

## Supplementary data

**Table S1.** Oligonucleotides used for qPCR analysis.

Gene Symbol	Accession No.	Primer Sequence Forward (5'-3') Reverse (5'-3')	Optimized qPCR Conditions (Annealing Temp/MgCl)
<b>Cytokines</b>			
<i>Ccr2</i>	NM_009915.2	GGTCTGGTTGGGTTGTAAA GTCTTTGAGGCTTGTGCTATG	59°C; 3 mM
<i>Cd68</i>	NM_001291058.1	CTCTTGCTGCCTCTCATCATT CTGGTAGGTTGATTGTCGTCTG	58 °C; 2,5 mM
<i>Cxcl1</i>	NM_008176.3	GTGTCAACCACTGTGCTAGT CACACATGTCCTCACCTAATAC	61 °C; 3.5 mM
<i>Cxcr7</i>	NM_001271607.1	GACCATGTAGGCCTCAGATTAG CAGCCGAGACTGGCATAAA	63 °C; 3.5 mM
<i>Il-1α</i>	NM_010554.4	CCTTACACCTACCAGAGTGATTT CCTTACACCTACCAGAGTGATTT	65 °C; 3 mM
<i>Il-1β</i>	NM_008361.4	ATGGGCAACCACTTACCTATTT GTTCTAGAGAGTGCTGCCTAATG	64 °C; 3 mM
<i>Il-6</i>	NM_031168.2	GATAAGCTGGAGTCACAGAAGG TTGCCGAGTAGATCTCAAAGTG	59 °C; 3.5 mM
<i>Il-14</i>	NM_001005506.3	CCTCACTTCAGCTACCTCTTAAA CTACAAGTGGATGGAGGGAAAAG	61 °C; 3.5 mM
<i>Mcp1</i>	NM_011333.3	CCTGGATCGGAACCAAATGA CGGGTCAACTTCACATTCAAAG	62 °C; 3 mM
<i>Tgfa</i>	NM_031199.4	CTTTAGGAAGGACCTGGGTTG GTGTGTCCAGGCTCCAAATA	66 °C; 3 mM
<i>Tnfa</i>	NM_013693.3	GTCTCAGAATGAGGCTGGATAAG CATTGCACCTCAGGGAAGAA	63 °C; 2.5 mM
<b>Fatty Acid Metabolism</b>			
<i>Cgl58</i>	NM_026179.2	ATGCTGTGGAATGAGGACATAG CATAGTGAGTGGCTGGTGAAA	59°C; 2,5mM
<i>Chreb</i>	NM_021455.5	CAGTCTCGGGATGAAATAGA CAAAGCGCTGATGTGTGATG	61°C; 2,5mM
<i>Cpt1α</i>	NM_013495.2	TCGAAACCCAGTGCCTTAAC AAGCAGCACCTCACATATC	58°C 2,5 mM
<i>Cyp21</i>	NM_009995.2	CTGGGTCGGAGCTTCATTT GTCTTGACTCTCTCCCTTGAC	59°C; 3,5mM
<i>Dgat1</i>	NM_010046.3	CCAACCATCTGATCTGGCTTAT GACTCAGCATTCACCAATCT	65°C; 3mM
<i>Glyk</i>	BC003767.1	GCACTAGAAGCTGTTTGTITCC GCTGGTCATTCCCTCATCTAC	58°C; 2,5 mM
<i>Hmgcs2</i>	NM_008256.4	CCTGTGAAGAGGGAGATGAAAG GCCCACAGTCTGAGAATAAGC	64°C; 3 mM
<i>Fabp1</i>	NM_017399.5	AAGTCAAGGCAGTCGTCAAG TGGTATTGGTGATTGTGTCTCC	59°C; 3,5mM
<i>Fitm2</i>	NM_173397.4	GACAGGAGGACAATGGCTAAT CCACACCAAAGGTACCTAGTAAG	56°C; 2,5mM
<i>Irs1</i>	NM_010570.4	GTCAGGGACACTCTGACTAAC TGCCAAGGAAAGACAGGATAAA	61°C; 2,5mM
<i>Irs2</i>	NM_001081212.2	CTGCTGCTCACTTTCCTATCA CCTGCCTCTTGGTTCCTTATC	61°C; 2,5mM
<i>Pgc1α</i>	NM_001127330.2	GCCTAAGTTTGAGTTTGCTGTG GCGGTCTCCACTGAGAATAATG	58°C 2,5 mM
<i>Ppara</i>	NM_011144.6	GCTCGTACAGGTCATCAAGAAG CTGCCATCTCAGGAAAGATCAG	59°C 2,5 mM

