

# **The mRNA-binding protein TTP promotes hepatocarcinogenesis but inhibits tumour progression in liver cancer**

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## **Supplementary material**

### **Supplementary Figures:**

**Figure S1: Hepatic fatty acids (FAs) in short-term sham- (control) or DEN-treated WT and *IsTtp*-KO mice**

**Figure S2: Hepatic fatty acids (FAs) in long-term sham- (control) or DEN-treated WT and *IsTtp*-KO mice**

**Figure S3: Effects of TTP overexpression on chemoresistance in hepatoma cells (normalised to control vector)**

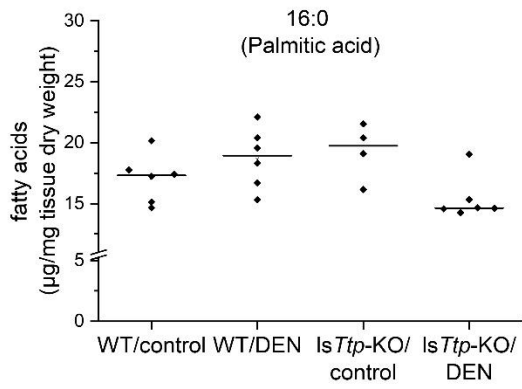
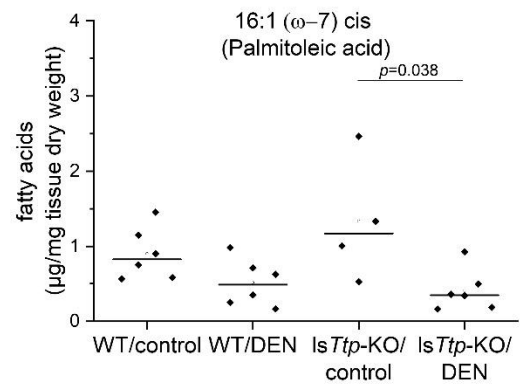
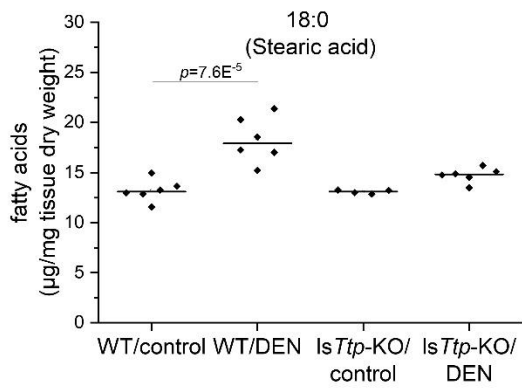
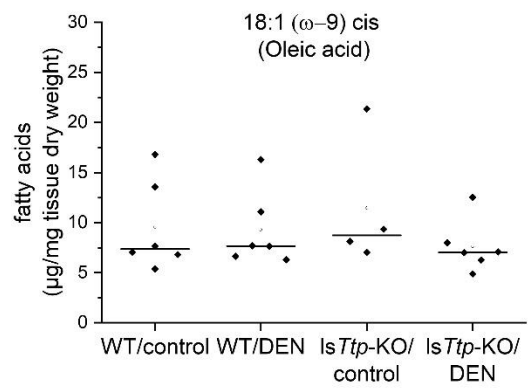
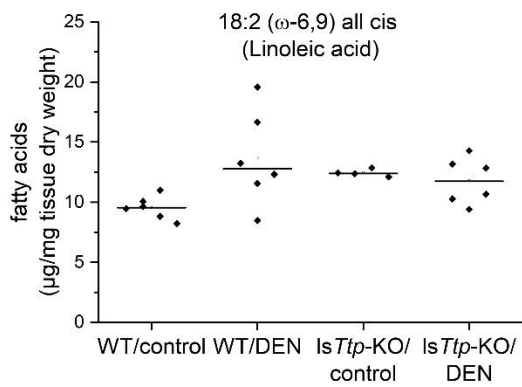
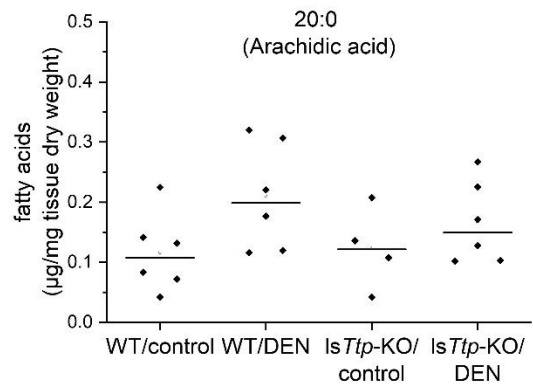
**Figure S4: *Zfp36* mRNA levels in *IsTtp*-KO and WT animals**

