Supplementary Table 1. Primers for quantitative real-time PCR

Gene		Sequences of primers (5’ to 3’)		GenBank Accession No.
ACSL1		For: CAGAACATGTGGGTGTCCAG Rev: GTTACCAACATGGGCTGCTT		NM_007981
ACSL3		For: AACCTGTCAGTTCCAAACCG Rev: GCCAATTATAGTGCCCCAGA		NM_028817.2
ACSL4		For: CTGGAAAGCAAACTGAAGGC Rev: AGGGATACGTTCACACTGGC		NM_207625.2
ACSL5		For: GATCGGGGTAAAGGGATCAT Rev: GGCAAGCTCTACTCGTTTGG		NM_027976.2
ACSL6		For: AAAGGCTAAGAGACCGGAGC Rev: GTCAGGAAAGGCTCAGTTGC		NM_144823.4
FATP2		For: ATTCGTGCCAAGTCTCTGCT Rev: GTAAAAGACGGACACGGCAT		NM_011978.2
FATP4		For: TATGGCTTCCCTGGTGTACTAT Rev: TTCTTCCGGATCACCACAGTCA		NM_011989
FATP5		For: GCCTATGCCACACCTCATTT Rev: ACCCGGACAACTTTGTGAAG		NM_009512.1
GPAT1		For: AGCAAGTCCTGCGCTATCAT Rev: CTCGTGTGGGTGATTGTGAC		NM_008149
GPAT2		For: AAGAAAGAGGTACAGCGTATCC Rev: GTGGAGAGCCCTCCTGCACAG		NM_001081089.1
GPAT3		For: CTTTGAAATCGGAGGAACCA Rev: TTTGCAAACTGAACTGCGTC		NM_172715.3
GPAT4		For: GGCATGGTGACGTACCTTCT Rev: GCTTCAGCCAGCACAAGAC		NM_018743.3

Supplementary Table 2. Physiological measurements and liver and plasma metabolites in male 12-14 wk old *Acsl1*$^{L-/-}$ and control mice

	14% fat diet	
	Control (n=11)	KO (n=11)
Physiologic parameters		
Body weight (g)	28.7 ± 0.4	28.6 ± 0.5
Liver weight (g)	1.35 ± 0.03	1.33 ± 0.03
Liver/body weight (%)	4.67 ± 0.07	4.61 ± 0.11
Inguinal adipose (g)	0.37 ± 0.04	0.29 ± 0.02
Inguinal adipose/body weight (%)	1.27 ± 0.13	1.01 ± 0.05
Gonadal adipose (g)	0.45 ± 0.04	0.38 ± 0.03
Gonadal adipose/body weight (%)	1.53 ± 0.14	1.31 ± 0.09
Brown adipose (g)	0.06 ± 0.004	0.06 ± 0.003
Brown adipose/body weight (%)	0.22 ± 0.01	0.21 ± 0.01
Plasma metabolites		
Glucose (mg/dl)	161 ± 9	171 ± 14
NEFA (mEq/l)	0.49 ± 0.10	0.44 ± 0.09
Insulin (ng/ml)	1.55 ± 0.17	1.32 ± 0.17
TAG (mg/dl)	61 ± 7	53 ± 5
Total cholesterol (mg/dl)	73 ± 2	72 ± 3
β-hydroxybutyrate (mM)	0.12 ± 0.01	0.14 ± 0.02
Liver metabolites		
TAG (mg/g liver)	6.8 ± 0.7	6.1 ± 0.6
Total cholesterol (mg/g liver)	2.5 ± 0.1	2.8 ± 0.2
Glycogen (μmol/g liver)	60 ± 11	67 ± 15

Mice were fasted for 4 h. Data represent means ± SEM. The number of animals in each group is indicated in parentheses. Statistical comparisons were determined by Student's t-test.

Supplementary Table 3. Physiological measurements and liver and plasma metabolites in 15-17 wk old male *Acsl1*$^{L-/-}$ and control mice after a 24 h fast.