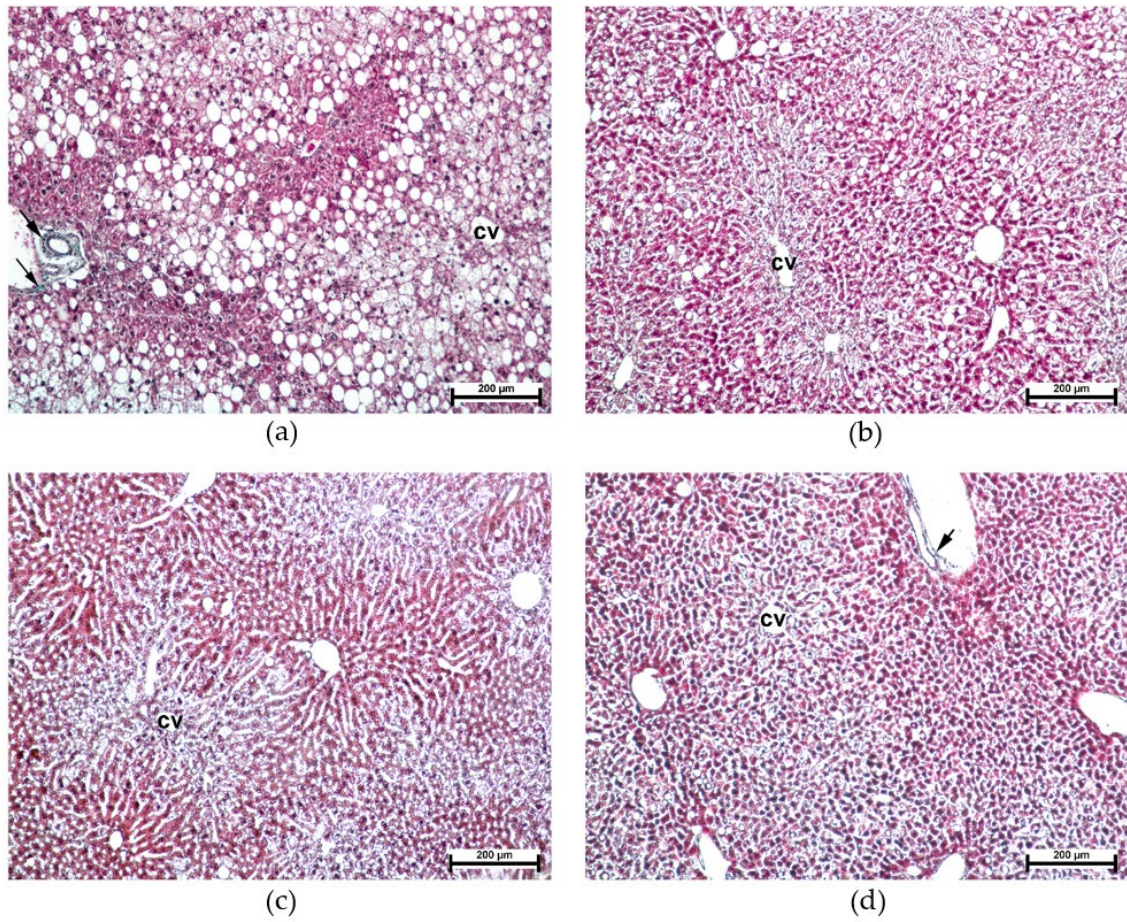
<i>Ppar<math>\gamma</math></i>	NM_0011273 30.2	GCCTAAGTTTGAGTTTGCTGTG GCGGTCTCCACTGAGAATAATG	59°C 2,5 mM
<i>Srebp1</i>	NM_011480. 4	AGCCCTCCACCAGGTAATAA GGGTTCCCAGTCTACTACTAA	61°C; 2,5mM
<b>Reference (housekeeping) genes</b>			
<i>Act<math>\beta</math></i>	NM_007393. 5	GCAAGCAGGAGTACGATGAG CCATGCCAATGTTGTCTCTT	61 °C; 3,5mM
$\beta$ 2m	NM_009735. 3	CCTGCAGAGTTAAGCATGACAGT TCATGATGCTTGATCACATGTCT	60 °C; 3 mM

