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SUPPLEMENTARY METHODS

Mouse behavioral analyses.

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SUPPLEMENTARY METHODS

Mouse behavioral analyses.

Behavioral parameters were tested in 8-10 weeks old mice (n = 16-20) as outlined below.

Grip strength test. To test for neuromuscular functions, grip strength was measured using Grip strength Test according to the protocol (Model GT3, Bioseb In Vivo Research Instruments). Maximal peak force (g) developed by the mouse while being on the grid was recorded and three independent tests were done for each mouse.

Rotarod. Motor coordination and balance were tested using motor driven rotating rod (Rotarod). The mice were placed on an accelerating rotarod where the speed was constantly increased from 4 to 40 revolutions per min, during a 5 min period. The time before the animal fell off from the rotarod was recorded. The animals were trained on the rotarod with constant speed (12 rpm) for up to 120 sec for three consecutive days prior to the test. The experiment was performed for four consecutive days with two sessions each day with 1 h interval.

Treadmill acute exhaustion assay. Acute exhaustion was tested on a motorized mouse treadmill (LE8710, Panlab) with an electric grid as a motivational stimulus. The mice were acclimatized to the treadmill for two days before the experiment: Day 1; 5 min without movement, afterwards the training protocol with 5 min at 5 m/min, 5 min with 7 m/min and 5 min with 9 m/min was conducted. Day 2; same as day 1, except the point without movement. On the following day, the acute exhaustion performance was tested with the following settings: 5 min with 6 m/min, then 2 min with 9 m/min and subsequent increasing the speed by 3 m/min every 2 min up to 30 m/min. The mice then run with the final speed and an incline increase by 5° every 10 min until exhaustion. Exhaustion was defined by entering the electric grid (0.2 mA) for more than 3 sec, two times on the grid for more than 1 sec or staying on the grid for more than 50% of the time.

Open field. To test for general activity, exploratory behavior and anxiety, open field tests were done. The mice were placed in a square with white enclosure, 50x50 cm with 50 cm high walls and an overhead camera with tracking system monitored the movement of the mice for 45 min.

Elevated zero maze. A related test is the elevated zero maze where the mice are challenged to open spaces and heights, and therefore to a larger extend monitors anxiety. The circular white maze with diameter of 60 cm and width of 5 cm, was elevated 60 cm above the floor. The platform was equally divided in four quadrants: two quadrants on opposite sides of the platform had enclosed walls and the two other quadrants were open with a 1 cm lip. An overhead camera with a tracking system monitored the mice. They were allowed to explore the maze for 5 min.