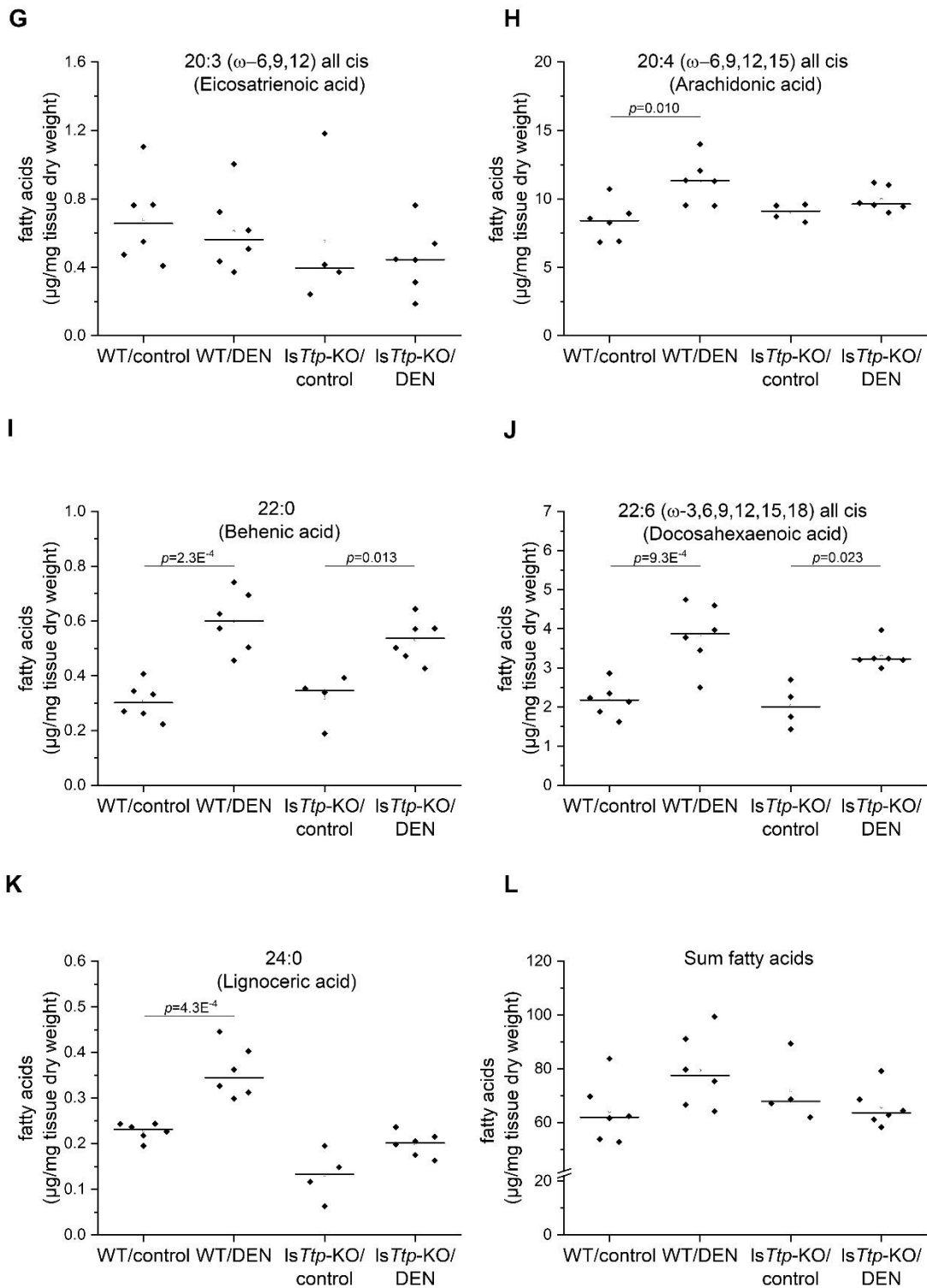
### **Supplementary Tables:**

**Table S1: Primer sequences of human and murine genes**

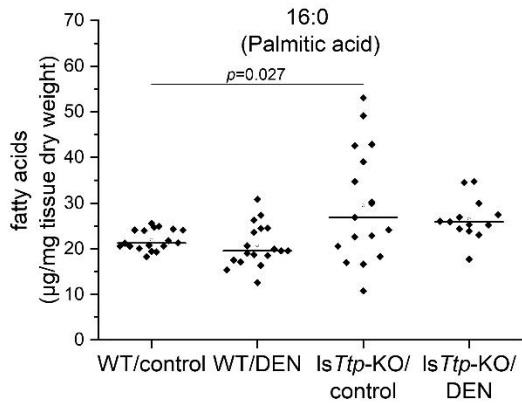
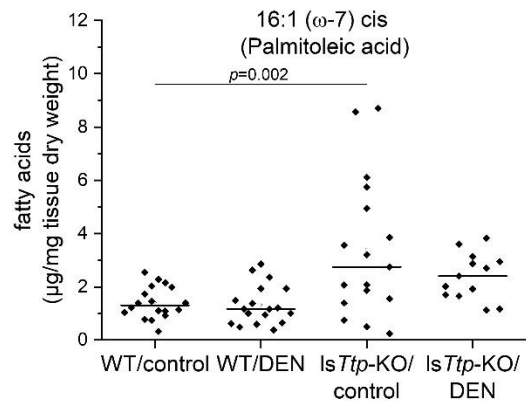
