Supporting Information (Materials and Methods)

Animals. Mice were maintained under a 12-hour light/12-hour dark cycle with free access to water and standard mouse diet (66% carbohydrate, 12% fat, 22% protein). *Oct1*$^{-/-}$ mice were generated as previously described (1) and backcrossed 10 generations to FVB/N or C57BL/6J background. *Oct1*$^{-/-,\ ob/ob}$ mice were generated by crossing *Oct1*$^{+/-}$ and *Oct1*$^{+/-,Ob/ob}$ mice. pLIVE In Vivo Expression vector (Mirus Bio LLC, Madison, WI, USA) was used to generate the liver-specific transgenic human *OCT1* mice. Human OCT1 was cloned to Nhe I (5') and Xho I (3') sites, and then was cut from the vector between Bgl I and Nde I. The linear fragment containing OCT1 was purified and then was injected to C57BL/6J mice (Genomic Core at Gladstone Institutes at UCSF, USA). The detailed vector construction was described in the supplementary materials (Supplementary Fig. S5A and S5B) (2). Unless mentioned otherwise, all experiments were performed in FVB mice with the exception of the experiments with the transgenic mice in which all mice strains were C57BL/6J, consistent with the background of the *OCT1* transgenic mice. Animal studies described herein were reviewed and approved by UCSF IACUC.

Animal experiments. All the animal studies were conducted in male mice. In general, unless otherwise specified, mice were 16 to 20 weeks old. For the thiamine tissue accumulation study, age-matched *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ male mouse littermates (10 to 14 weeks) fasted for 16 hours were given an intravenous dose (2 mg/kg) with 0.2 μCi/g of [^{3}H] thiamine (American Radiolabeled Chemicals, St. Louis, MO, USA) and sacrificed at early time point (5 min) or 1 hour later. Tissues were removed, weighed and homogenized with lysis buffer (1% SDS and 1 N NaOH). For pharmacokinetic studies, after the same dose of thiamine, mice were placed in metabolic cages and tail blood samples were collected at various times points. Plasma was obtained following centrifugation of the blood samples, and total thiamine products (radioactive thiamine plus thiamine metabolites) were measured with a scintillation counter. For the thiamine and metformin interaction study, *Oct1*$^{+/+}$ mice fasted for 16 hours were given an intravenous dose (2 mg/kg) with 0.1 μCi/kg of [^{3}H] thiamine with or without 50 mg/kg metformin. For chronic dosing studies, metformin was administered daily 100 mg/kg intraperitoneally and plasma and tissue levels of thiamine and its metabolites were quantified at the end of 7 days. Blood was drawn at various time points. 10 μl of plasma was analyzed by scintillation counting at different time points ranging from 2.5 min to 4 hours. For the ORO staining and Western blotting, *Oct1*$^{+/+}$, *Oct1*$^{-/-}$, *Oct1*$^{+/+,\ ob/ob}$, *Oct1*$^{-/-,\ ob/ob}$, *Oct1*$^{+/+,\ OCT1tg}$ and *Oct1*$^{-/-,\ OCT1tg}$ male mouse littermates were fasted for 16 hours. Mice were fasted for 16 hours for the plasma triglyceride (TG)/insulin/glucose characterization studies,

but not the MRI study, which was performed in unfasted mice. All diets were obtained from Harlan Laboratories, Inc (Madison, WI, USA): normal chow (2016); high fat diet (consisting of 55% calories from fat; 25% from carbohydrate and 15% from protein) (**TD.93075)**, thiamine deficiency food (TD.81029). Pair-fed control chow was modified from thiamine deficiency food (TD.81029) by adding 20 mg/kg thiamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) (TD.120472). For high fat diet treatment, mice were kept on high fat diets for 16 weeks starting at 4-week old. For studies using thiamine deficient diets, 12- to 16-week old mice were fed thiamine deficiency food for 3 weeks or 1 week before the characterization.

Cell culture. HEK-293 Flip-in cells were cultured as described (1, 3). Primary hepatocytes (12- to 16-week old mice) were isolated from adult mice by a modified version of the collagenase method in the UCSF Liver Center. Hepatocytes were maintained in Dulbecco's Modified Eagle's Medium (DMEM H-21) (4.5g/Liter Glucose) (UCSF cell culture facility, USA) medium with 1X ITS (Insulin-Transferrin-Selenium), 1X penicillin/streptomycin and 10% fetal bovine serum (Life technologies, Grand Island, NY, USA) for 6 hours allowing the cells to attach to the plate bottom. After the cells attached, the medium was switched to DMEM H-21 with 1X penicillin/streptomycin for 16 hours, followed by drug treatment as described below (Foretz et al., 2010). After serum fasting, hepatocytes were cultured in glucose-free DMEM H-21 (UCSF cell culture facility) containing antibiotics alone or with 100 μM Bt2-cAMP (Sigma-Aldrich), with or without various doses of metformin (Sigma-Aldrich) or AICAR (Toronto Research Chemicals, Toronto, Ontario, Canada) for 6 hours before harvesting. For the thiamine effect on the AMPK activation study, primary hepatocytes were fasted by described above. Then various percentages of DMEM H-21 medium (thiamine, 4mg/L) were blended with thiamine-deficient DME H21 (thiamine, 0 mg/L) to adjust the thiamine levels in the medium. After 6 hours incubation, cells were harvested and subject to the assays. For glycolysis studies, hepatocytes were exposed to lonidamine (400 μM) for 8 hours and then nucleotides and P-AMPK were measured.

TG, glucose, insulin and lactate assays. Lipids were extracted from mouse liver tissue or human liver tissues purchased from Asterand (Detroit, MI, USA) and Capital Biosciences (Rockville, MD, USA) using the Folch method (4). Extracts were evaporated under a stream of nitrogen and resuspended in chloroform:methanol (2:1) containing 0.01% butyrated hydroxyltoluene. Aliquots were dried and resuspended in 1-butanol containing 0.01% butyrated hydroxytoluene for measurement of total TG (BioVision, Milpitas, CA, USA). Results were reported as milligrams of TG per gram liver. Glucose was measured using the OneTouch® Blood Glucose Meters (Lifescan,

Milpitas, CA, USA). Insulin from plasma was measured using the ELISA assay following the manufacturer's protocol (EMD Millipore, Billerica, MA, USA). Lactate assays were conducted with a kit from BioVision.

Metabolomics study. Metabolomic studies were performed in the Metabolomics Core of the UC Davis Genome Center (Davis, CA, USA). Metabolomics methods for highly polar metabolites were applied to supernatants obtained from sonicated samples using methanol as extraction solvent from mouse liver, HEK293 cell lines and hepatocytes from *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice (5). Hydrophilic interaction chromatography (HILIC-LC/ESI-MS) analysis was performed with the use of a modified silica-based column (Luna HILIC Diol, 150 X 3mm, 3 mm particle size; Phenomenex, Torrance, CA, USA). The mobile phases were 100 mM ammonium formate (pH 4.0) (A) and acetonitrile (B) (flow rate 0.4ml/min at 408°C). After a 2-min isocratic run at 3% A, a sequential ramping scheme was followed up to 40% A for total injection time of 20 min. The injection volume was set to 10 μl. The entire effluent from the HPLC column was directed into the ESI source of an LTQ linear ion trap (LIT) mass spectrometer (Thermo Fisher) operated under Xcalibur software (v1.4, Thermo Fisher). Full scan spectra were acquired from 100–1500 amu at unit mass resolution with maximum injection time set to 200 ms in one micro scan. Acquisition was performed in both positive/negative switching modes. A sucrose tune file in negative/positive modes at high LC flow rate was used during all of the LC/ESIMS acquisitions on the LTQ mass spectrometer. Exported data sets in netCDF format were further converted and loaded into the MarkerView 1.1 software (Applied Biosystems, Foster City, CA, USA). Peak alignment and peak picking parameters were adjusted in accordance with the chromatography quality (peak width, baseline, background noise, etc.). Principle component analysis (PCA) was performed. Data point reduction was applied to remove components possessing low relative variances of metabolite abundance. Modified peak lists were exported into Statistica Data Miner (StatSoft, Tulsa, OK, USA) for further analysis.

To quantify the TMP and TPP level in mouse liver by LC/MS, 200 μl of saline solution was added to 25 mg of liver. Liver was homogenized and 1 mL of acetonitrile containing 1 μM of internal standard (IS) to precipitate protein. The mixture was kept on ice for 5 minutes then vortexed for 5 minutes. The mixture was centrifuged at 15,000 rpm for 10 minutes and 800 μL of clear supernatant was transferred to the glass tubes. The supernatant was evaporated then the residue is reconstituted in 200 μL of water before transferred to the new centrifuge 1.5 mL tube. The constituted solution was centrifuged at 15,000 rpm for 10 minutes. The supernatant was used for LC-MS/MS analysis. Standard curves for TMP and TPP were constructed.

