Supplementary Material

Hepatocyte-specific lysosomal acid lipase deficiency protects mice from diet-induced obesity but promotes hepatic inflammation

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Materials and Methods

Energy metabolism *in vivo*

Weight-matched female control and *Liv-Lipa*$^{-/-}$ mice fed chow and HF/HCD were housed in climate-controlled metabolic cages in a regular light-dark cycle (12 h light, 12 h dark) with free access to food and water. Mice were acclimatized for 48 h before experiments. O_2 consumption, CO_2 production, and locomotor activity (using infrared sensor frames) were measured every 15 min by indirect calorimetry system (TSE PhenoMaster, TSE Systems, Bad Homburg, Germany). Carbohydrate and lipid oxidation rates were determined as described [1] and converted from mg/h into kcal/h. To determine fecal energy content measurement, feces of single housed mice were collected every day for two weeks. The feces were dried and grounded,1 g of feces was pressed into a tablet and burned in an adiabatic oxygen bomb calorimeter C4000 A (IKA Analysentechnik, Stauffen, Germany). Each measurement was performed in duplicate.

VLDL secretion

Female mice were fed a HF/HCD for 8 weeks before the experiment was performed. Twelve hour fasted mice were injected intraperitoneally with 500 mg/kg body weight of tyloxapol in PBS. Blood was drawn from v.facialis every other hour post-injection and plasma was separated by centrifugation at 7,000 rpm for 7 min at 4°C. Triglyceride concentrationswere determined enzymatically, according to manufacturer's instructions (DiaSys, Holzheim, Germany).

Table S1: Primers used for real-time PCR

Gene	Forward Primer	Reverse Primer
Ccl3	TGTACCATGACACTCTGCAAC	CAACGATGAATTGGCGTGGAA
Ccl4	TTCCTGCTGTTTCTCTTACACCT	CTGTCTGCCTCTTTTGGTCAG
Ccl5	GCTGCTTTGCCTACCTCTCC	TCAGTGACAAACACGACTGC
Col3a	GGGGACCAGGGCGACCACT	CAGGTGAACCCGGCAAGAACG
Cyclophilin A	CCATCCAGCCATTCAGTCTT	TTCCAGGATTCATGTGCCAG
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
G6Pase	CGACTCGCTATCTCCAAGTGA	GGGCGTTGTCCAAACAGAAT
Gpdh	CTCGCCATCGCCCTCACTG	ACCGCTCACTCGCTCTTTGC
Il4	TGGATCTGGGAGCATCAAGGT	TGGAAGTGCGGATGTAGTCAG
Il6	TCTATACCACTTCACAAGTCGGA	GAATTGCCATTGCACAACTCTTT
Ckt19	GTTCAGTACGCATTGGGTCAG	GAGGACGAGGTCACGAAGC
Lipa	GCAAAGGTCCCAGACCAGTT	TCATCAAAACTGAAGGCCCAGA
Mcp1	ACTGAAGCCAGCTCTCTCTTCCTC	TTCCTTCTTGGGGTCAGCACGAC
Mcsf1	ATGAGCAGGAGTATTGCCAAGG	TCCATTCCCAATCATGTGGCTA
Mdh	GAACCAATCAGAGTCCTTGTGAC	GGCACAGTCTTGCAGTTCCA
Pcx	AATGTCCGGCGTCTGGAGTA	ACGCACGAAACACTCGGAT
Pdhb	AAGAGGCGTTTTCACCGCTC	GTCACCGTATTTCTTCCACAGG
Pepck	AAGCATTCAACGCCAGGTTC	TGCAGGCACTTGATGAACTC
Pfkl	GGAGGCGAGAACATCAAGCC	GCACTGCCAATAATGGTGCC
Tgfb	CCGCTTTAAGTAGTTCTGTTCGT	AGCCGTGGGGTCCTTTCTGTG
Tnfa	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG