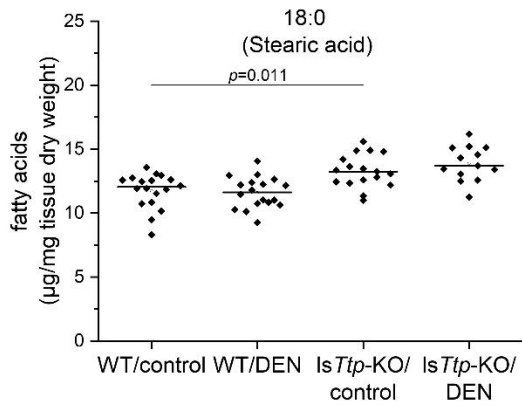
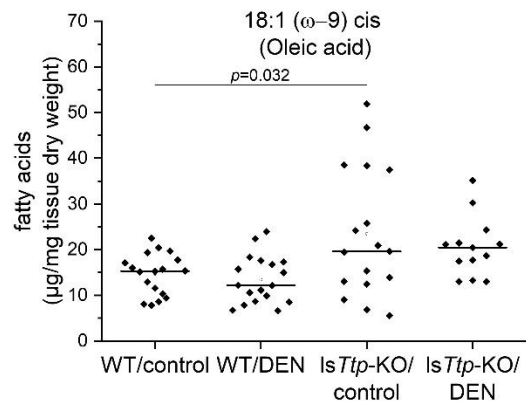
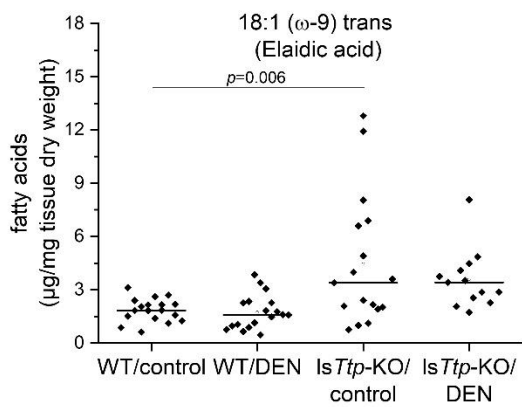
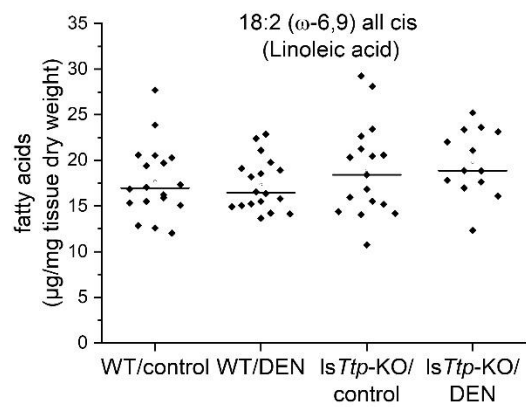
### **Other Supplementary Material:**