Quantification of thiamine, thiamine monophosphate and thiamine pyrophosphate in mouse plasma by LC/MS/MS method. Molecular MS Diagnostics, Inc. (Cranston, Rhode Island, USA) developed the methods for quantification of thiamine, thiamine monophosphate and thiamine pyrophosphate (thiamine diphosphate) in mouse plasma samples. All chemicals and reagents used in this analysis were HPLC grade or reagent grade. Plasma samples were extracted by aliquoting 100 μL of plasma sample in to a 1.5 mL Eppendorf vial. 400 μL of internal standard (20 ng/mL carbutamide) in methanol with 0.1% formic acid were added to each vial, and then vortexed each sample for 30 seconds. The samples were then centrifuged at 13,500 rpms for 5 minutes to spin down all precipitated plasma proteins. All 200 μL of the supernatant was decanted to an appropriate labeled 1 mL HPLC sample vial. The samples were evaporated to dryness under heated Nitrogen gas (60 °C) for 15 minutes, resuspended in 50 μL of mobile phase A, and injected (10 μL) onto the HPLC-MS/MS system for analysis. Tandem-mass spectrometry (MS/MS) was performed on a triple stage quadrupole from Perkin Elmer SCIEX API 4000 (Sciex, Concord, Ontario, Canada) with an atmospheric pressure ionization (API) chamber. The HPLC column is coupled to the mass spectrometer. The analytical column used was Phenomenex, Synergi 4 μm Hydro-RP-80A, 4 μm particle, 50 x 4.6 mm, part number 00B-4375-E0. A reversed phase chromatography was used. The following is the chromatography conditions and operation conditions for detecting thiamine and the metabolites.

Mobile Phases:

Mobile Phase A:	DI water with 0.01% Trifluoroacetic acid(v/v)
Mobile Phase B:	MeOH with 0.01% Trifluoroacetic acid(v/v)
Column Temperature:	Ambient
Injection Volume:	10 μL
LC Method:	Gradient
Run Time:	5.0 minutes
Thiamine retention time:	1.2 min ± 0.2 min
Thiamine Monophosphate retention time:	1.0 min±0.2min
Thiamine Diphosphate:	0.9 min±0.2min
Carbutamide (IS) retention time:	2.4 min ± 0.2 min
Flow rate:	700 μL/min
Autosampler:	Samples 8-10 C temperature Solvent 1 Wash Mobile Phase B Solvent Wash 2 Mobile Phase A

Detail of LC Gradient method

Step	Start	Sec	Flow	Grad	%B	Comments
1	0.00	45	0.700	Hold	0	Load Sample
2	0.75	75	0.700	Ramp Linear	0	Start Gradient on analytical column into MS
3	2.0	6	0.700	Hold	100	Hold
4	2.60	36	0.700	Hold	100	Immediate Step Down, Re-equilibrate

5	2.61	6	0.700	Hold	0	Re-equilibrate
6	4.75	132	0.700		0	Re-equilibrate

Mode of operation: positive ion mode, multiple reaction monitoring (MRM) of the transitions from $[M+H]^+$ to a suitable daughter ion

Q1 Mass (amu)	Q3 Mass	Dwell (msec)	DP	FP	EP	CE
265.10 (Thiamine)	122.20	50	50	200	10.00	25
345.20 (Thiamine Monophosphate)	122.10	50	50	280	10.00	30
425.20 (Thiamine Diphosphate)	122.20	50	50	280	10.00	30
272.7 (IS)	74.0	50	50	200	10	25

Over-expressing human OCT1 in *C. elegans* and Huh7. The constructs of pINT:GFP, pINT::GFP::OCT1 and pdaf-1::RFP and fat staining were prepared following a previously described method (6, 7). Huh7 cells were transfected using a lipofectamine-mediated method (Life Technologies). 48-hours following transfection, cells were harvested and subject to various analysis.

mRNA Expression Levels. Taqman gene expression assays for all human and mouse genes were performed using Applied Biosystems 7500 Fast Real-Time PCR System using inventoried probes (Applied Biosystems, Foster City, CA, USA). The relative expression level of each mRNA transcript was calculated by the comparative method (ΔΔCt method) β-Actin was used as housekeeping gene for normalization (3).

Western blotting, tissue staining and flow cytometry. For Western blotting, cultured cells or tissues were analyzed using established procedures (3). The primary antibodies were anti -AMPK complex,-P-ACC, -ACC, -LKB1, -PDH, -FASN, -β-Actin (Cell Signaling Technology, Danvers, MA), -OGDH, -TPK1 and -TKT (Proteintech, Chicago, IL, USA). The secondary anti-mouse or rabbit HRP antibodies were obtained from Cell Signaling Technology. For the quantification of Western blot bands, the ImageJ method was used (http://rsb.info.nih.gov/ij/index.html). For immunostaining, tissue sections from frozen or paraffin-embedded mouse tissue slides were used (3). The antibodies were anti-glutamine synthetase (GS) (Millipore, Billerica, MA, USA), -human OCT1 (Abcam, Cambridge, MA), -β-Gal and –pyruvate dehydrogenase (PDH) (Cell signaling). The secondary antibodies Alexa Fluor® 488 or 594 anti-rabbit or mouse were from life technologies. For paraffin embedded sections, the tissue sections were placed in a rack, and the following dewax procedures were performed: 1. Xylene: 2 x 3 min; 2. Xylene 1:1 with 100% ethanol: 3 min;

3. 100% ethanol: 2 x 3 min; 4. 95% ethanol: 3 min; 5. 70 % ethanol: 3 min; 6. 50 % ethanol: 3 min; 7. Running cold tap water to rinse. For the frozen tissue sections, sections were soaked in 1XPBS for 15 min at room temperature before the staining. Prior to antibody application, sections were steamed in 0.01 M citric acid buffer (pH 6.0) for 15 min then cooled in 1X PBS for 15 min at room temperature. Sections were blocked in 10% lamb serum and 0.5% triton-X100 for 45 min, then incubated with rabbit primary antibodies for 1 hour or overnight at 4 °C. Following the 1st antibody incubation, sections were washed in PBS and then incubated for 1 hour with secondary antibodies following 3X10 min washes in 1XPBS. The resulting sections were placed on coverslips with Aqueous Mounting Medium (Thermal Fisher). The nucleus was stained with diamidino-2-phenylindole (DAPI). 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal) (Sigma) staining followed a published method (8), Briefly, tissue samples were fixed in 1% paraformaldehyde in 1× PBS for 60 min and then washed several times in rinse buffer (0.1 M NaPi [pH 7.4], 2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02% NP-40). Samples were then stained in 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal) buffer (5 mM potassium ferrocyanide [$K_4Fe(CN)_6$], 5 mM potassium ferricyanide [$K_3Fe(CN)_6$], 1 mg of X-Gal/ml) for 24 h in an incubator set at 30°C. Stained samples were rinsed in 10% formalin and stored in 1× PBS prior to mounting and imaging. All the pictures were taken using a Retiga CCD-cooled camera and associated QCapture Pro software (QImaging Surrey, BC Canada). For flow cytometry, the primary hepatocytes were fixed with 1% PFA for 30 minutes and analyzed on a BD FACSCalibur Flow Cytometer (BD Bioscience, San Jose, CA, USA).

ELISA Assays for P-AMPK and P-ACC. ELISA kits to quantify P-AMPK and P-ACC were used in experiments with hepatocytes. The ELISA kits are available from Cell Signalling Technology: P-AMPK ELISA (PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Kit) and P-ACC ELISA (PathScan® Phospho-Acetyl-CoA Carboxylase (Ser79) Sandwich ELISA Kit). The protocols described in their product inserts were followed. For maximum effect, the amount of protein required for optimum results are 25 μg for P-AMPK and 75 μg for P-ACC.

Transport studies. Transport kinetic studies were performed in transfected HEK293 cell lines as described previously using radiolabeled substrates and unlabeled inhibitors (1). V_{max}, K_m and IC_{50} values were assessed by fitting the data using GraphPad Prism version 4.02 (GraphPad).