SUPPLEMENTARY FIGURES

Figure S1

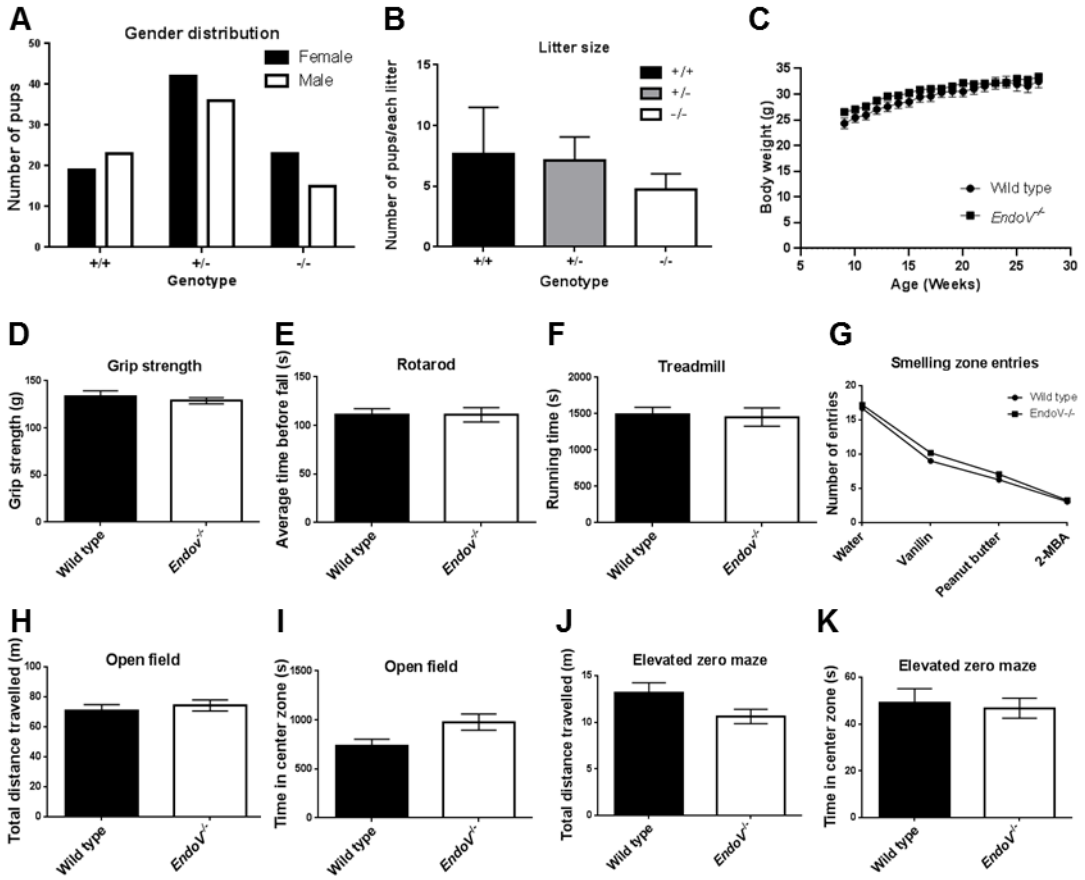

Figure S1 Breeding and behavioral analysis of wild type and *EndoV*$^{-/-}$ mice.
(**A**) Gender distribution, (**B**) litter size in *EndoV*$^{-/-}$ breeding and (**C**) growth of *EndoV*$^{-/-}$ mice. Wild type and *Endo*V$^{-/-}$ mice were tested for (**D**) grip strength, (**E**) balance/coordination with rotarod, (**F**) endurance on a treadmill, (**G**) olfactory function, exploratory behavior, anxiety and general activity by open field (**H**, **I**) and elevated zero maze (**J**, **K**) (n=16-20). g=”grip force”, s= time in seconds, m= distance in meters. Graphs are shown as means ± SEM.