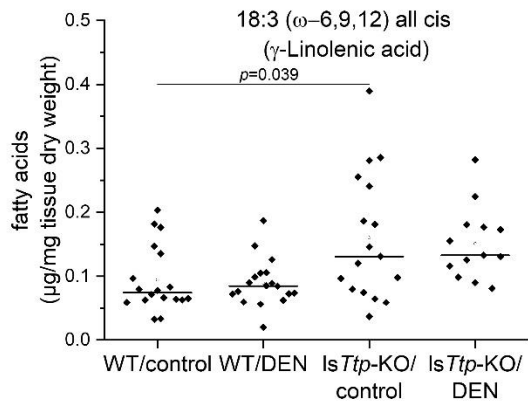
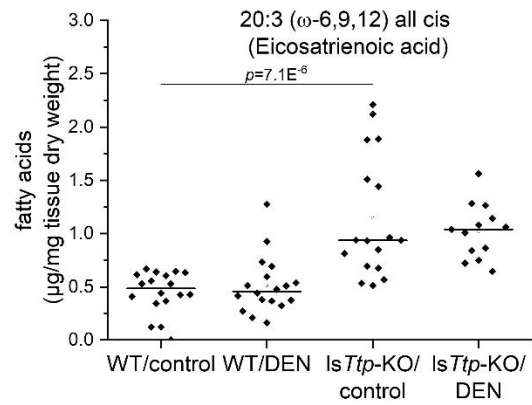
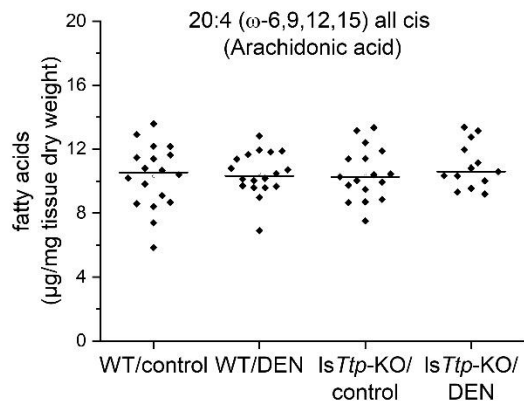
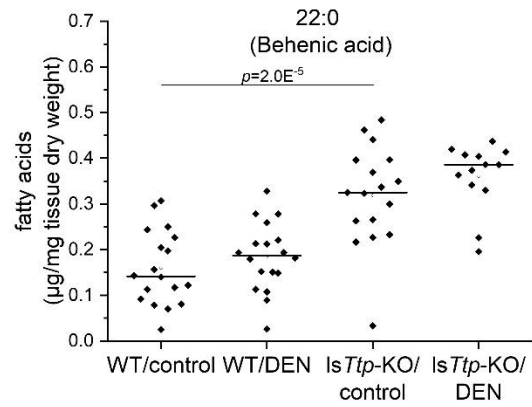
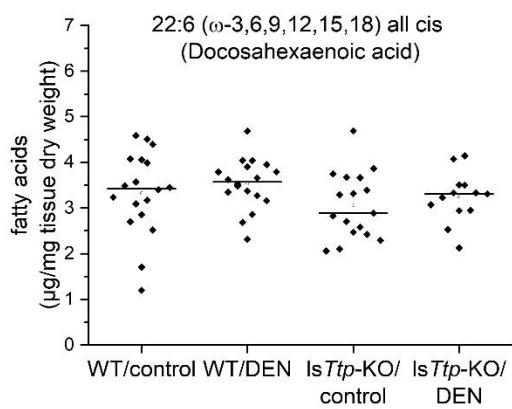
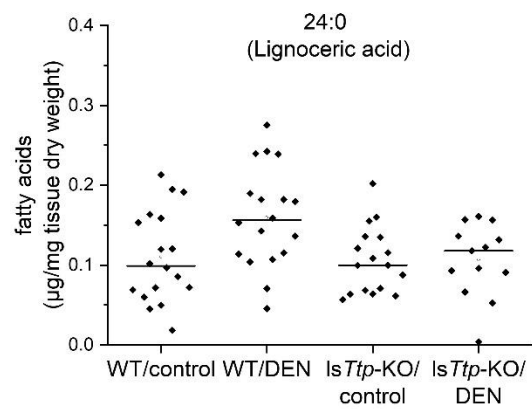
**ARE in TTP target genes**