*Ccr2*- chemokine (C-C motif) receptor 2; *Cd68*- mouse CD68 antigen; *Cgl58*- abhydrolase domain containing 5; *Chreb*- carbohydrate response element binding protein; *Cpt1 $\alpha$* - carnitine palmitoyltransferase I; *Cxcl1*- C-X-C motif chemokine ligand 1; *Cxcr7*- atypical chemokine receptor 3; *Cyp21*- cytochrome P450, family 21, subfamily a, polypeptide 1; *Dgat1*- diacylglycerol O-acyltransferase 1; *Fabp1*-fatty acid binding protein 1; *Fitm2*- fat storage-inducing transmembrane protein 2; *Glyk*- glycerol kinase; *Hgmcs2*- hydroxymethylglutaryl-CoA synthase; *Il-1 $\alpha$* - interleukin 1 alpha; *Il-1 $\beta$* - interleukin 1 beta; *Il-6*- interleukin 6; *Il-14*- interleukin 14; *Irs1*- insulin receptor substrate 1; *Irs2*- insulin receptor substrate 2; *Mcp1* - monocyte chemoattractant protein-1; *Srebp1*- sterol regulatory element binding transcription factor 1; *Tgf $\beta$* - tumor growth factor beta; *Tnfa*- tumor necrosis factor alpha; *Pgc1 $\alpha$* - Ppar $\gamma$  coactivator; *Ppar $\alpha$*  - peroxisome proliferator activated receptor alpha VI; *Ppar  $\gamma$* -peroxisome proliferator activated receptor gamma; *Act $\beta$*  – actin beta;  $\beta$ 2m – beta 2 microglobulin

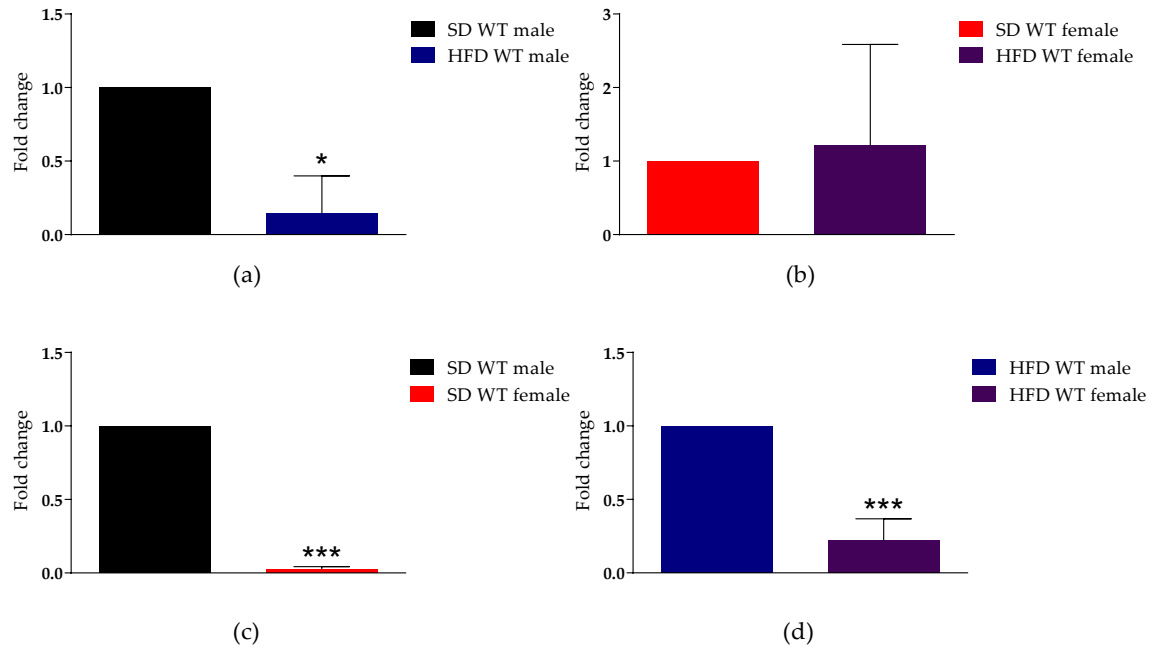
**Table S2.** Fatty acid (average) content of abdominal fat in mice on high fat diet.