Free Fatty Acid (FFA) β-Oxidation, liver enzyme and Nucleotide Assays. FFA oxidation was assessed in mouse hepatocytes by measuring the production of $^{14}CO_2$ from [1-^{14}C] palmitic acid

following a published method (9) except the William E medium was replaced with DME H-21. The specific activity of [1-^{14}C] palmitic acid (Perkin Elmer) used in the study was 60 mCi/mmol. AMPKi is an acronym for AMPK inhibitor, also known as compound C. It is a cell-permeable pyrrazolopyrimidine compound that inhibits KDR/VEGFR2, ALK2/BMPR-I, AMPK kinase activity (IC_{50} = 25.1, 148, and 234.6 nM, respectively) (http://www.millipore.com/catalogue/item/171260-1mg). For FFA β-oxidation in liver homogenates, a Potter-Elvejhem homogenizer was used with 0.5 g of liver manually homogenized in 10 mL of ice-cold isolation buffer (220 mM mannitol, 70 mM sucrose, 2 mM HEPES, 0.1 mM EDTA pH 7.4). Aliquots of homogenate (300 µl) were added to 1.7 ml of reaction medium (50 mM sucrose, 150 mM Tris-HCl, 20 mM KH_2PO4, 10 mM $MgCl_2$-$6H_2O$, 2 mM EDTA, 1 mM L-carnitine, 0.2 mM CoA, 2 mM NAD, 0.1 mM malate, 10 mM ATP, 1 mM Palmitate complexed to fatty acid-free albumin at a 5:1 molar ratio in DME H21, 0.3 µCi of [1-^{14}C]-palmitate (Amersham Biosciences), pH 7.4. Reactions performed in a sealed flask were allowed to proceed for 30 min in a shaking water bath at 37°C. Incubations were terminated by the addition of 1 ml of 3 M perchloric acid to the reaction medium to precipitate protein and non metabolized palmitate and then further incubated at room temperature for 2 h for collection of $^{14}CO_2$ into a suspended well containing 500 µl of ethanolamine. Blanks were prepared by acidification of the reaction medium immediately after the addition of the homogenate. Radioactivity in CO_2 was quantified by liquid scintillation spectrometry.

Serum liver enzyme assays were performed at the clinical chemistry laboratory of the San Francisco General Hospital (San Francisco, CA, USA). ATP production from HEK293 cells or primary hepatocytes was assayed using CellTiter-Glo® luminescent cell viability assay kit (Promega, Madison,USA). A thiamine-deficient DME H21 was used and various thiamine amounts were added from 0 to 4 mg/L (UCSF cell culture facility). Mice were fasted for 16 hours and then liver was collected for hepatic ATP, ADP, and AMP determination. To determine the adenine nucleotide levels, bioluminescent assays were used for quantifying the concentrations of AMP, ADP and ATP (Promega). Protocols from the manufacturer (Promega) were followed to determine the nucleotides levels (ATP: Promega Kinase-Glo®; ADP: ADP-Glo®; and AMP: AMP-Glo®). Liver samples or cells were extracted using lysis buffer provided by the manufacturer.

Magnetic resonance imaging (MRI) of mice liver. Mice fed high fat diets (55% fat calories) for 16 weeks were studied. All ^{1}H magnetic resonance imaging (MRI) was conducted on a 14.1T wide-bore micro-imaging system (Agilent Systems, Cupertino, CA) equipped with a 40mm ^{1}H transmit/receive volume RF coil. Liver volume was quantified using a multi-slice T2-weighted spin

echo sequence (TE=7ms, TR=1.8s) with a FOV of 30 x 30 mm (matrix size of 256 x 192). Percent fat content was assessed using a 2-point Dixon method approach (10) with data acquired with analogues parameters. The liver was segmented in both sequences and these regions of interest were used to calculate both volume and percent fat.

Data analysis. Data are expressed as mean ± standard error of the mean (Mean ± SEM). For statistical analysis, the Student's t-test was used. The data were analyzed using GraphPad Prism 4.0 (GraphPad Software Inc.). A *P* value less than 0.05 was considered statistically significant, *$P<0.05$, **$P<0.01$ and ***$P<0.001$. For pharmacokinetic studies, area under the plasma concentration time curve (AUC) and half-life ($t_{1/2}$) were calculated from the data by model independent methods using WinNonlin (1).

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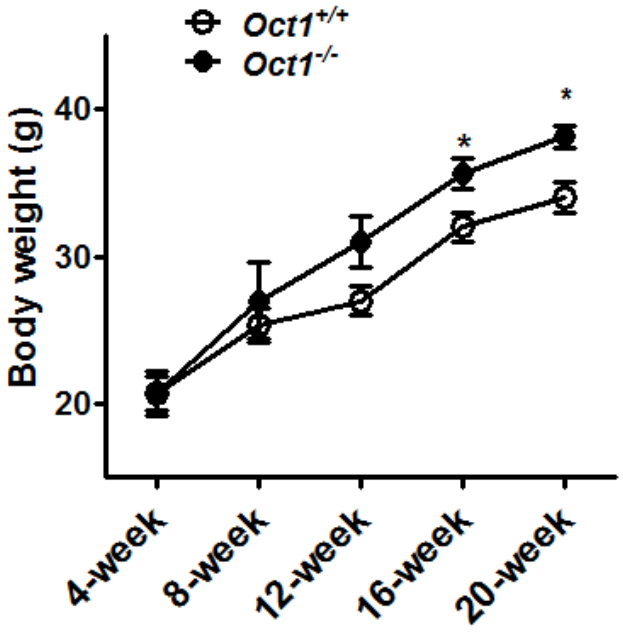
Supporting Information (Supplementary Figures)

Figure S1

S1A



S1B

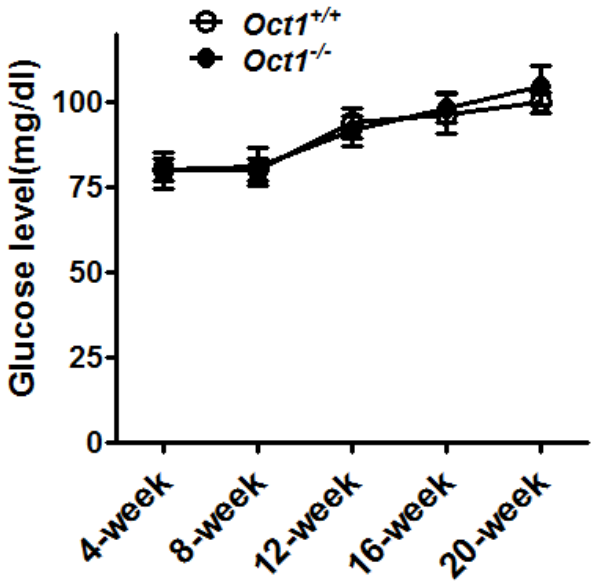
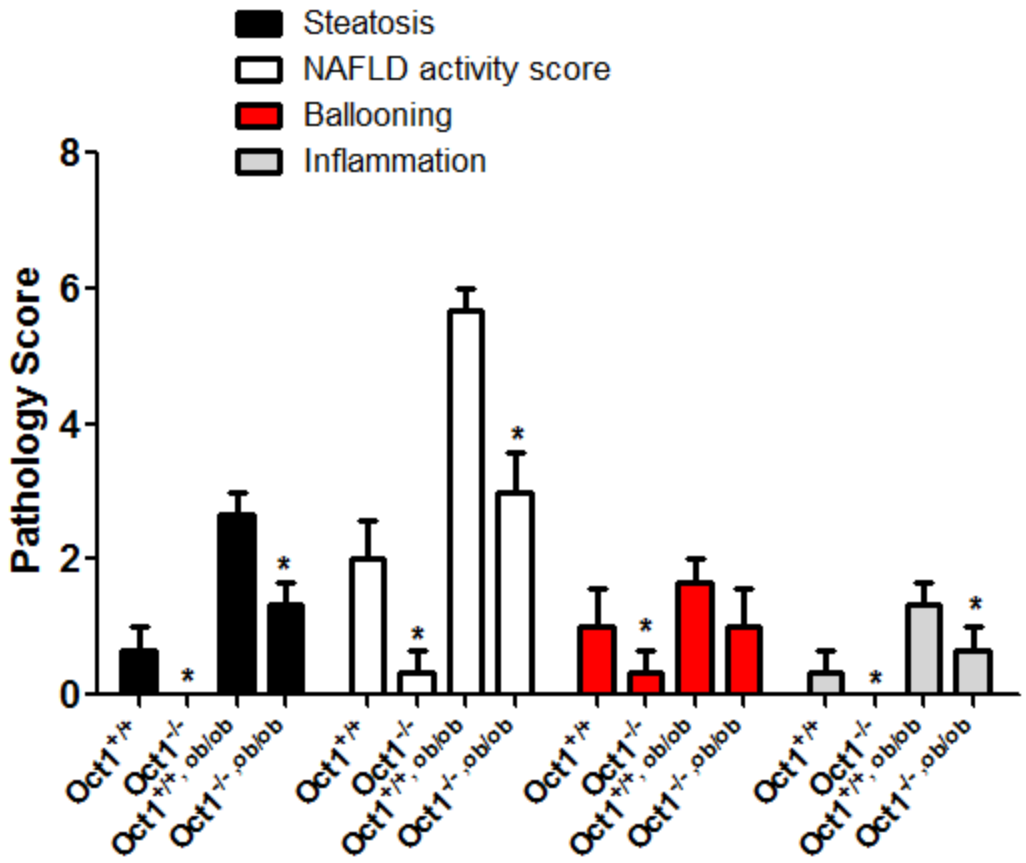