Figure S2

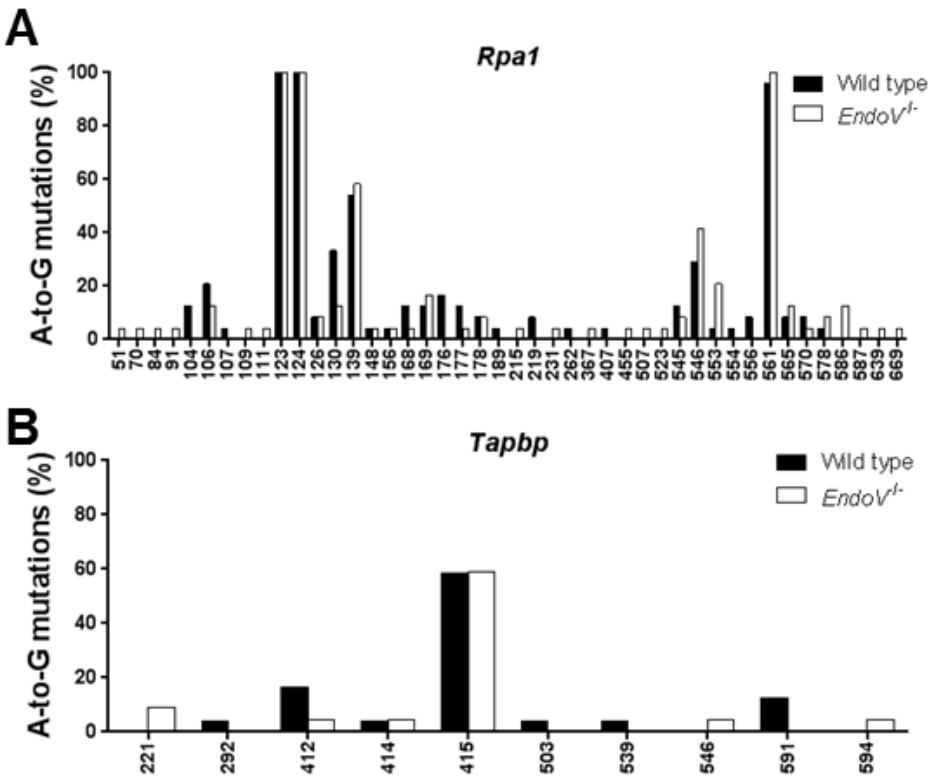

Figure S2 3'UTR hyperediting in *Rpa1* and *Tapbp*.
Mapping positions and level of A-G mutations in (**A**) *Rpa1* and (**B**) *Tapbp* 3'UTRs in wild type and *EndoV*$^{-/-}$ mice by direct DNA sequencing. For each genotype, 24 individual clones obtained by subcloning of PCR products from cDNA, were sequenced. A-to-G mutations were calculated as the number of clones with Gs (in %) at the given positions. The numbers on the X axis refer to the nucleotide position where 1 corresponds to chr11:75300454 for *Rpa1* and chr17: 33928142 for *Tapbp* in the mouse GRCm38/mm10.

Figure S3

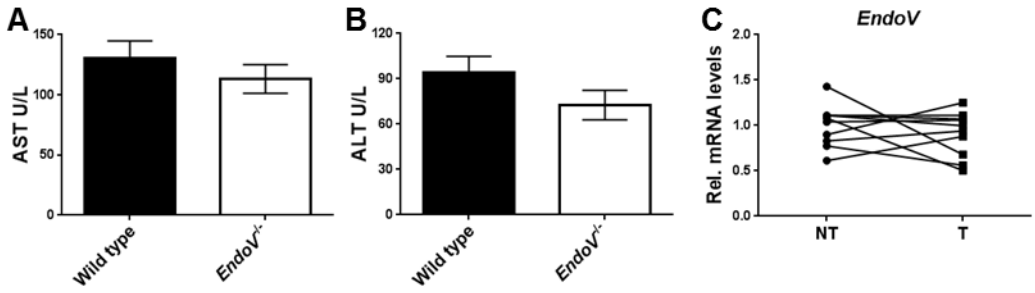

Figure S3 Analyses of DEN-treated mice.
(**A**) AST and (**B**) ALT values for the DEN-treated mice (n=28-30). Graphs are shown as means ±SEM. (**C**) RT-qPCR of *EndoV* mRNA in non-tumor (NT) and tumor (T) tissue samples using wild type DEN-treated mice (n=10). Samples are related to the average of NT samples which was set as 1. Lines depict *EndoV* mRNA levels in NT and T samples within each animal analyzed.