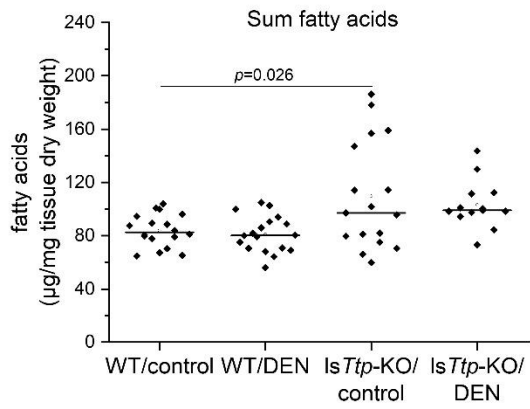
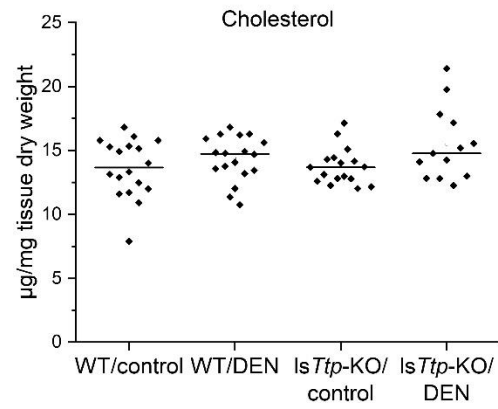
**A****B****C****D****E****F**



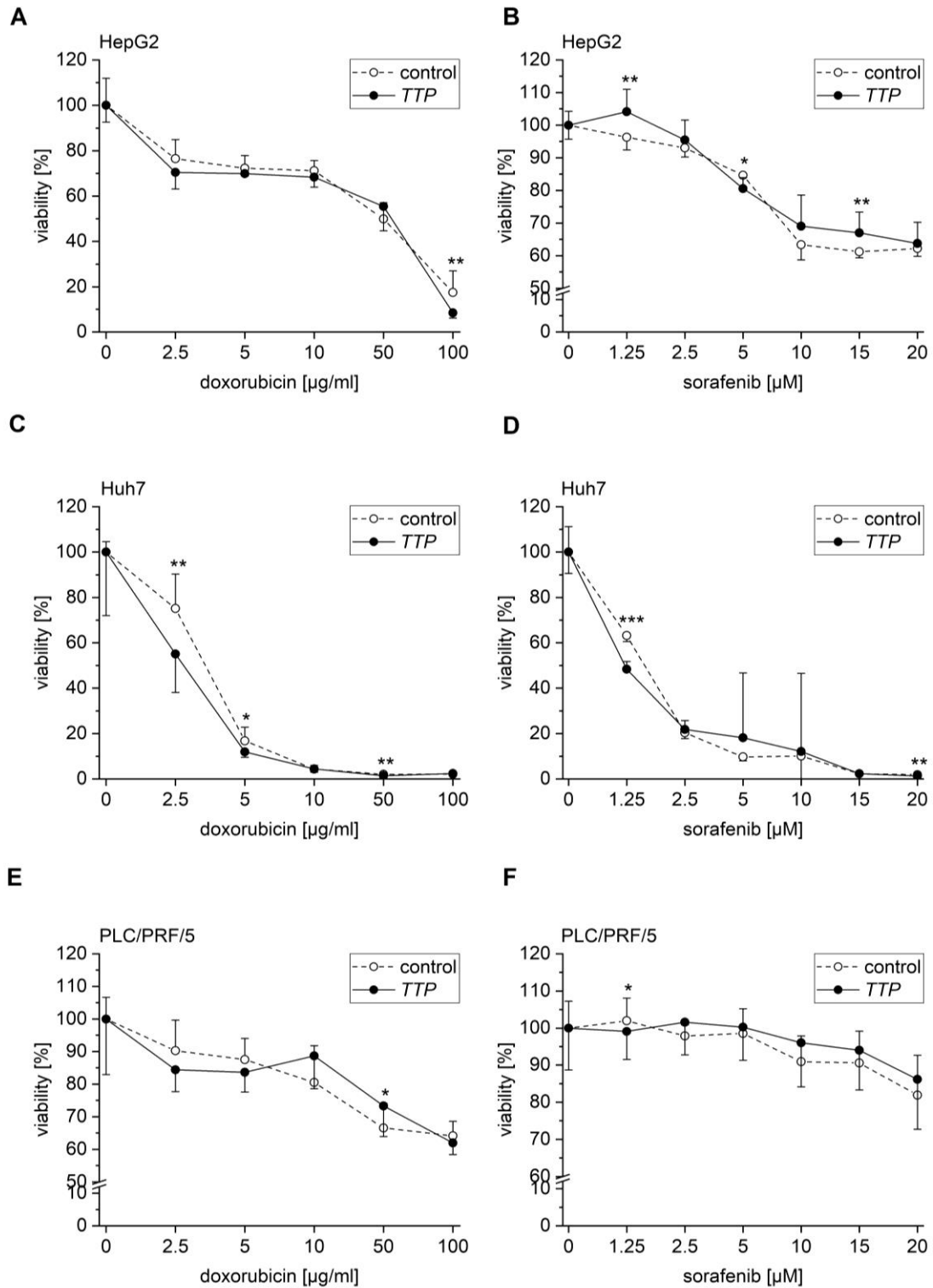
**Supplementary Figure S1. Hepatic fatty acids (FAs) in short-term sham- (control) or DEN-treated WT and *IsTtp-KO* mice.**  $n = 6$  (WT/control),  $6$  (WT/DEN),  $4$  (*IsTtp-KO*/control),  $6$  (*IsTtp-KO*/DEN). Rhombi illustrate single data points, horizontal black lines illustrate median and white rectangles illustrate means. Significant p values ( $\alpha < 0.05$ ) are shown.