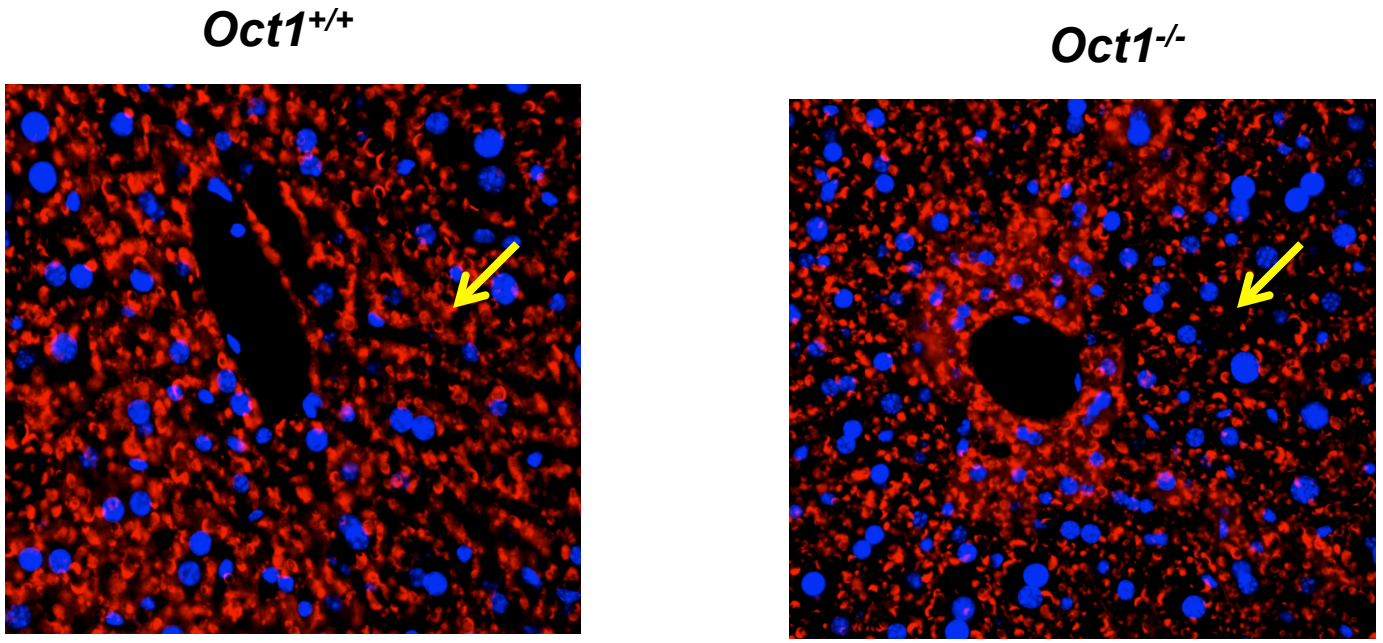
Figure S1. Oct1 deficiency results in changes in liver characteristics including triglyceride levels. *(A)* Time course of body weight of *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice from 4-weeks to 20-weeks. Mice were maintained with free access to water and normal mouse diet. *(B)* Plasma glucose levels from 4-weeks to 20-weeks (n=10/genotype).

S1C



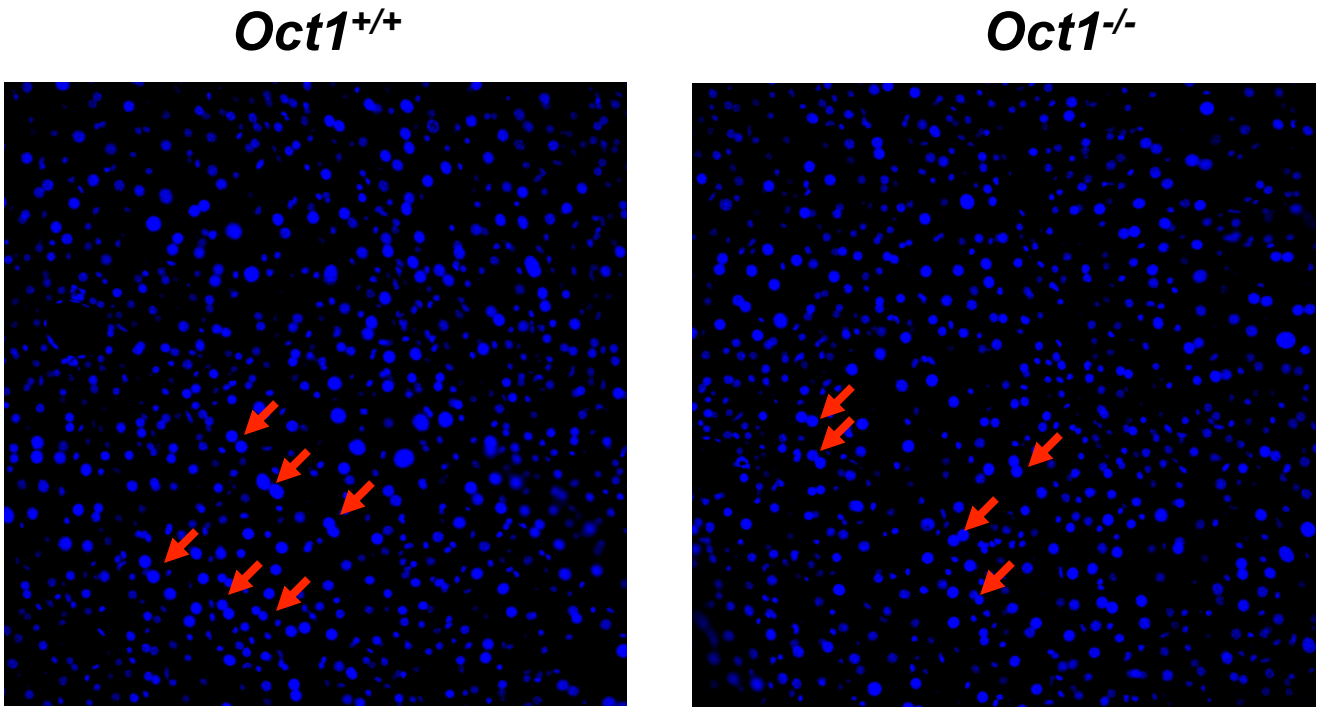
(C) The pathology scores of the livers according to the minimal Kleiner/ Brunt criteria (steatosis, NAFLD, ballooning, inflammation). The steatosis and NAFLD in *Oct1*$^{-/-}$ or *Oct1*$^{-/-,ob/ob}$ mouse livers improved over their wildtype counterpart. *P<0.05, compared with *Oct1*$^{+/+}$ or *Oct1*$^{+/+,\ ob/ob}$ mouse livers.

S1D



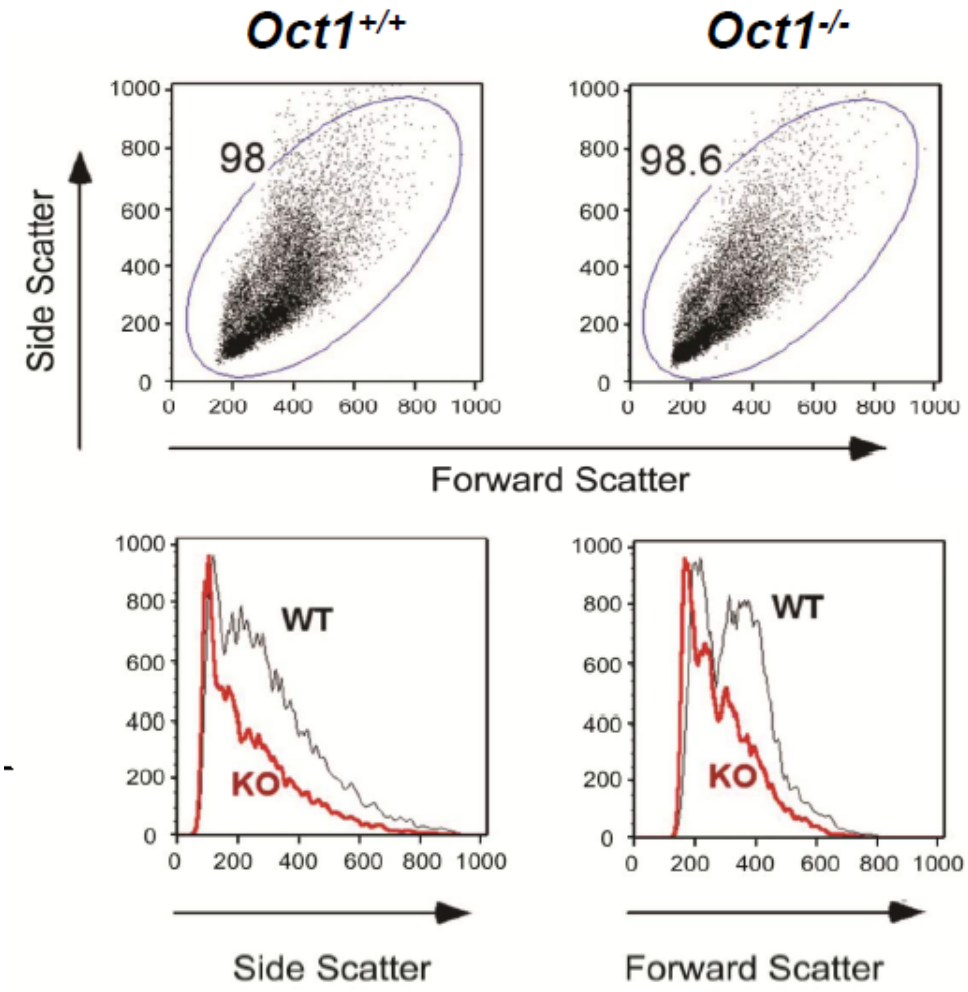
(D) Representative tight junction staining of *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mouse livers with ZO-1. In the area of central vein, the *Oct1*$^{-/-}$ mouse livers showed a slightly looser pattern than wildtype mice (yellow arrow), suggesting that there may be polarity change by losing Oct1.