Main fatty acids (g of fatty acids/100 g of total fatty acids)	Groups			
	WT $\sigma$	Tff3-/- $\sigma$	WT $\eta$	Tff3-/- $\eta$
C 14:0	0.77 *	0.78 †	0.99	1.10
C 16:0	19.01 *	18.45 †	20.06	21.44
C 16:1	5.85	5.90	6.33	5.85
C 18:0	3.76	3.20 †	4.17	4.63
C 18:1	54.77	54.97 †	52.17	50.02
C 18:2, n-6	13.19	14.06	13.12	13.61
C 18:3, n-3	0.48	0.48 †	0.59	0.64
C 20:1, n-9	0.51	0.53	0.49	0.53
C 20:2, n-6	0.18 *	0.19 †	0.25	0.27
C 20:4, n-6	0.25 *	0.21 †	0.36	0.37
C 22:6, n-3	0.09 *	0.08 †	0.17	0.18
$\Sigma$ SFA <sup>1</sup>	24.03	22.87 †	25.72	27.68
$\Sigma$ MUFA <sup>2</sup>	61.45	61.74 †	59.33	56.73
$\Sigma$ PUFA <sup>3</sup>	14.47 ‡	15.33	14.89	15.50
n-6/n-3 PUFA <sup>4</sup>	21.30 : 1 *	22.56 : 1 †	15.88 : 1	15.09 : 1

<sup>1</sup> Saturated fatty acids. <sup>2</sup> Monounsaturated fatty acids. <sup>3</sup> Polyunsaturated fatty acids. <sup>4</sup> Ratio of omega -6 and omega -3 polyunsaturated fatty acids. Results are presented as mean and were analysed using general linear models (GLM) procedures of the SAS/STAT module (SAS Institute Inc., Cary, NC, USA), the differences being determined by a Tukey–Kramer multiple comparison test, taking into consideration the genotype as the main effect, separately for male and female mice. Statistical significance was considered at  $p < 0.05$ . \*—WT  $\sigma$  vs WT  $\eta$  (sex related diff.); † - Tff3-/-  $\sigma$  vs Tff3-/-  $\eta$  (sex related diff.); ‡ - WT  $\sigma$  vs Tff3-/-  $\sigma$  (gene related diff.).

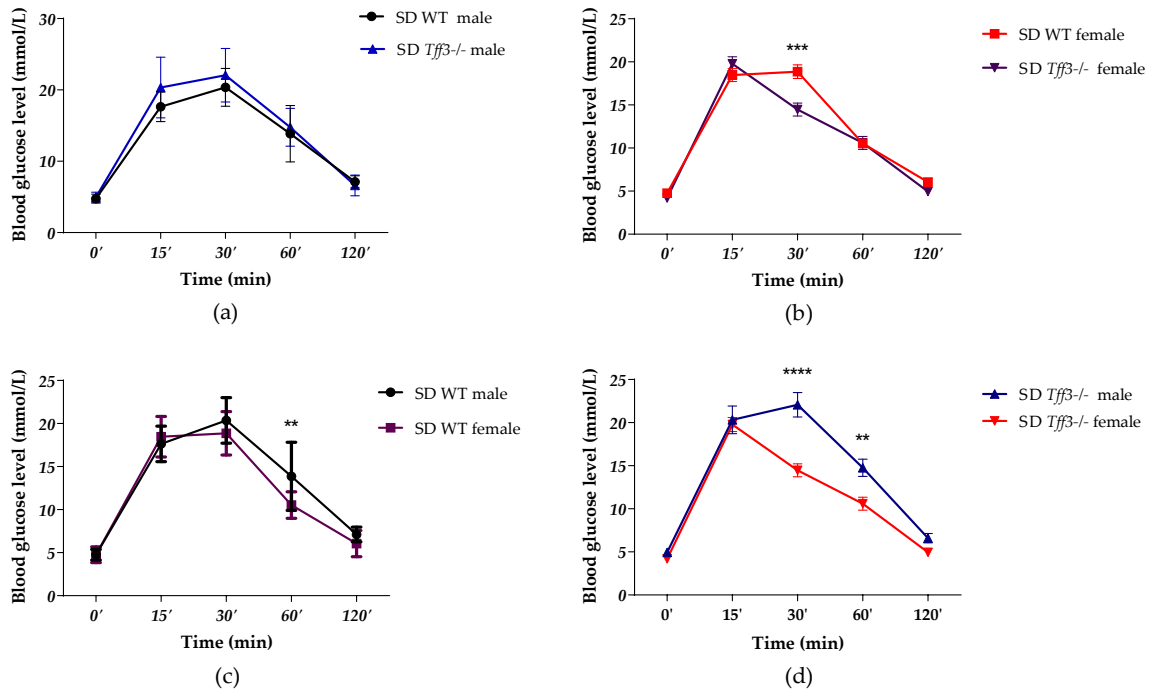


**Figure S1. Masson-Goldner staining of livers from male and female WT and *Tff3*<sup>-/-</sup> mice on HFD, (a) WT male (b) WT female (c) *Tff3*<sup>-/-</sup> male (d) *Tff3*<sup>-/-</sup> female. Collagen fibers (green; arrows); cv, central vein. Scale bars 200 μm.**