Figure S1

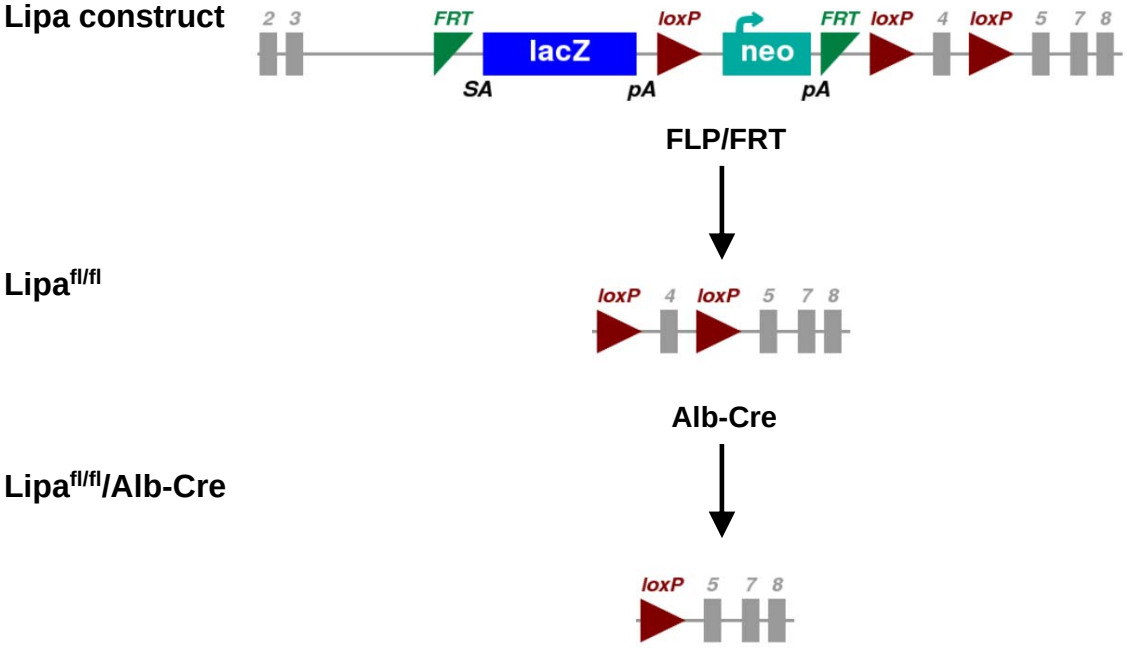
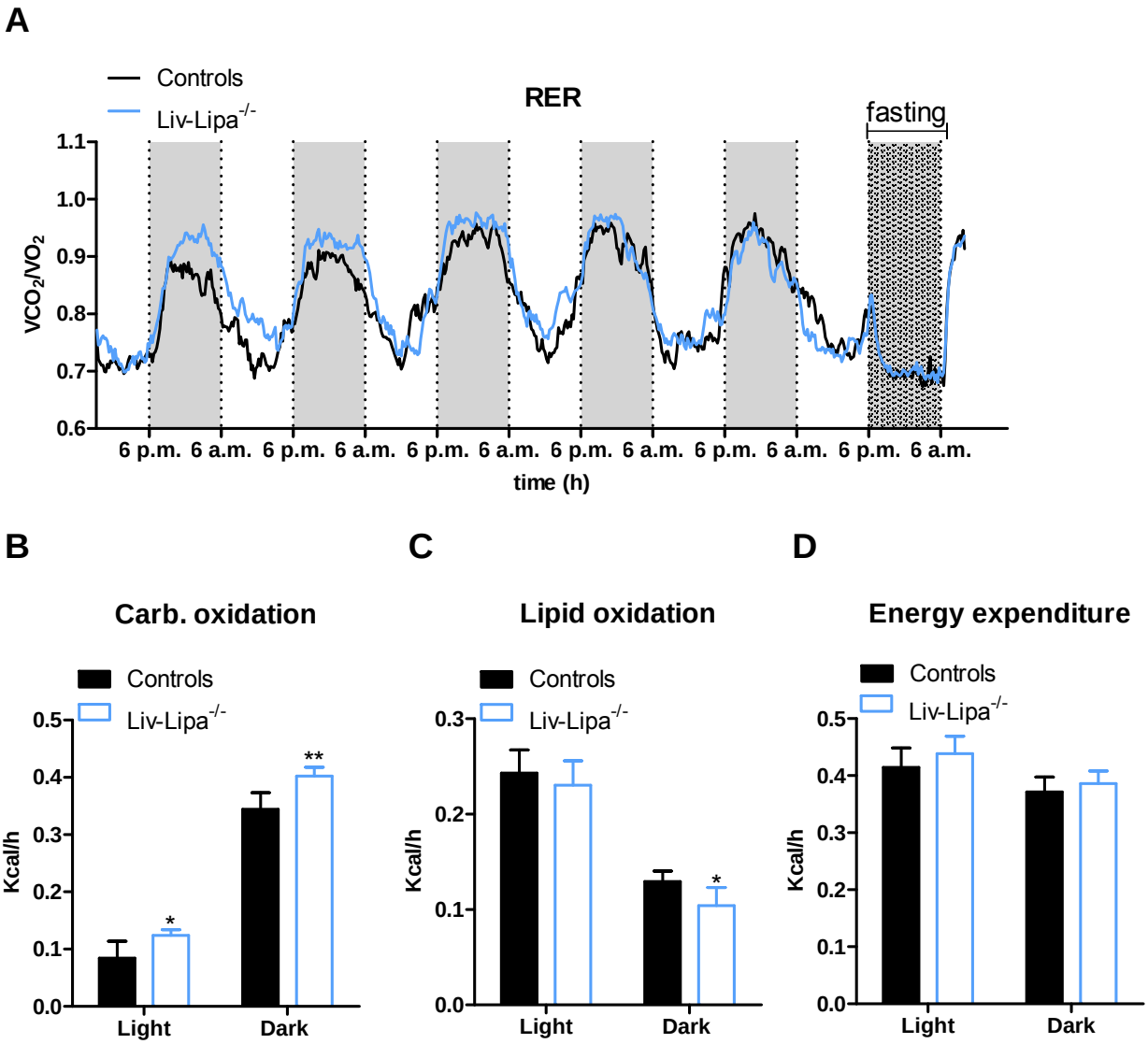