S1E



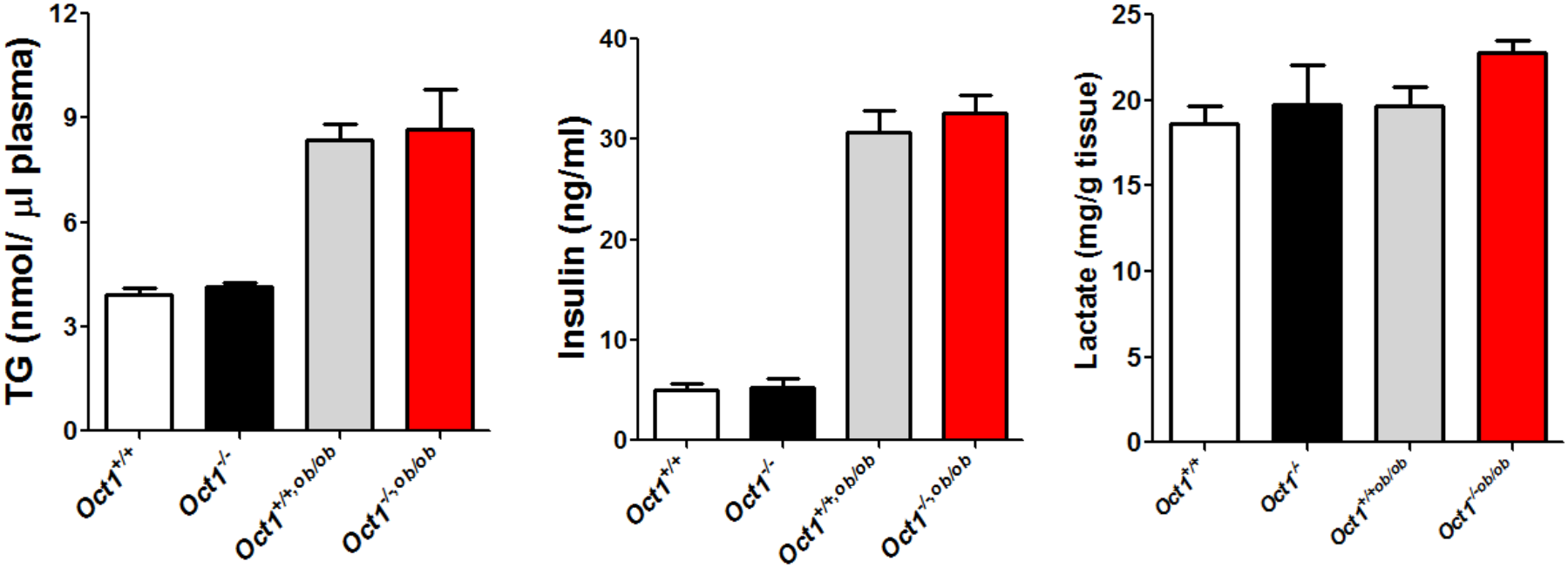
(E) Representative DAPI staining of *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mouse livers. The ploidy of the cells showed no differences between of *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mouse livers, as most hepatocytes were normally binucleate in both mouse lines. Red arrows indicate some of the binucleate cells.

S1F



(F) Representative flow cytometry scans of cell size of primary hepatocytes from *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice. Numbers on the Y- and X- axes indicate cell numbers. Data are mean ± SEM. *$p< 0.05$, **$p <0.01$, ***$p < 0.001$.

S1G



(G) Triglyceride (TG), insulin and lactate levels from the plasma of *Oct1*$^{+/+}$, *Oct1*$^{-/-}$, *Oct1*$^{+/+,ob/ob}$ and *Oct1*$^{-/-,ob/ob}$ mice at 16-weeks (lean mice=10/genotype, ob/ob mice=6/genotype). Mice were fasted for 18 hours and then TG, insulin and lactate were measured respectively.

Figure S2

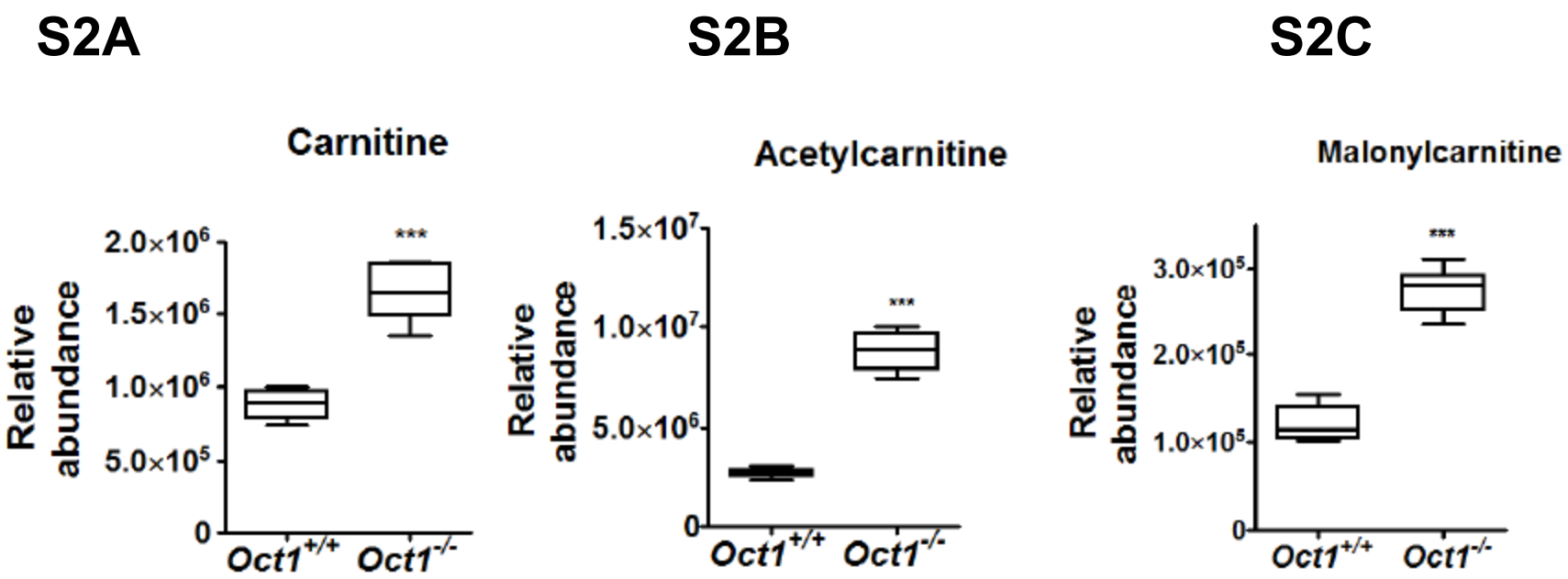
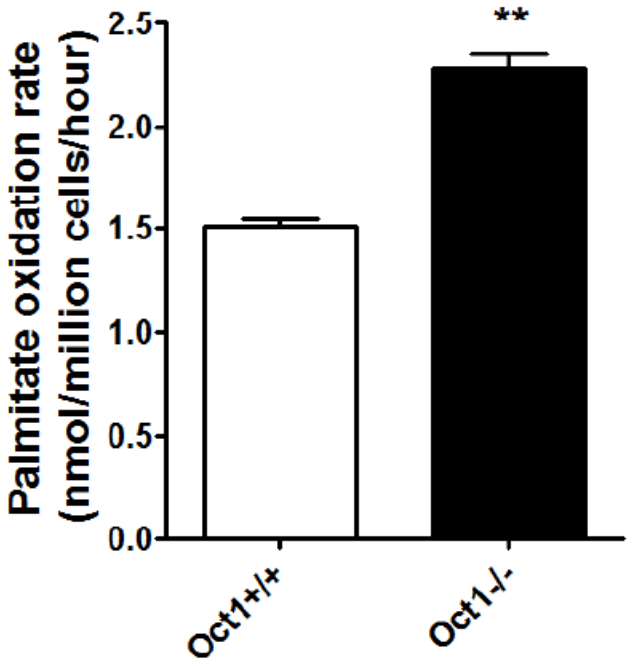
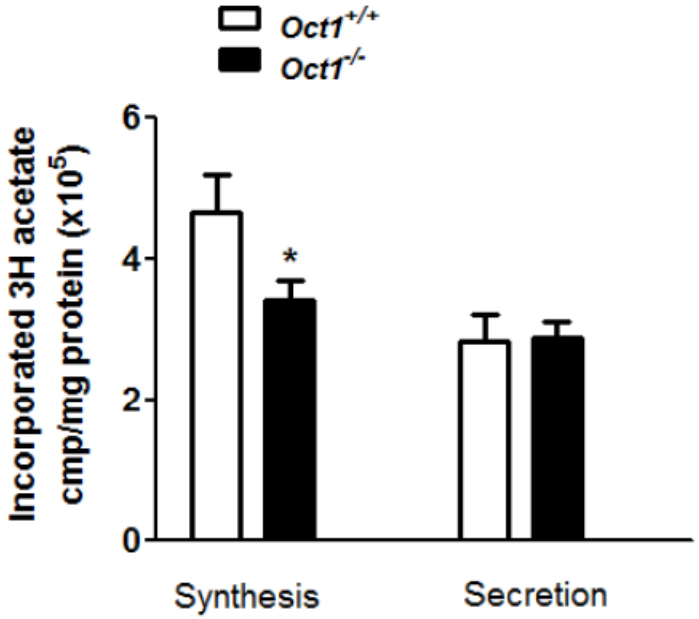