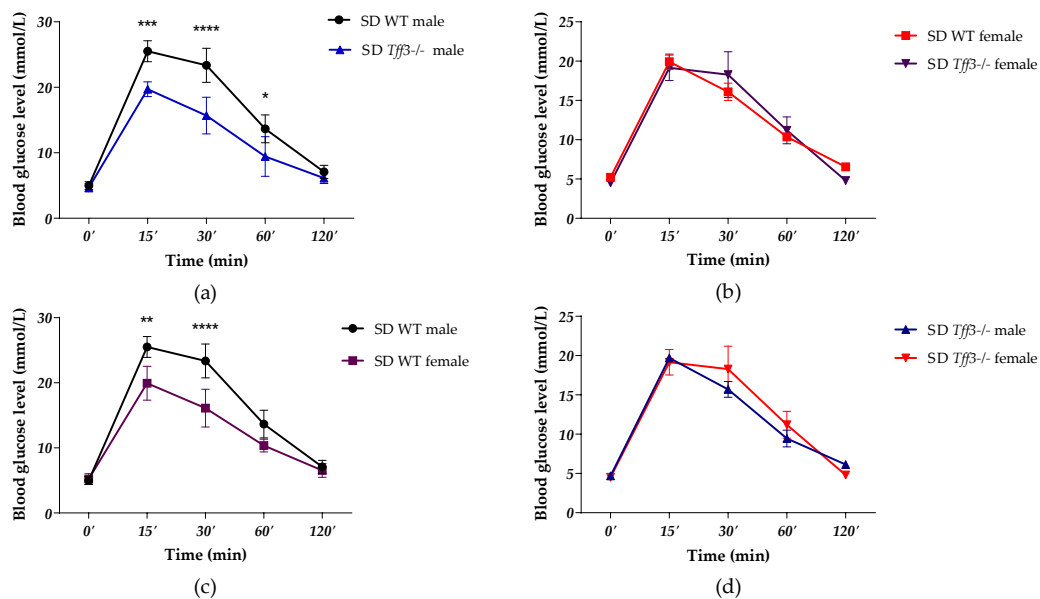


**Figure S2. Effect of long-term treatment with HFD and effect of gender on the expression of *Tff3* in the liver tissue of WT mice.** We performed qPCR for all animal groups ( $n = 5$ ) using the SYBR green detection system. Ct values were analyzed using REST© software and results are expressed as fold change and SEM. *Tff3* gene expression of WT male fed with long term HFD compared to age matched (9-month-old) WT male fed with SD (a) WT female HFD compared to WT female SD (b) WT female fed with SD compared to WT male SD (c) and WT female fed with HFD compared to WT male fed with HFD (d). Statistically significant time points marked as \*  $p \leq 0.05$ ; \*\*\*  $p \leq 0.001$ .