Figure S1: Generation of *Liv-Lipa*$^{-/-}$ mice. Mice carrying the *Lipa*$^{tm1a(EUCOMM)Hmgu}$*/Biat* construct on a C57BL/6N background were generated using an ES cell line from the European Conditional Mouse Mutagenesis Program (EUCOMM). This construct is a so-called targeted trap allele, as it has been generated by targeting yet functions as a gene-trap knock-out. The floxed mouse (*Lipa*$^{fl/fl}$) with a conditional Lipa allele was generated by breeding *Lipa*$^{tm1a(EUCOMM)Hmgu}$*/Biat* mice with mice expressing FLP recombinase. Mice heterozygous for the floxed allele were bred together to obtain homozygous *Lipa*$^{fl/fl}$ mice that served as controls. The hepatocyte-specific null allele was generated by crossing *Lal*$^{fl/fl}$ mice with mice expressing Cre recombinase under the control of the albumin promoter to generate *Liv-Lipa*$^{+/-}$ mice. Mice containing the hepatocyte-specific deletion were then bred homozygously to produce *Liv-Lipa*$^{-/-}$ mice. The schematic illustration is a copy from the website of EUCOMM.

Figure S2


A
Controls
Liv-Lipa-/-
RER
fasting
1.1
1.0
0.9
0.8
0.7
0.6
VCO2/VO2
6 p.m. 6 a.m. 6 p.m. 6 a.m. 6 p.m. 6 a.m. 6 p.m. 6 a.m. 6 p.m. 6 a.m. 6 p.m. 6 a.m.
time (h)
B
Carb. oxidation
Controls
Liv-Lipa-/-
0.5
0.4
0.3
0.2
0.1
0.0
Kcal/h
*
**
Light
Dark
C
Lipid oxidation
Controls
Liv-Lipa-/-
0.3
0.2
0.1
0.0
Kcal/h
*
Light
Dark
D
Energy expenditure
Controls
Liv-Lipa-/-
0.5
0.4
0.3
0.2
0.1
0.0
Kcal/h
Light
Dark

