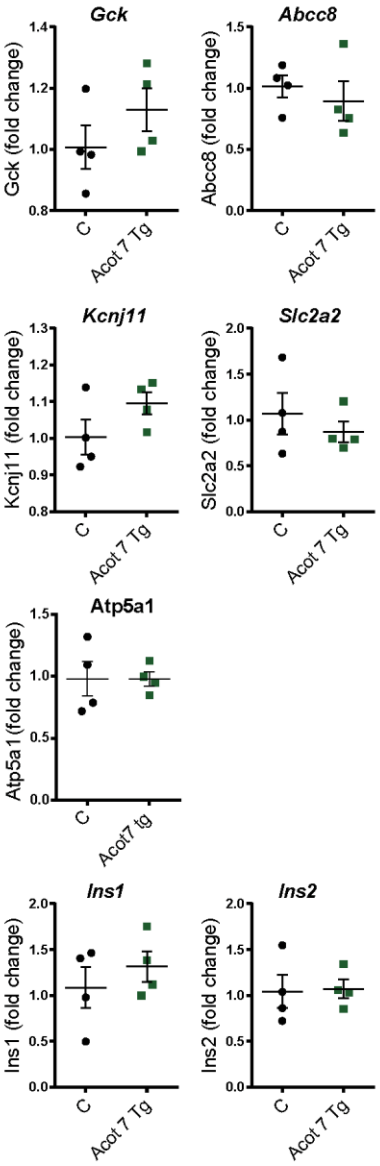
Gck
Gck (fold change)
1.4
1.2
1.0
0.8
C
Acot 7 Tg
Abcc8
Abcc8 (fold change)
1.5
1.0
0.5
0.0
C
Acot 7 Tg
Kcnj11
Kcnj11 (fold change)
1.3
1.2
1.1
1.0
0.9
0.8
C
Acot 7 Tg
Slc2a2
Slc2a2 (fold change)
2.0
1.5
1.0
0.5
0.0
C
Acot 7 Tg
Atp5a1
Atp5a1 (fold change)
1.5
1.0
0.5
0.0
C
Acot7 tg
Ins1
Ins1 (fold change)
2.0
1.5
1.0
0.5
0.0
C
Acot 7 Tg
Ins2
Ins2 (fold change)
2.0
1.5
1.0
0.5
0.0
C
Acot 7 Tg

Figure S5.