Figure S4

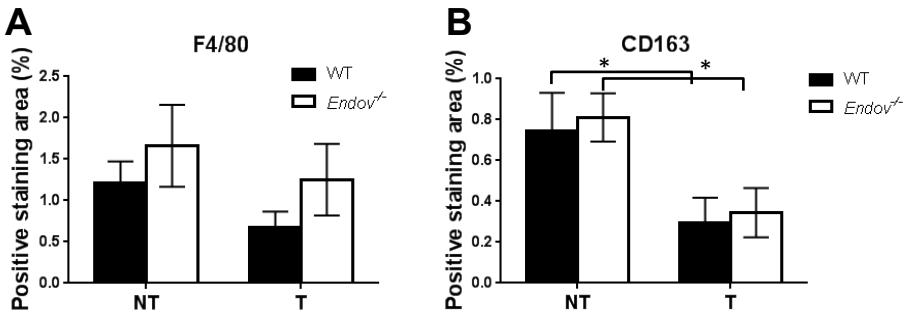

Figure S4 Inflammatory cells in the livers of the DEN mice.
(**A**) Percentage of F4/80 positive cells and (**B**) CD163 positive cells, in non-tumor (NT) and tumor (T) liver samples from DEN-treated wild type (WT) and *EndoV*$^{-/-}$ mice. Quantification was performed by Z9 (n=6). Graphs are shown as means ±SEM. *P <0.05 by paired Student's t-test.

Figure S5

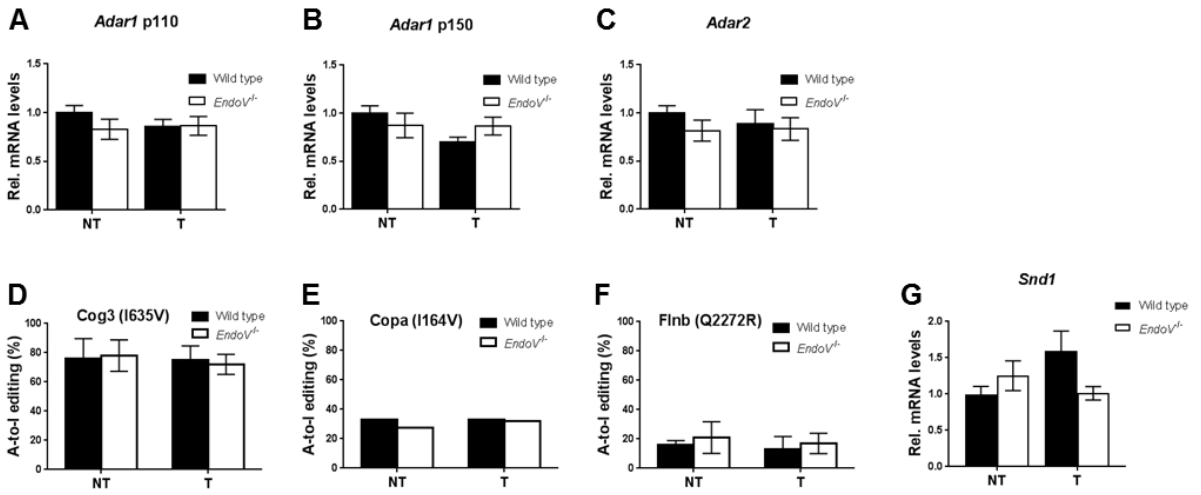

Figure S5 Evaluation of A-to-I editing in DEN mice.
Expression of (**A**) *Adar1* p110, (**B**) *Adar1* p150 and (**C**) *Adar2* mRNA in the DEN-treated mice as measured by RT-qPCR. The values are related to the average of wild type non-tumor (NT) samples which was set as 1. T= tumor, (n=8-10). A-to-I editing levels of specific positions in (**D**) *Cog3*(I635V; n=5), (**E**) *Copa*(I164V; n=2, no SEM) and (**F**) *Flnb*(Q2272R; n=5) mRNA in tumor and non-tumor tissue from DEN-treated mice as measured by reversed transcription and DNA sequencing of PCR products as in Figure 2B. (**G**) *Snd1* mRNA levels measured by RT-qPCR. The values in RT-qPCR are related to the average of wild type non-tumor (NT) samples which was set as 1. Graphs are shown as means ±SEM.