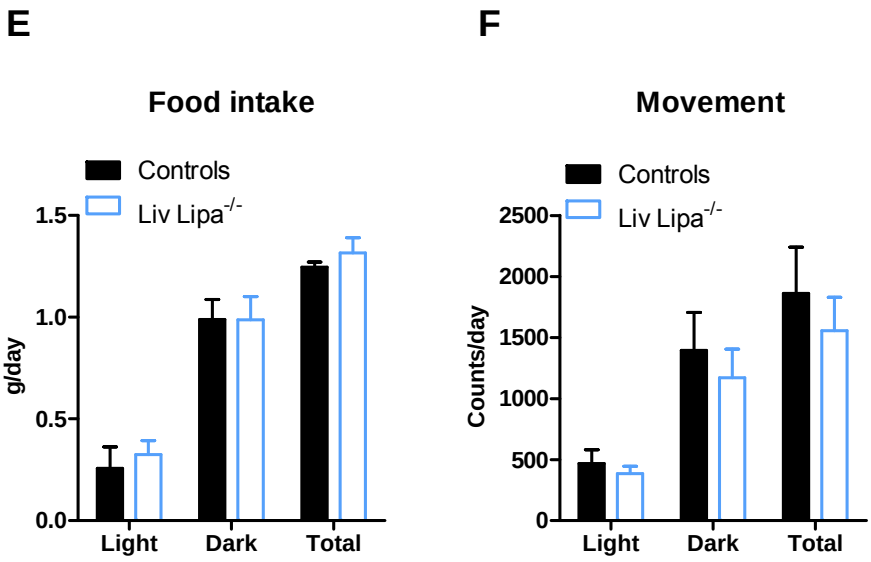


Figure S2: Increased carbohydrate oxidation in *Liv-Lipa*$^{-/-}$ mice. Female control and *Liv-Lipa*$^{-/-}$ mice were housed at room temperature in metabolic cages with free access to chow diet and water. (**A**) Respiratory exchange ratio (RER), (**B**) carbohydrate oxidation, (**C**) lipid oxidation, and (**D**) energy expenditure were measured by indirect gas calorimetry. (**E**) Food intake and (**F**) daily locomotor activity (n=4-7). Gray-shaded areas represent dark phases (6 p.m. - 6 a.m.); non-shaded light phases (6 a.m. - 6 p.m.). Data represent mean + SD; $p < 0.05$ (*), $p \leq 0.01$ (**); (**B-F**) Student's unpaired t-test.