	24 h fast	
	Control (n=9)	KO (n=13)
Physiologic parameters		
Initial body weight (g)	31.3 ± 0.7	32.4 ± 0.5
Weight loss during 24 h fast (g)	3.07 ± 0.16	3.04 ± 0.10
Liver weight (g)	1.13 ± 0.02	1.17 ± 0.02
Liver/body weight (%)	4.00 ± 0.10	4.01 ± 0.06
Inguinal adipose (g)	0.25 ± 0.03	0.28 ± 0.03
Inguinal adipose/body weight (%)	0.87 ± 0.09	0.96 ± 0.09
Gonadal adipose (g)	0.40 ± 0.05	0.46 ± 0.06
Gonadal adipose/body weight (%)	1.38 ± 0.17	1.55 ± 0.19
Brown adipose (g)	0.05 ± 0.002	0.05 ± 0.002
Brown adipose/body weight (%)	0.18 ± 0.01	0.18 ± 0.01
Plasma metabolites		
Glucose (mg/dl)	121 ± 13	126 ± 7
NEFA (mEq/l)	0.58 ± 0.07	0.64 ± 0.04
Insulin (ng/ml)	0.19 ± 0.10	0.39 ± 0.11
TAG (mg/dl)	39 ± 4	47 ± 7
Total cholesterol (mg/dl)	55 ± 2	55 ± 2
β-hydroxybutyrate (mM)	0.66 ± 0.09	0.61 ± 0.06
Liver metabolites		
TAG (mg/g liver)	15.6 ± 1.7	15.3 ± 1.8
Total cholesterol (mg/g liver)	7.2 ± 0.5	6.8 ± 0.3
Glycogen (μmol/g liver)	0.51 ± 0.17	0.72 ± 0.34

Mice were fasted for 24 h. Data represent means ± SEM. The number of animals in each group is indicated in parentheses. Statistical comparisons were determined by Student's t-test.

Supplementary Table 4. Physiologic measurements and plasma metabolites in 22-26 week old male *Acsl1*$^{L-/-}$ and control mice fed a high-fat or a control diet for 14-18 weeks

	10% fat diet (control)		45% fat diet (high fat)	
	Control (n=11)	KO (n=12)	Control (n=13)	KO (n=11)
Physiologic parameters[1]				
Body weight (g)	30.7 ± 0.7	29.8 ± 0.6	37.2 ± 1.6 ‡	39.3 ± 1.7 ‡
Liver weight (g)	1.20 ± 0.05	1.20 ± 0.05	1.27 ± 0.06	1.28 ± 0.08
Liver/body weight (%)	3.92 ± 0.15	4.04 ± 0.16	3.35 ± 0.12 ‡	3.27 ± 0.12 §
Inguinal adipose (g)	0.46 ± 0.03	0.39 ± 0.03	1.29 ± 0.18 ‡	1.48 ± 0.19 ‡
Inguinal adipose/body wt %	1.50 ± 0.07	1.32 ± 0.10	3.11 ± 0.36 §	3.62 ± 0.37 §
Gonadal adipose (g)	0.55 ± 0.03	0.49 ± 0.04	1.43 ± 0.16 ‡	1.75 ± 0.21 ‡
Gonadal adipose/body wt (%)	1.79 ± 0.10	1.64 ± 0.11	3.67 ± 0.37 §	4.36 ± 0.43 §
Brown adipose (g)	0.10 ± 0.01	0.09 ± 0.01	0.14 ± 0.02 †	0.15 ± 0.02 †
Brown adipose/body wt (%)	0.31 ± 0.01	0.31 ± 0.02	0.36 ± 0.03	0.36 ± 0.03
Total fat (% of body wt)	11.7 ± 0.9	11.3 ± 0.8	26.4 ± 2.1 §	24.9 ± 1.9 §
Lean mass (% of body wt)	79.6 ± 0.9	79.4 ± 0.9	67.1 ± 1.7 §	68.0 ± 1.6 §
Plasma metabolites[1]				
Glucose (mg/dl)	179 ± 9	166 ± 8	176 ± 10	168 ± 6
NEFA (mEq/l)	0.33 ± 0.03	0.29 ± 0.03	0.35 ± 0.03	0.42 ± 0.05 †
Insulin (ng/ml)	1.03 ± 0.31	0.58 ± 0.11	1.73 ± 0.35	2.73 ± 0.57 ‡
TAG (mg/dl)	27 ± 2	30 ± 2	29 ± 3	26 ± 2
Total cholesterol (mg/dl)	79 ± 3	78 ± 3	99 ± 4 ‡	112 ± 5 ‡*
β-hydroxybutyrate (mM)	0.10 ± 0.01	0.11 ± 0.01	0.14 ± 0.04	0.20 ± 0.04
Liver metabolites[1]				
TAG (mg/g liver)	29.9 ± 3.3	22.4 ± 2.2	41.2 ± 7.8	53.5 ± 4.7 †
Total cholesterol (mg/g liver)	8.2 ± 0.7	7.2 ± 0.7	6.7 ± 1.1	8.5 ± 0.7
Glycogen (μmol/g liver)	92 ± 24	103 ± 41	44 ± 8 †	40 ± 11 †

† $p<0.05$, ‡ $p<0.01$, § $p<0.001$ compared with 10% fat control diet in the same genotype (Two-way ANOVA)

* $p<0.05$ compared with control mice fed the same diet (Two-way ANOVA)