Figure S6

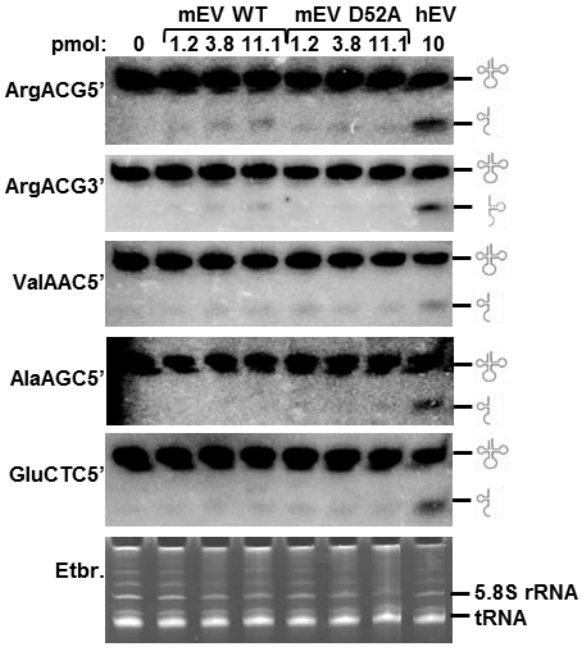

Figure S6 Cleavage of tRNA by recombinant mEndoV.
Northern blots of small RNA isolated from human U373 cells incubated with recombinant mouse MBP-EndoV wild type (WT) or inactive mutant D52A (0, 1.2, 3.8 or 11.1 pmol) using probes for ArgACG5', ArgACG3', ValAAC5', AlaAGC5' and GluCTC5' tRNA. Human EndoV (hEV, 10 pmol) was included as a positive control. An ethidium bromide stained gel at the bottom shows equal loading of small RNA. Glyphs to the right of the membranes indicate full-length and fragment tRNA species.

Figure S7

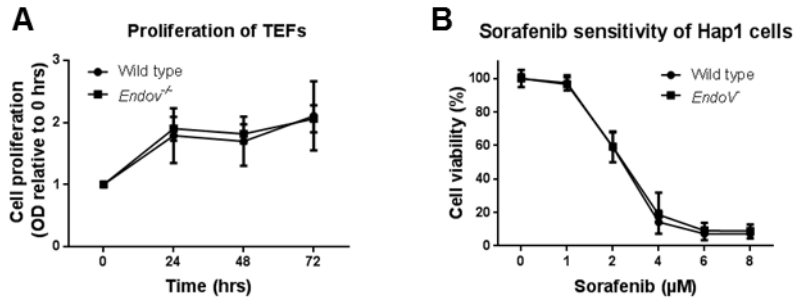

Figure S7 Analyses of mouse and human cells with or without EndoV.
(**A**) Proliferation of wild type and *EndoV*$^{-/-}$ tail fibroblasts. Values are related to the starting time point (0 h). Graph is shown as mean ±SEM (n=3). (**B**) Viability as measured by MTT of wild type and *EndoV*$^{-}$ Hap1 cells after sorafenib exposure for 24 h (n=6). Values are related to the average of the untreated samples (0 µM). Graph is shown as mean ±SD.

SUPPLEMENTARY TABLES

Table S1
Primers.