**A****B****C****D****E****F**

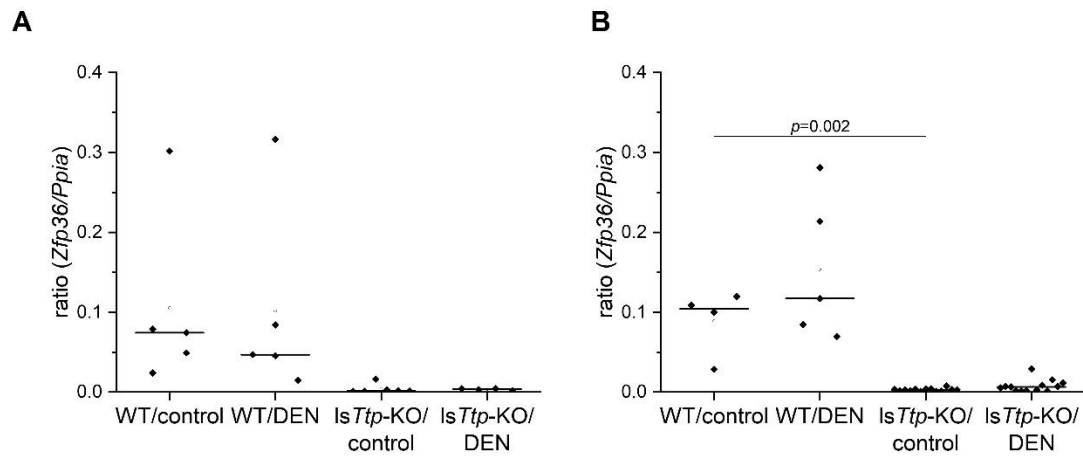
**G****H****I****J****K****L**

**M****N**

**Supplementary Figure S2. Hepatic fatty acids (FAs) in long-term sham- (control) or DEN-treated WT and *IsTtp*-KO mice.** n = 18 (WT/control), 18 (WT/DEN), 17 (*IsTtp*-KO/control), 13 (*IsTtp*-KO/DEN). Rhombi illustrate single data points, horizontal black lines illustrate median and white rectangles illustrate means. Significant p values ( $\alpha < 0.05$ ) are shown.



**Supplementary Figure S3. Effects of TTP overexpression on chemoresistance in hepatoma cells (normalised to control vector).** Cells were transfected with either *TTP* (gene name *ZFP36*) or a control vector. 24 h after transfection, cells were treated with different concentrations of doxorubicin or sorafenib for 24 h. Cell viability was determined *via* MTT assay. Both groups (*TTP* and control vector) are normalised to the viability of the control vector transfected cells without addition of doxorubicin or sorafenib (=100%). (A): HepG2 cells treated with doxorubicin. (B): HepG2 cells treated with sorafenib. (C): Huh7 cells treated with doxorubicin. (D): Huh7 cells treated with sorafenib. (E): PLC/PRF/5 cells treated with doxorubicin. (F): PLC/PRF/5 cells treated with sorafenib. n = 3; quadruplicates. Statistical difference: \*: p ≤ 0.05; \*\*: p ≤ 0.01; \*\*\*: p ≤ 0.001.