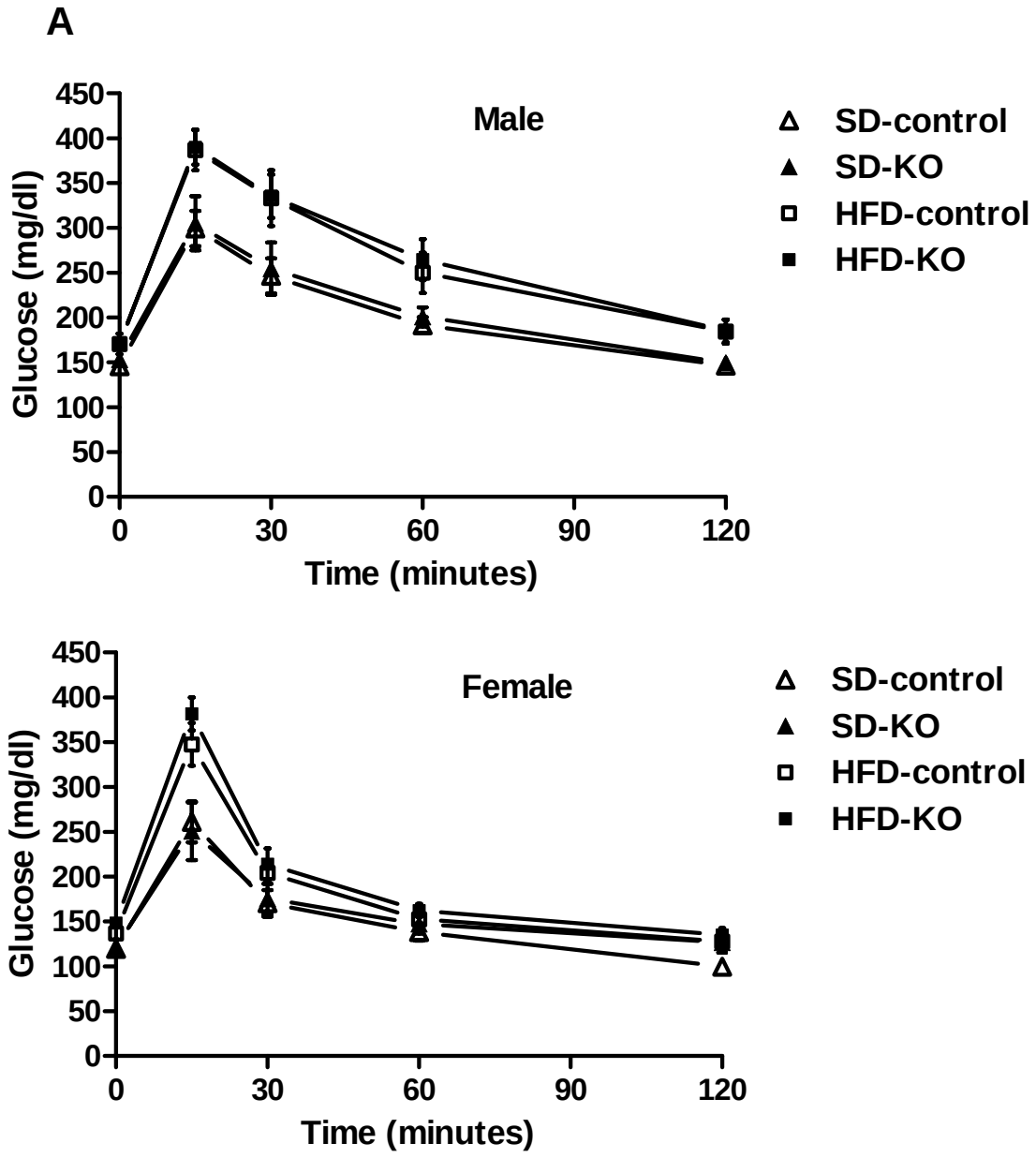
[1] Samples were obtained after a 4 h fast. Data represent means ± SEM. The number of animals in each group is indicated in parentheses.