Name	5'→3' sequence	Note
Genotyping		
EndoV forward	agccaggagtacaaggagca	
EndoV reverse	gcaagtgggtgttggagagt	
EndoV$^{-/-}$ reverse (*Neo*)	ggggaacttcctgactaggg	
Site specific editing		
Cog3 forward	ccacagttcgaaggacactt	
Cog3 reverse	ctcctccagctgctctacaa	
Copa forward	gcacttggattatatccgtacaa	
Copa reverse	cagcacaagacacattgttgtaat	
Flnb forward	gctgttgagggccctagtaa	
Flnb reverse	gctctcctacacgtactttgaaa	
3'UTR analyses		
*Rpa1*_3UTR forward	ctccacaatgaagatcctctag	PCR: 3'UTR region (770nt)
*Rpa1*_3UTR reverse	ggcaaccgaacggtaactg	PCR: 3'UTR region (770nt)
*Tapbp*_3UTR forward	gtcacagtaaagaagttctcgtc	PCR: 3'UTR region (692nt)
*Tapbp*_3UTR reverse	tcttctcaaccctgggactc	PCR: 3'UTR region (692nt)
M13 forward	gtaaaacgacggccagtg	Sequencing
*Ctn*_Fwr region forward	tgagcccagttgaaggattt	PCR: 3'UTR region (653nt)
*Ctn*_Fwr region reverse	agggcaactagcatttgtgg	PCR: 3'UTR region (653nt)
*Ctn*_IR2 region forward	agcactgtgagtcagcagaa	PCR: 3'UTR region (597nt)
*Ctn*_IR2 region reverse	ctcatctcaccagccccaag	PCR: 3'UTR region (597nt)
*Ctn*_Fwr_seq forward	gtttagagattctcctatctatc	Sequencing
*Ctn*_IR2_seq reverse	ccaacctgttaaagtgctgct	Sequencing
Northern blot analyses		
tRNA AlaAGC5'	cgagcgctctaccatttgagctaatccccc	human/mouse
tRNA ArgACG5'	acgcgttatccattgcgccactggccc	human/mouse
tRNA ArgACG3'	cgagccagccaggagtcgaacctggaat	human/mouse
tRNA GluCTC5'	gccgaatcctaaccactagaccaccaggga	human/mouse
tRNA LeuCAG3'	gtgtcaggagtgggattcg	mouse
tRNA LeuAAG5'	agacgcattatccattgagccactggccc	human
tRNA LysCCT5'	atgctctaccgactgagctagccgggc	human/mouse
tRNA SerAGA5'	catcgccttaaccactcggccacgactac	human/mouse
tRNA ValAAC5'	ggcgaacgtgataaccactacactacggaaac	human/mouse

Table S2
Metabolic parameters.

20 months old mice	Wild type (n=20) ± SEM	*EndoV*$^{-/-}$ (n=17) ± SEM
Body weight (g)	42.3 ± 1.6	42.1 ± 1.8
TAG-liver (mg/g tissue)	38.5 ± 6.9	36.3 ± 8.3
TAG-plasma (mg/dl)	100.4 ± 9.5	116.2 ± 7.8
NEFA-plasma (mmol/l)	1007 ± 59	1193 ± 116
Glucose-full blood (mmol/L)	8.8 ± 0.3	9.2 ± 0.3
C-peptide-plasma (ng/ml)	0.80 ± 0.11	0.70 ± 0.12
% Liver/body weight ratio	5.2 ± 0.2	5.2 ± 0.2
% Spleen/body weight ratio	0.24 ± 0.02	0.25 ± 0.01

Table S3
The most up- and down regulated genes in *EndoV*$^{-/-}$ (*EV*$^{-/-}$) mice livers compared to wild type (WT).

Upregulated genes	p-value	Fold change *EV*$^{-/-}$ vs. WT	Downregulated genes	p-value	Fold change *EV*$^{-/-}$ vs. WT
Serpina1e	0,037	3,64	*Slc25a47*	0,018	-1,96
Tmem254b	0,021	2,20	*Cyp2a5*	0,008	-1,81
Tat	0,029	1,60	*1500017E21Rik*	0,003	-1,68
Csad	0,040	1,54	*Apol9a*	0,006	-1,65
Acss2	0,020	1,48	*Lhpp*	0,043	-1,57
Apom	0,001	1,43	*Oat*	0,016	-1,55
1810008I18Rik	0,012	1,40	*Creld2*	0,049	-1,50
Tob1	0,020	1,38	*Apol9b*	0,018	-1,42
BC005537	0,044	1,38	*Ifi27*	0,032	-1,41
C4b	0,033	1,36	*Lect2*	0,014	-1,36
Ces1d	0,024	1,35	*Cd59a*	0,005	-1,35
Ces2a	0,026	1,35	*Neat1*	0,014	-1,35
Srebf1	0,036	1,33	*Hmgcs2*	0,044	-1,32
Sardh	0,013	1,31	*H2-T9*	0,006	-1,31
Afmid	0,032	1,29	*Tmed9*	0,047	-1,30