**Supplementary Figure S4: *Zfp36* mRNA levels in *IsTtp*-KO and WT animals.** *Zfp36* / *Ppia* mRNA ratio in wild type (WT) and *IsTtp*-KO (KO) animals injected with either 0.9% NaCl as sham-treatment of DEN following the short-term (A) or long-term (B) protocol.



gene	forward primer sequence 5' → 3'	reverse primer sequence 5' → 3'	gene bank accession no.	AT [°C]	product size [bp]	primer concentration [μM]
<b>ACTB</b>	TGCGTGACATTA AGGAGAAG	GTCAGGCAG CTCGTAGCTCT	NM_001101.3	60	107	0.2
<b>BCL2</b>	ACAACATCGCC CTGTGGATGAC	ACTTGTGGCTC AGATAGGCACC	NM_000633.2; NM_000657.2	64	206	0.25
<b>E2F1</b>	AGTTCATCAGC CTTTCCCACC	CTCCAAGCCCT GTCAGAAATCC	NM_005225.2	59	133	0.25
<b>ELAVL1</b>	GGTGACATCGG GAGAACGAA	CCAAGCTGTGT CCTGCTACT	NM_001419.2	60	142	0.25
<b>IGFBP1</b>	GCCTCCATCAA GATTGCACCAC	AGCTTCACTTCC TCCTTGGGAC	NM_006546.3; NM_001160423.1	62	145	0.2
<b>IGFBP3</b>	TCCCACCCAATT TGTTGGAGCC	GCAGCCCCCGC ATTTTCTTTAC	NM_006547.2	62	113	0.2
<b>MYC</b>	AGCCACAGCAT ACATCCTGTCC	CTCGTCGTTTCC GCAACAAGTC	NM_002467.5; NM_001354870.1	56	79	0.2
<b>NEAT1_</b> <b>v1/v2</b>	TGCTACAAGGT GGGGAAGACTG	CCCACACCCCA AACAAAACAA	NR_131012.1; NR_028272.1	60	185	0.25
<b>NEAT1_</b> <b>v2</b>	TTTCAAAGGGA GCAGCAAGGG	ACGGCACAGGC AAATAAGACAC	NR_131012.1	64	199	0.25
<b>ZFP36</b>	TCGCCACCCCA AATACAA	TTCGCTAGGGT TGTGGAT	NM_003407.3	60	99	0.25
<b>VEGFA</b>	CGCTTACTCTCA CCTGCTTCTG	GGTCAACCACT CACACACACAC	NM_001171623.1	60	240	0.25
<b>XIAP</b>	AATAGTGCCAC GCAGTCTACA	CAGATGGCCTG TCTAAGGCAA	NM_001167.3; NM_001204401.1	64	102	0.25
<b>Csnk2a2</b>	GTAAAGGACCC TGTGTCAAAGA	GTCAGGATCTG GTAGAGTTGCT	NM_009974.3	60	85	0.4
<b>Zfp36</b>	CTTCATCCACAA CCCCACC	CAGGGAAGGGC CAGAAAAG	NM_011756.4	59	138	0.25
<b>Ppia</b>	GCGTCTCCTTC GAGCTGTTT	CACCCTGGCAC ATGAATCCT	NM_008907.1	60	138	0.5

Supplementary Table 1: Primer sequences of human and murine genes.

Supplementary Material 1: ARE in TTP target genes.

The following motifs are enriched in TTP targets (Mukherjee et al., 2014): **ATTTA** (blue), **TTTTT** (yellow), **CTTTT** (fat), **TTTTC** (bordeaux), **CTTTC** (purple), **TATTTATT** (green), **TTATTTATT** (underlined) and **TATT** (orange).

*BCL2* variant alpha (NM\_000633.2)

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### MYC variant 1 (NM\_002467.5)

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VEGFA variant 1 (NM\_001171623.1)

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### IGF2BP1 variant 1 (NM\_006546.3)

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**IGF2BP3 (NM\_006547.2)**

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### NEAT1 variant 2 (NR\_131012.1)

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