Figure S3

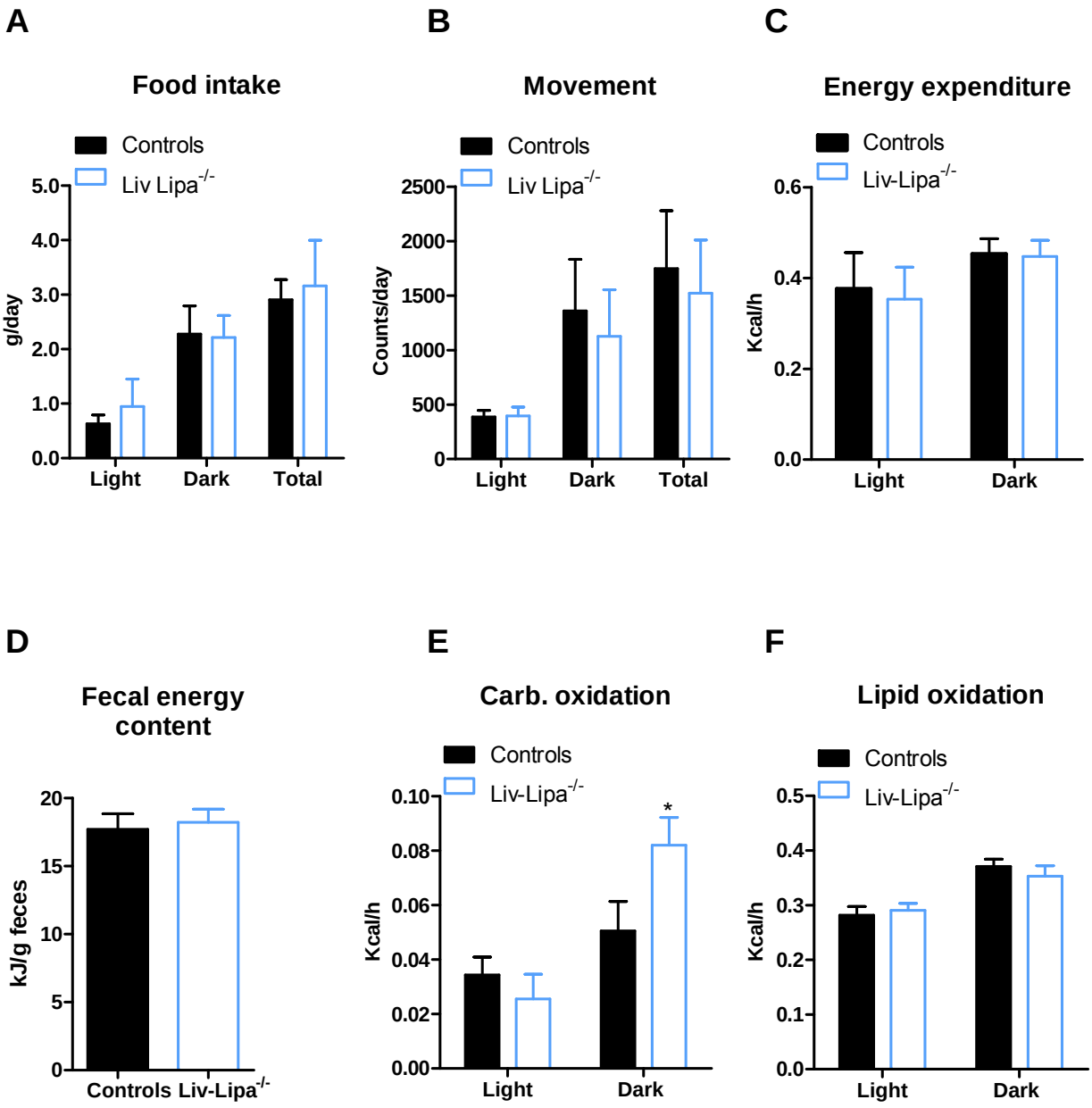


Figure S3: Comparable metabolic parameters in control and *Liv-Lipa*$^{-/-}$ mice fed HF/HCD. After 4 weeks on HF/HCD, female control and *Liv-Lipa*$^{-/-}$ mice were housed in metabolic cages with free access to diet and water (n=4-6) (**A**) Food intake, (**B**) daily locomotor activity, (**C**) and energy expenditure were measured by indirect gas calorimetry. (**D**) Fecal energy content was measured using an adiabatic oxygen bomb calorimeter (n=5-7). Quantification of (**E**) carbohydrate oxidation, and (**F**) lipid oxidation. Data represent mean + SD; $p < 0.05$ (*). (**A-F**) Student's unpaired t-test.