Figure S2. *Oct1*$^{-/-}$ mouse liver exhibit increased fatty acid β-oxidation and AMPK activation. *(A-C)* Results of metabolomic studies of liver extracts from *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice (n=6/genotype). Carnitine, acetylcarnitine and malonylcarnitine represent three of the most significantly elevated metabolites in hepatic extracts from *Oct1*$^{-/-}$ mice.

S2D

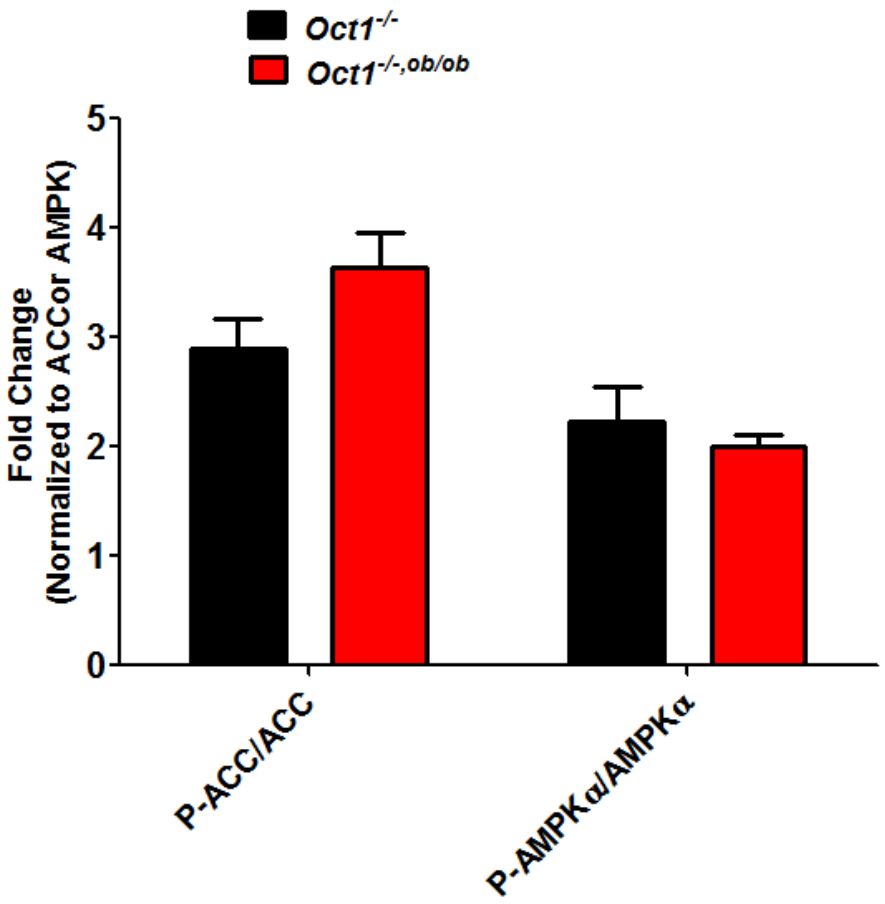


S2E



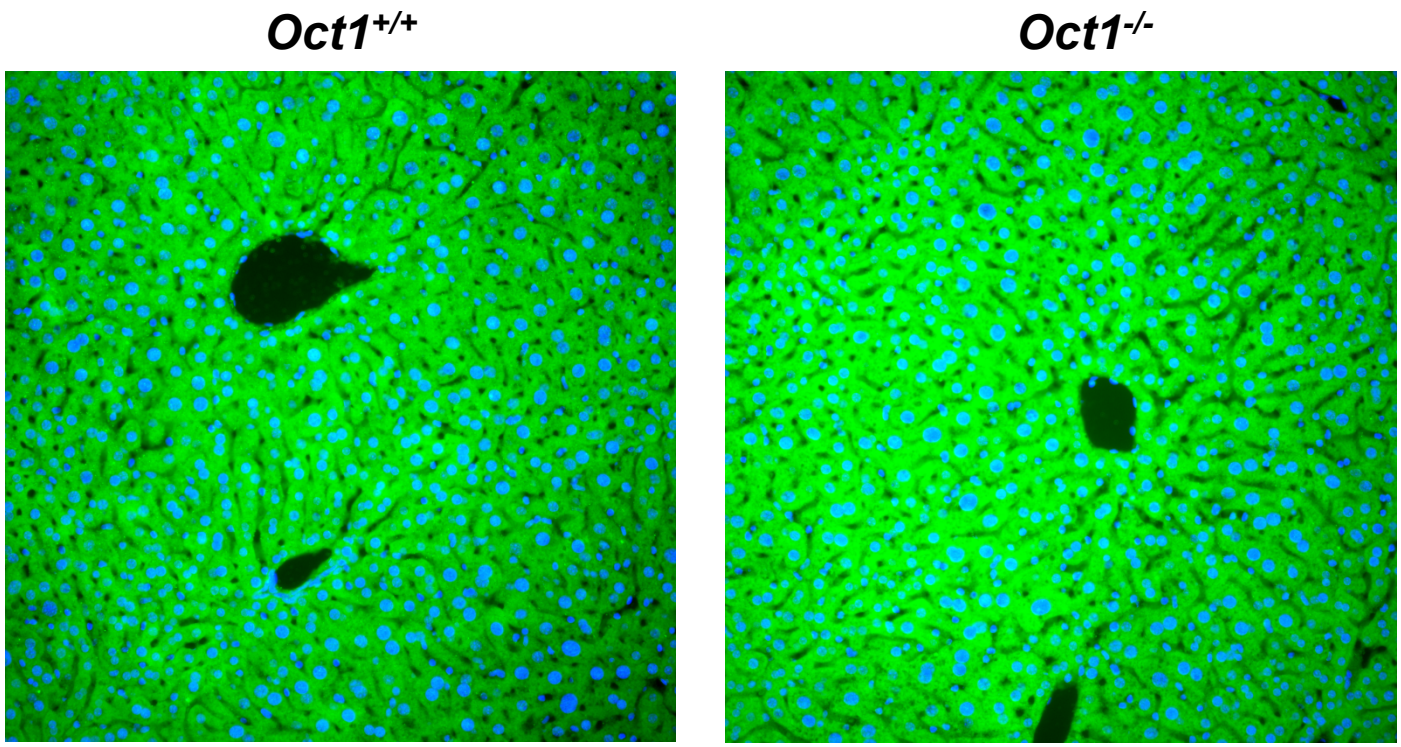
(D and E) Free fatty acid β-oxidation rates (D) and fatty acid synthesis and secretion (E) in primary hepatocytes from *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice (n=6/genotype).