Table S4
Gene expression analyses of DEN-treated mice.
(*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001)

Gene	Ratio (Tumor/Non-tumor) ± SEM	
	Wild type (n=28)	*EndoV*$^{-/-}$(n=29)
Apoptosis		
Bax	1.1 ± 0.2	1.2 ± 0.1
BclXL	1.8 ± 0.2 *	1.5 ± 0.2
Cell invasion/signaling pathways		
Cdh1	2.2 ± 0.2 ***	2.4 ± 0.2 ***
Fos	1.8 ± 0.7	1.7 ± 0.2
Jun	1.2 ± 0.1	1.2 ± 0.1
Mmp9	1.0 ± 0.3	0.7 ± 0.4
Mmp12	3.0 ± 0.6 *	3.8 ± 1.1
Myc	1.0 ± 0.3	0.7 ± 0.3
Ppia	1.0 ± 0.1	0.9 ± 0.1
Insulin/glucose signaling and metabolism		
Atp5B	0.8 ± 0.1 *	0.8 ± 0.1
Ghr	0.5 ± 0.1 **	0.6 ± 0.1 **
Glut1	1.1 ± 0.2	1.2 ± 0.2
G6pdx	3.9 ± 0.5 **	2.2 ± 0.2 **
Hk2	1.6 ± 0.4	0.7 ± 0.2
Igf1	0.4 ± 0.1 **	0.4 ± 0.0 ***
Igf2	5.4 ± 3.4	4.4 ± 2.1
Irs1	1.0 ± 0.1	0.7 ± 0.1 **
Pkm2	1.4 ± 0.2 *	1.3 ± 0.1
Srebf1	0.7 ± 0.1	0.7 ± 0.2
Fat metabolism		
Acaca	1.1 ± 0.1	1.3 ± 0.2
Acadm	0.7 ± 0.1	0.7 ± 0.1 *
Acox1	0.5 ± 0.1 **	0.6 ± 0.1 **
Cpt1a	1.1 ± 0.1	1.3 ± 0.2
Fasn	0.9 ± 0.2	1.4 ± 0.3
Mgll	0.8 ± 0.3	0.5 ± 0.3 *
INF pathway		
Bst2	0.6 ± 0.2	0.3 ± 0.1 ***
Ifit1	0.7 ± 0.2	0.4 ± 0.1 ***
Irf1	0.8 ± 0.1	0.8 ± 0.1 *
Pkr	0.7 ± 0.1 *	0.7 ± 0.1 **
Rsad2	0.7 ± 0.2	0.6 ± 0.1 **
Tnfα	1.7 ± 0.3	1.3 ± 0.3
Usp18	0.6 ± 0.2	0.7 ± 0.2
Other		
Serpina1e	1.2 ± 0.1	1.2 ±0.1
Slc25a47	1.0 ±0.4	0.7 ±0.1

Table S5
List of tRNA fragments in the RT-qPCR array.
(database: http://genome.bioch.virginia.edu/trfdb/index.php).