Figure S4

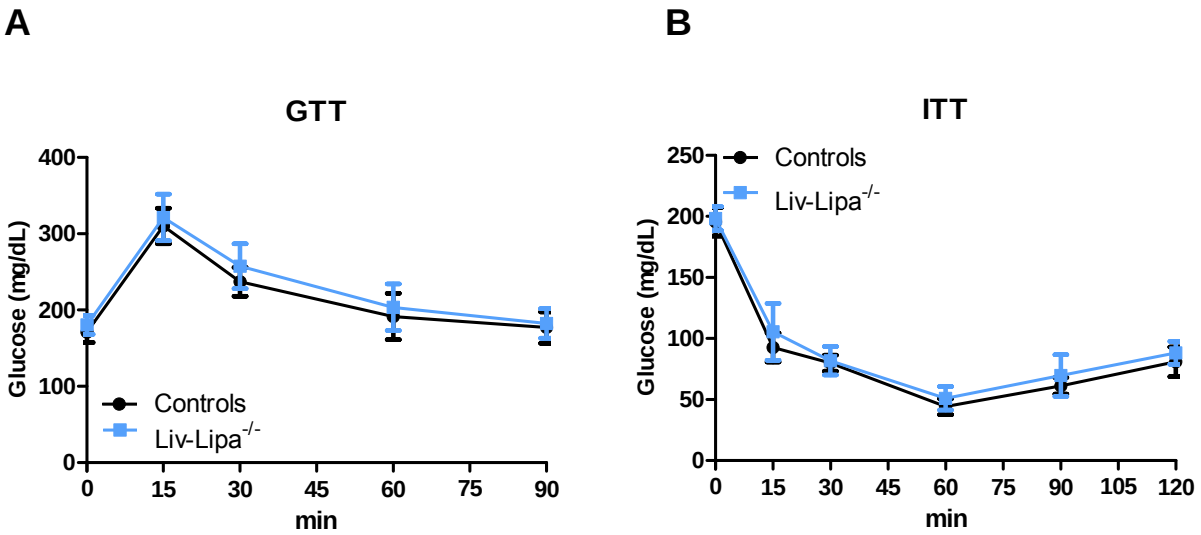


Figure S4: Comparable glucose clearance in chow diet-fed *Liv-Lipa*$^{-/-}$ mice. Plasma glucose concentrations in (**A**) 6 h-fasted (n=4-5) and (**B**) 4 h-fasted male control and *Liv-Lipa*$^{-/-}$ mice (n=5) after i.p. injection of (**A**) glucose (2 g/kg) and (**B**) insulin (0.25 U/kg), respectively. Data represent mean + SD. (**A, B**) ANOVA.