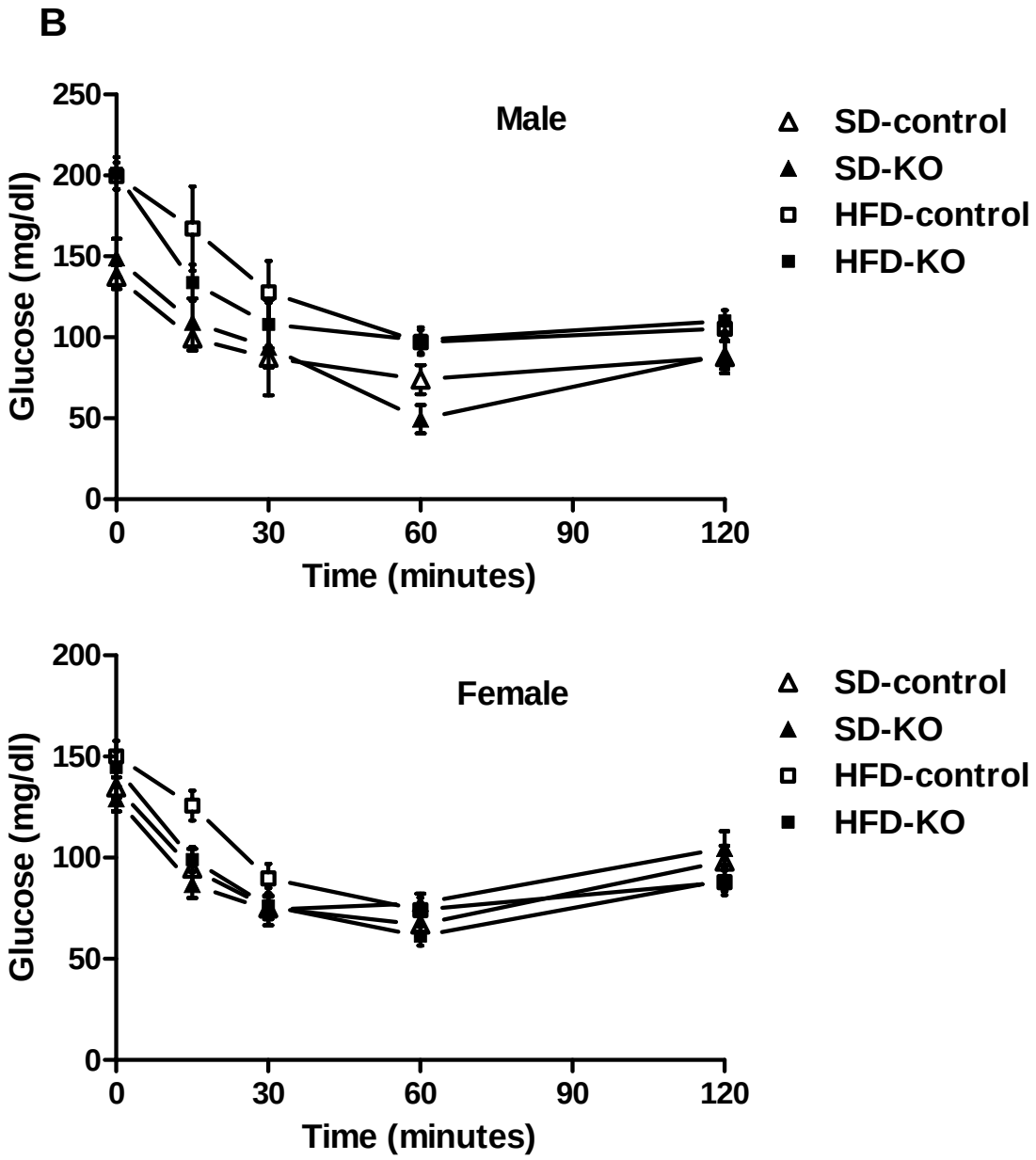
Supplementary Figures

Supplementary Fig. 1. *Acsl1*$^{L-/-}$ mice were not protected from insulin resistance induced by a high-fat diet . OGTT and ITT were performed in control and *Acsl1*$^{L-/-}$ mice after they were fed either a matched 10% fat diet (SD, Research Diets D12450B) or a 45% fat diet (HFD, Research Diets D12451) for 12 -14 weeks. Mice (20 weeks old) were fasted for 4 h before the tests. **A) OGTT in *Acsl1*$^{L-/-}$ mice**. Male and female mice were gavaged with 2.5 g glucose/kg body weight and blood glucose was measured at the indicated time points. Data show means ± SEM from 6-12 mice per group. **B) ITT in *Acsl1*$^{L-/-}$ mice.** Male and female mice (22 weeks old) were given 0.75 U insulin/kg body weight IP and blood glucose was measured at the indicated time points. Data show means ± SEM from 10-14 mice per group.

Supplementary Fig. 2. **Insulin stimulated phosphorylation of AKT in *Acsl1*$^{L-/-}$ and control mice.** 5 mo old male mice fed a standard chow diet (Prolab Isopro®RMH 3000, LabDiet) were fasted overnight (16h), anesthetized, and injected with either phosphate buffered saline (PBS) or insulin (1 U/kg body weight) via the portal vein. Livers were freeze-clamped 2 min later and analyzed for AKT serine473 phosphorylation by Western blot with antibody against phosphorylated AKT and total AKT. Densitometry analysis of phosphorylated AKT immunoblots were normalized to total AKT. Data show means ± SEM from 5-9 mice per group. ** p <0.01 versus PBS treated mice in the same genotype (Two-way ANOVA).

Supplementary Fig. 1





Supplementary Fig. 1, cont.

Supplementary Fig. 2