S2F



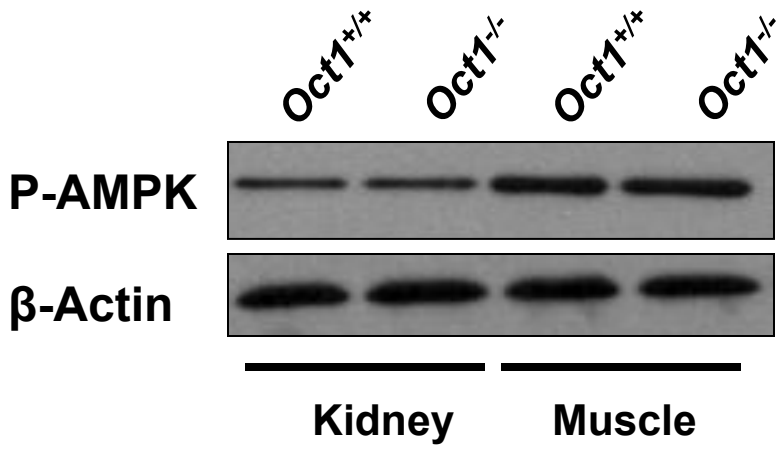
(F) Ratios of P-ACC to ACC and P-AMPKα normalized to AMPKα in livers from *Oct1*$^{-/-}$ and *Oct1*$^{-/-,ob/ob}$.

S2G



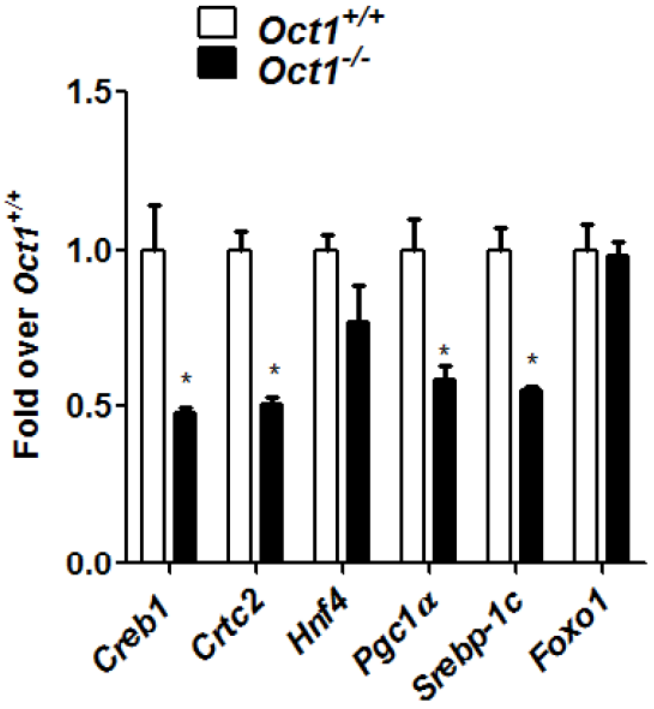
(G) Representative P-AMPK staining of *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mouse livers. The *Oct1*$^{-/-}$ mouse livers showed a globally higher AMPK activation than wildtype mice.

S2H



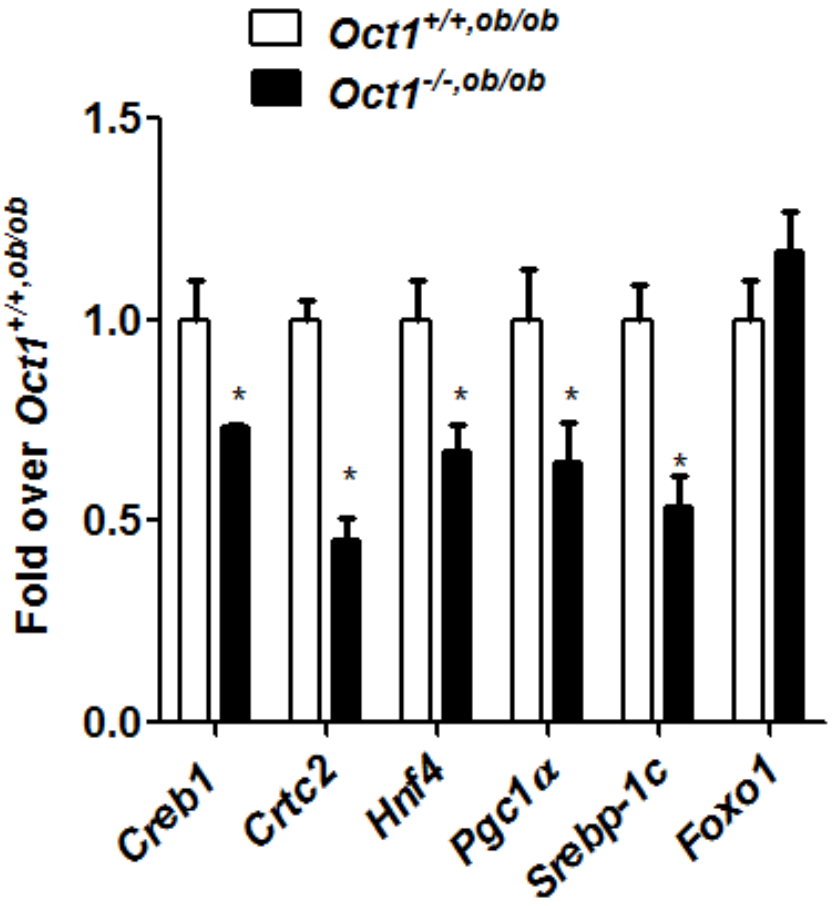
(H) AMPK activation in kidney and muscle of *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice. P-AMPK level in kidney and muscle showed no apparent difference between *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice.

S2I



(I) mRNA expression level of a group of critical transcriptional factors in lipogenesis and gluconeogenesis. Creb1, cAMP responsive element binding protein 1; Crtc 2, CREB regulated transcription coactivator 2; Hnf4α, hepatocyte nuclear factor 4, α; Pgc1α , peroxisome proliferator-activated receptor gamma, coactivator 1 α; Srebp-1c, sterol regulatory element binding protein 1c; Foxo 1, Forkhead box protein O1. The corresponding gene expression levels in WT were set as 1, n=6/genotype. Data are expressed as mean ± SEM. **p< 0.05, **p <0.01, ***p < 0.001*.

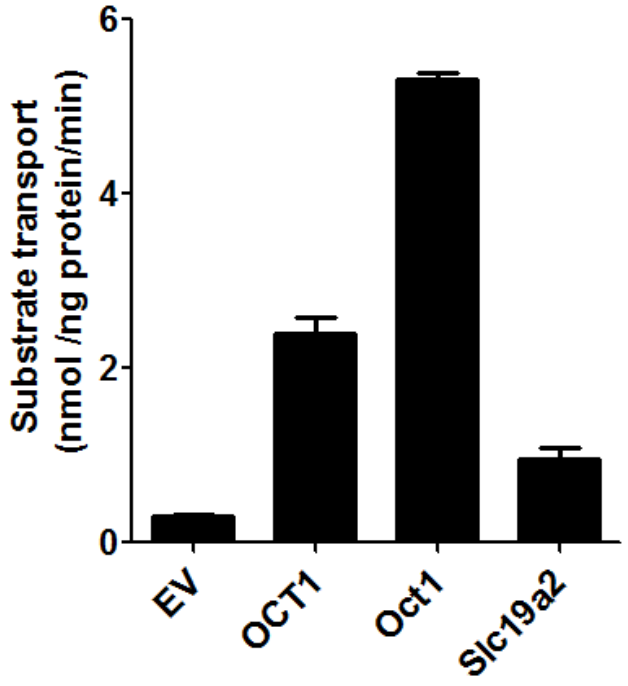
S2J



(J) Expression level of transcriptional factors relative to lipogenesis and gluconeogenesis in *Oct1*$^{+/+,ob/ob}$ and *Oct1*$^{-/-,\ ob/ob}$ mouse livers. Generally, these transcriptional factors were expressed lower in *Oct1*$^{-/-,\ ob/ob}$ mouse livers except *Foxo1* than those of *Oct1*$^{+/+,ob/ob}$ mice, similar to the observations of lean *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice.