Figure S5

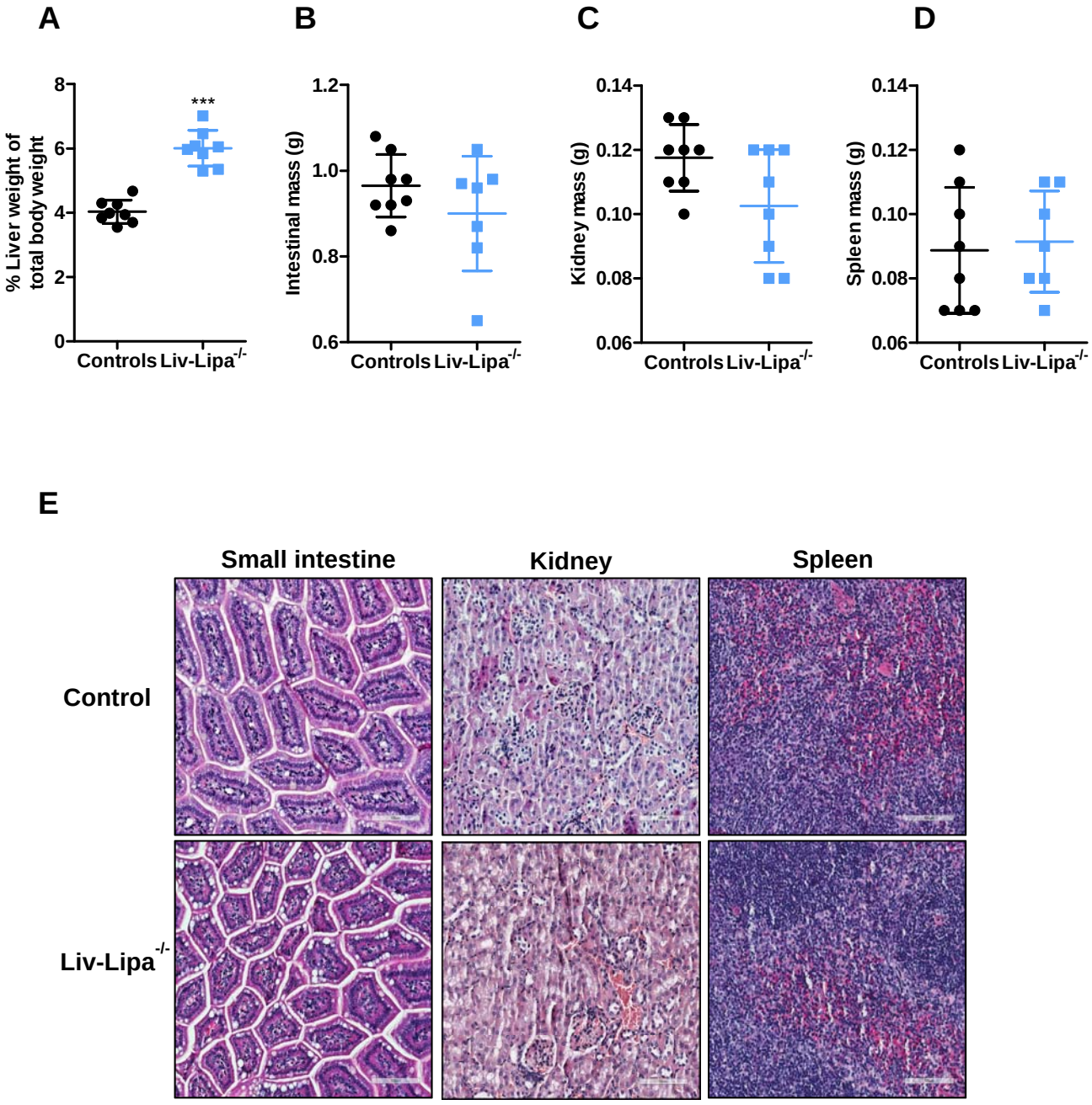


Figure S5: Tissue weights and morphology in HF/HCD-fed *Liv-Lipa*$^{-/-}$ and control mice. (**A**) Liver weight relative to body weight (n=8). (**B**) Total intestine, (**C**) kidney, and (**D**) spleen weight of female control and *Liv-Lipa*$^{-/-}$ mice fed HF/HCD for 10 weeks. (**E**) Representative H&E stainings of small intestine (jejunum), kidney, and spleen sections from 10 week HF/HCD-fed male control and *Liv-Lipa*$^{-/-}$ mice (scale bar, 100 µm). Data represent mean ± SD (n=8); p ≤ 0.001 (***). (**A-C**) Student's unpaired t-test.

Figure S6

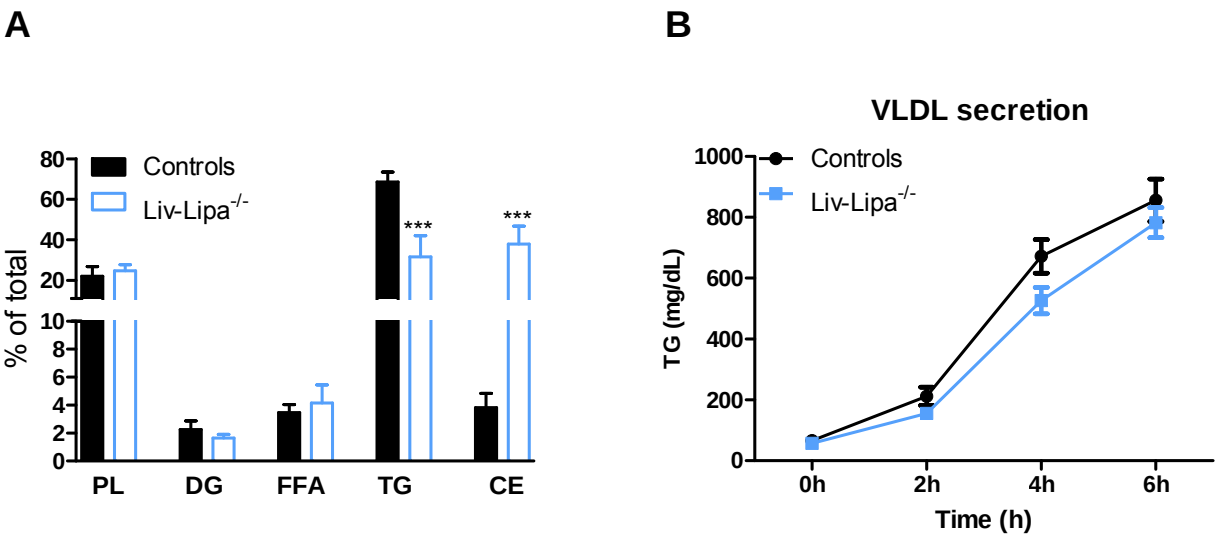


Figure S6: (**A**) Percentage distribution of lipid species in control and *Liv-Lipa*$^{-/-}$ mice measured after TLC separation by GC. (**B**) Female control and *Liv-Lipa*$^{-/-}$ mice were fed HF/HCD for 8 weeks prior to the experiment. VLDL secretion was measured in 8 h-fasted mice after tyloxapol injection. Data represent mean + SD (n=8); $p \leq 0.001$ (***). (**A**) Student's unpaired t-test (**B**) ANOVA.

References

[1] E. Ferrannini, The theoretical bases of indirect calorimetry: a review, Metabolism. 37 (1988) 287-301.