(A) GTT 21-week-old Standard diet

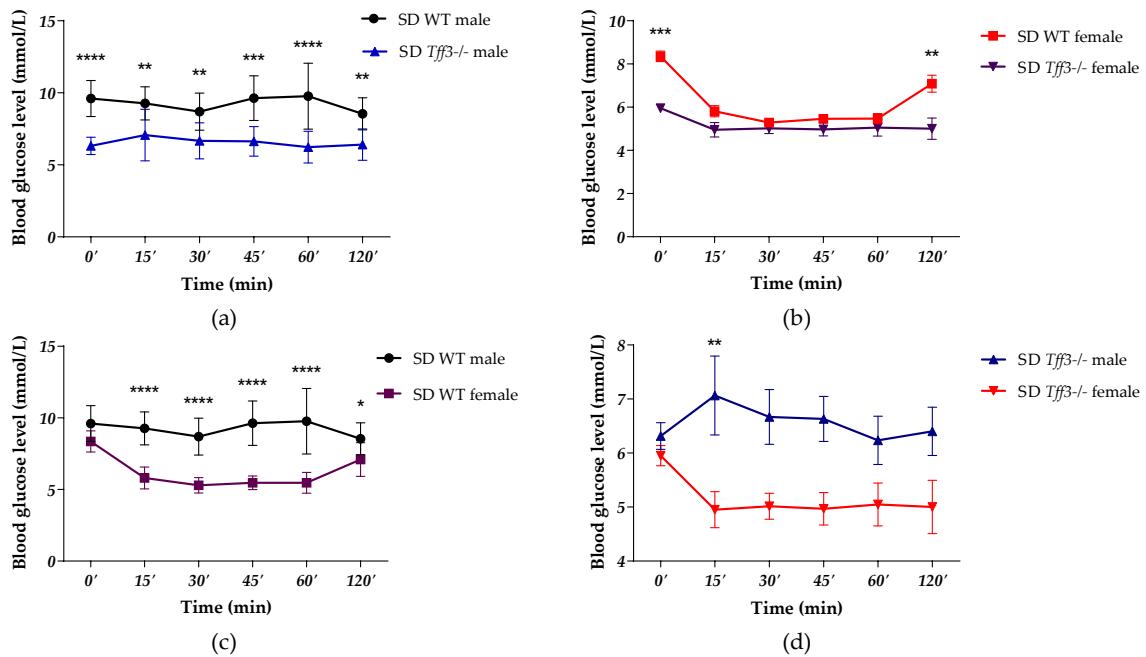


(B) 36 weeks old Standard diet

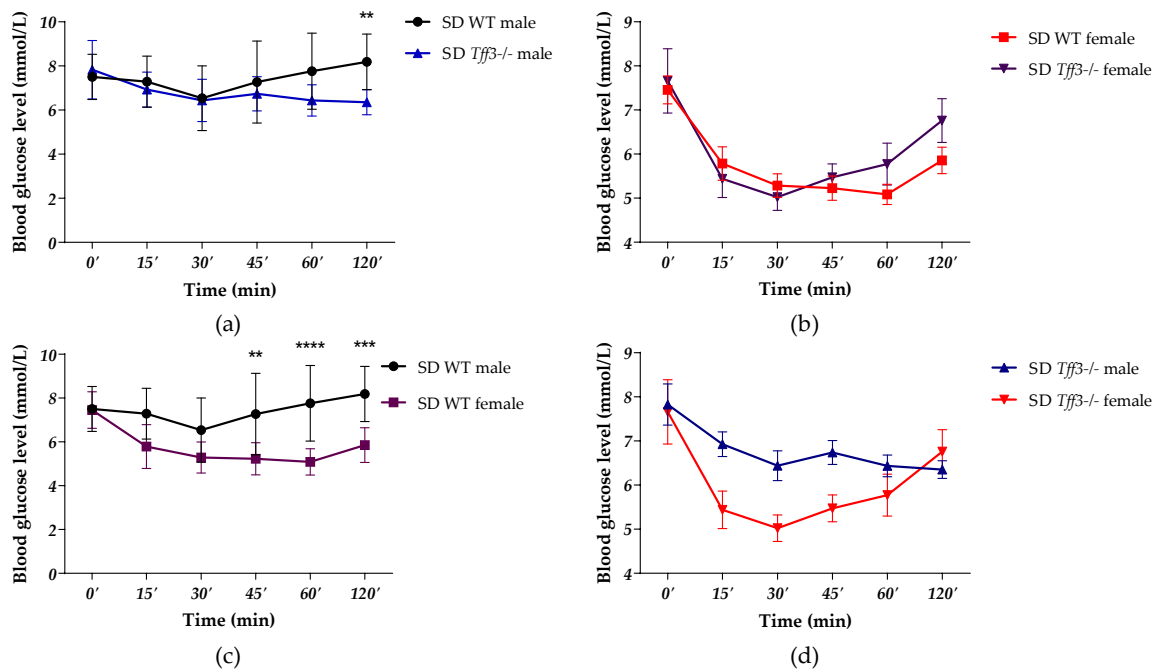


**Figure S3. Intraperitoneal glucose tolerance (GTT) performed on 21 (A)-and 36 (B) week-old WT and *Tff3*<sup>-/-</sup> mice (male and female) on standard diet (SD).** Blood glucose levels were measured at time points 0, 15, 30, 60 and 120 min after glucose injection (2 mg/g body mass) and showed as (a) WT male compared to *Tff3*<sup>-/-</sup> male, (b) WT female compared to *Tff3*<sup>-/-</sup> female (c) WT male compared to WT female and (d) *Tff3*<sup>-/-</sup> male compared to *Tff3*<sup>-/-</sup> female; 2 way ANOVA (Tukey post hoc test) was used for statistical analysis and significant time points are marked as \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; \*\*\*\*  $p \leq 0.0001$