Mouse tRF (88)			
tRF-1			
1003	**SerGCT**	3033b	**IleTAT**
1006	**HisATG**	3034a	**ArgTCT**
1008	**CysGCA**	3036a	**AlaTGC**
1009	**CysGCA**	3036b/3037b	**AlaTGC, AlaAGC**
1010	**PheGAA**	3038a	**IleAAT, AlaTGC, AlaCGC**
1015	**CysGCA**	3038b	**IleAAT**
1016	**ValTAC**	3039a	**AlaAGC**
1019	**TyrGTA**	3041b/3042b	**GlyTCC**
1020	**MetCAT**	3043a	**GlyGCC**
1026	**CysGCA**	3043b	**GlyGCC**
1035	**ThrCGT**	3044a	**GlyCCC**
tRF-3		3044b	**GlyCCC**
3001b	**AsnGTT**	3045a	**GluCTC**
3002a/3035a	**TrpCCA**	3046a	**TyrGTA, GluTTC**
3003a	**ProTGG, ArgTCG, ArgCCG**	3047b	**MetCAT**
3004b	**CysGCA, ProCGG, ProAGG**	3048a	**IleTAT**
3005a	**ThrTGT**	3050a	**CysGCA**
3006a	**GlnCTG**	3051a	**PheGAA**
3009a	**ValCAC**	3052a	**AspGTC, SerGGA**
3009b	**ValCAC, ValAAC, GlyACC**	**tRF-5**	
3010a	**LeuTAA, ValAAC, GlyACC**	5001a/5001b/5010a	**MetCAT, ArgACG**
3010b	**LeuTAA**	5002b/5004b	**GlyGCC, GlyGCC, GlyCCC**
3011a	**SerTGA**	5005a/5006b	**LysTTT, LysCTT**
3011b	**SerTGA**	5005b	**LysTTT**
3012b	**MetCAT**	5006a	**LysCTT**
3017a	**LeuCAG**	5006c	**LysCTT**
3017b	**LeuCAG**	5007a	**GlyCCC**
3019a	**ThrAGT**	5009a	**ProTGG, ProCGG, ProAGG**
3019b/3020b	**ThrAGT**	5009b	**ProTGG, ProCGG, ProAGG**
3021a	**SerGCT, ThrAGT**	5011a	**AlaTGC, ProCGG, ProAGG**
3023b	**HisGTG**	5011b/5012b	**AlaTGC, ProCGG, ProAGG, CysGCA**
3024b/3046b	**TyrGTA**	5012b	**CysGCA**
3025a	**SerTGA**	5013a	**LeuTAG, LeuAAG**
3025b	**SerTGA, SerAGA**	5013b	**LeuTAG, LeuAAG**
3026a	**SerCGA, SerAGA**	5013b/5017b/5018b	**LeuTAG, LeuAAG, LeuCAG, LeuCAA**
3027a	**SerCGA**	5014a	**ValTAC**
3028b/3029b	**GluTTC**	5014a/5015a	**ValTAC, GlnCTG**
3028b/3029b/ 3040b/3045b	**GluTTC**	5014b	**ValTAC**
3029a	**GluTTC, AspGTC, GluTTC**	5016a	**SerTGA, SerAGA**
3030a	**ArgACG**	5019a	**ValCAC, ValAAC, GlyACC**
3031a	**LysCTT**	5019b	**ValCAC, ValAAC**
3031b	**LysCTT**	5020b/5021a	**GluTTC**
3032a	**ValTAC**	5022a	**GluTTC, GluCTC**
3032b	**ValTAC**	5022b	**GluCTC**
3033a	**IleTAT**	5023b/5024b	**AspGTC**

Table S6

tRNA fragments with significant different levels in the liver samples.

KO=*EndoV*$^{-/-}$, WT= wild type, NT= non tumor and T= tumor.

1'tRFs: orange color; 3'tRF: blue color; 5'tRF: red color

Upregulated tRFs: grey background; down-regulated tRFs: white background

KO NT vs WT NT	WT T vs WT NT	KO T vs WT T
CysGCA	TyrGTA	HisATG
AspGTC	SerTGA	TyrGTA
GluTTC/AspGTC	MetCAT	IleAAT
GluCTC/GluTTC	ValTAC	ThrAGT
GlyCCC	AlaTGC/ProCGG/ProAGG/CysGCA	GlyCCC
IleTAT	ValTAC	
LeuTAA	ValTAC/GlnCTG	
SerCGA	ValCAC/ValAAC/GlyAAC	
AspGTC		
GlyGCC, GlyCCC		