### (A) ITT 23-week-old Standard diet

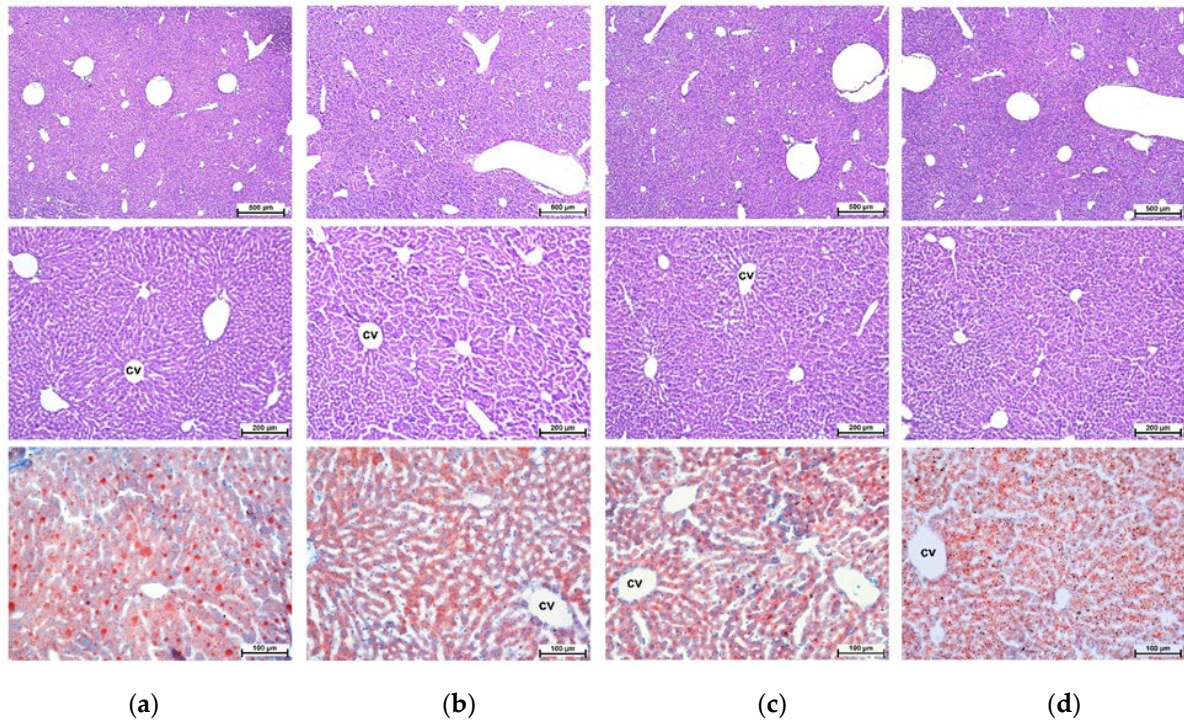


### (B) ITT 38-week-old Standard diet



**Figure S4 . Intraperitoneal insuline tolerance (ITT) test performed on 21 (A)-and 36 (B) week-old WT and *Tff3*<sup>-/-</sup> mice (male and female) on standard diet (SD).** Blood glucose levels were measured at time points 0, 15, 30, 60 and 120 min after glucose injection (2 mg/g body mass) and showed as (a) WT male compared to *Tff3*<sup>-/-</sup> male, (b) WT female compared to *Tff3*<sup>-/-</sup> female (c) WT male compared to WT female and (d) *Tff3*<sup>-/-</sup> male compared to *Tff3*<sup>-/-</sup> female. *Tff3* deficient mice at age of 23 weeks fasting for 4h have lower starting blood glucose level (Fig.S4A). Glucose level after longer fasting hours 16h (GTT) is not affected by *Tff3* deficiency. 2 way ANOVA (Tukey post hoc test) was used for statistical analysis and significant time points are marked as \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; \*\*\*\*  $p \leq 0.0001$ .





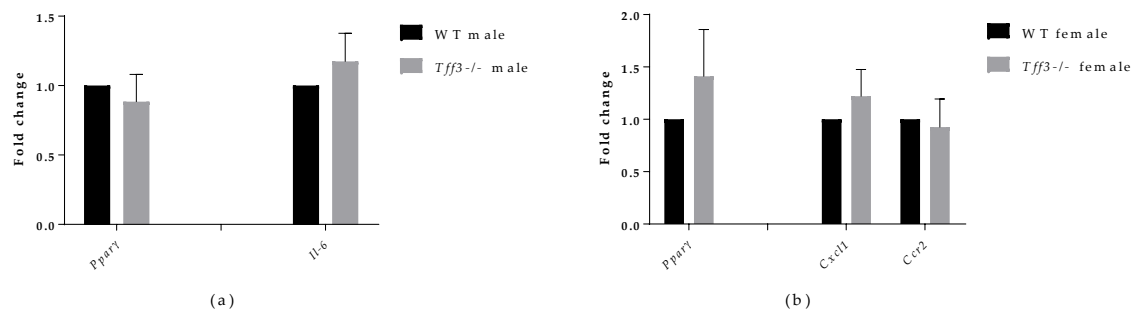
**Figure S5. Histologic evaluation of livers from standard diet (SD) male and female WT and *Tff3*<sup>-/-</sup> mice, (a) WT male (b) *Tff3*<sup>-/-</sup> male (c) WT female (d) *Tff3*<sup>-/-</sup> female. Representative liver sections were stained with HE (upper and middle row panels) and Oil Red O (bottom row panels). Scale bars 500  $\mu$ m (upper row panels), 200  $\mu$ m (middle) and 100  $\mu$ m (bottom row panels). HE- hematoxylin eosin, cv-central vein.**

**Table S3.** Fat (average  $\pm$  standard error) and Fatty acid content (average) of liver mice on standard diet.

Main fatty acids (g of fatty acids/100 g of total fatty acids)	WT ♂	Tff3-/- ♂	WT ♀	Tff3-/- ♀
C 14:0	0.60	0.55	0.74	0.66
C 16:0	22.94 ‡	25.43	22.71 §	24.27
C 16:1	7.28	5.88	5.74	5.44
C 18:0	3.68	5.46	3.84	5.59
C 18:1	25.68 ‡*	21.15†	31.55 §	28.18
C 18:2, n-6	23.35	21.56	21.81	18.40
C 18:3, n-6	0.51	0.42	0.71	0.56
C 18:3, n-3	1.06	0.94	0.92	0.74
C 20:1, n-9	0.33 *	0.28†	0.24	0.22
C 20:3, n-6	0.58 *	0.72	0.35	0.58
C 20:4, n-6	4.85	6.88	4.05	5.88
C 20:5, n-3	0.77 *	0.80	0.49	0.71
C 22:4, n-6	0.16	0.20	0.16	0.19
C 22:5, n-6	0.09	0.11	0.08	0.08
C 22:5, n-3	0.61 *	0.67	0.39	0.50
C 22:6, n-3	6.27 *	7.72	5.02	6.89
Σ SFA <sup>1</sup>	28.03 ‡	32.26	28.09 §	31.24
Σ MUFA <sup>2</sup>	33.35 ‡	27.37	37.58 §	33.90
Σ PUFA <sup>3</sup>	38.46 *	40.17 †	34.16	34.67
n-6 PUFA	29.18	29.62 †	28.56	25.27
n-3 PUFA	8.89	10.28	7.00	9.00
n-6/n-3 PUFA	3.39 : 1	2.92 : 1	3.80 : 1	2.91 : 1
Fat content (g/100 g liver)	15.0 $\pm$ 1.72 ‡*	9.9 $\pm$ 1.88	19.7 $\pm$ 1.72 §	13.9 $\pm$ 1.88

<sup>1</sup> Saturated fatty acids. <sup>2</sup> Monounsaturated fatty acids. <sup>3</sup> Polyunsaturated fatty acids. Results are presented as mean and as mean  $\pm$  standard error (for fat content) and were analysed using general linear models (GLM) procedures of the SAS/STAT module (SAS Institute Inc., Cary, NC, USA), the differences being determined by a Tukey–Kramer multiple comparison test, taking into consideration the genotype as the main effect, separately for male and female mice. Statistical significance was considered at  $p \leq 0.05$ . \*—WT ♂ vs WT ♀ (sex related diff.); † - Tff3-/- ♂ vs Tff3-/- ♀ (sex related diff.); ‡ - WT ♂ vs Tff3-/- ♂ (gene related diff.); § - WT ♀ vs Tff3-/- ♀ (gene related diff.)





**Figure S6. Effect of *Tff3* deficiency on *Pparγ*, *Il-6*, *Cxcl1* and *Ccr2* gene expression in liver of SD-fed mice.** We performed qPCR using the SYBR green detection system. Ct values were analyzed using REST© software and results are expressed as fold change. (a) *Pparγ* and *Il-6* gene expression in liver of *Tff3*<sup>-/-</sup> male mice compared to WT male mice (both fed with SD) (b) *Pparγ*, *Cxcl1*, *Ccr2* gene expression in liver of *Tff3*<sup>-/-</sup> female mice compared to WT female mice (both fed with SD)