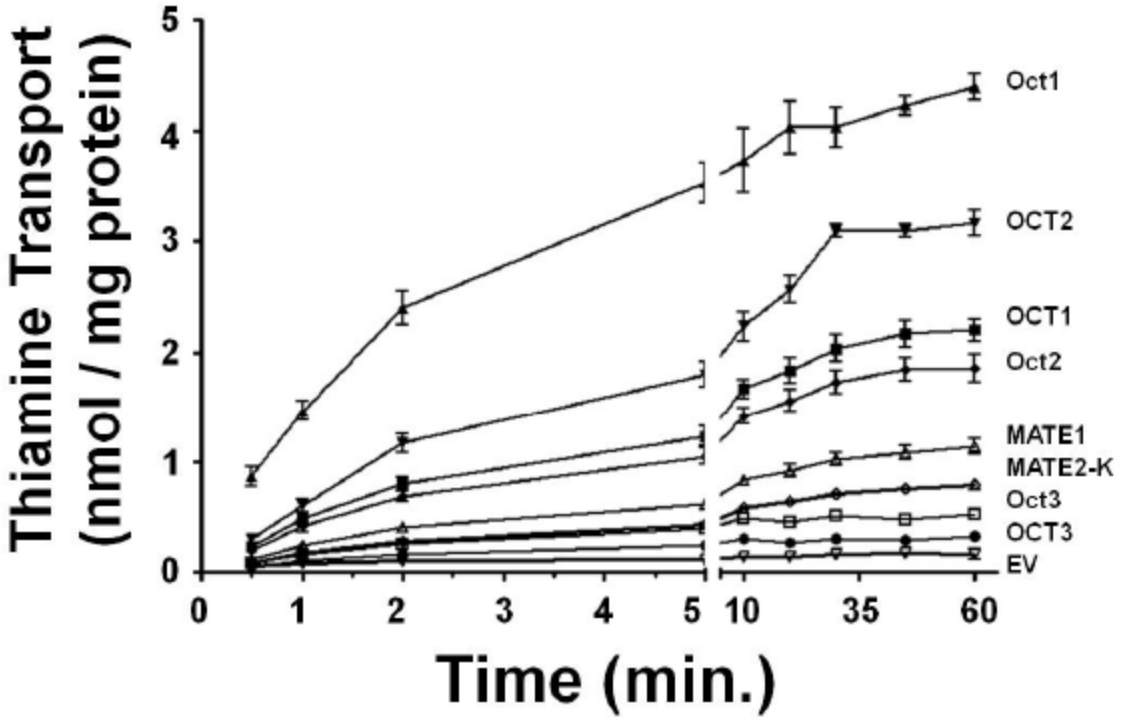
Figure S3

S3A



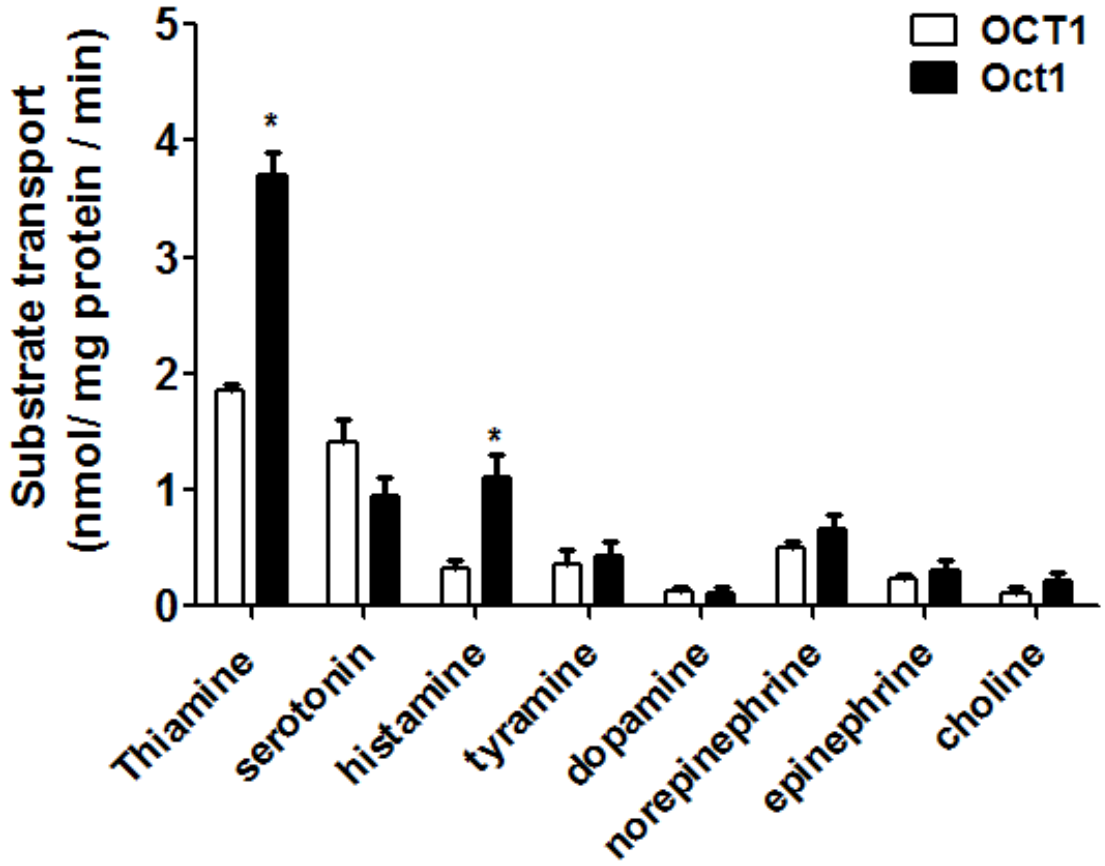
Figures S3. Thiamine uptake by various thiamine transporters. *(A)* Uptake of thiamine (100 nM) by human OCT1, mouse Oct1 and Slc19a2. 100nM [^{3}H]thiamine and 90 nM cold thiamine were added to the uptake solution. Uptake was terminated at 10 min.

S3B



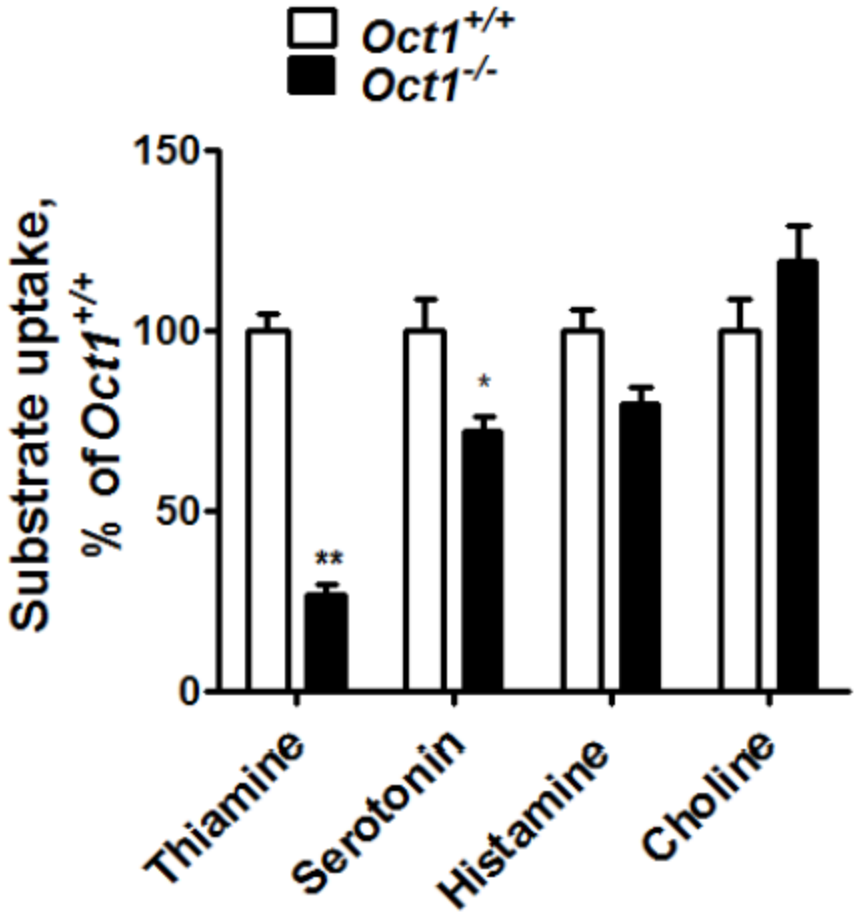
(B) Time course of [^{3}H]thiamine uptake by human OCT1-3, MATE1 and MATE2-K, mouse Oct1-3.

S3C



(C) Endogenous substrate uptake by HEK-OCT1 and HEK-Oct1 cells**.** Substrates were incubated for 10 min at 37 °C. Data are expressed as specific activity (activity of HEK293-OCT1 or Oct1 reference – activity of HEK293-EV, mean ± SEM). *$P<0.05$, compared to human OCT1.

S3D



(D) Uptake of various endogenous amines by *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ primary hepatocytes. All data are mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure S4

S4A

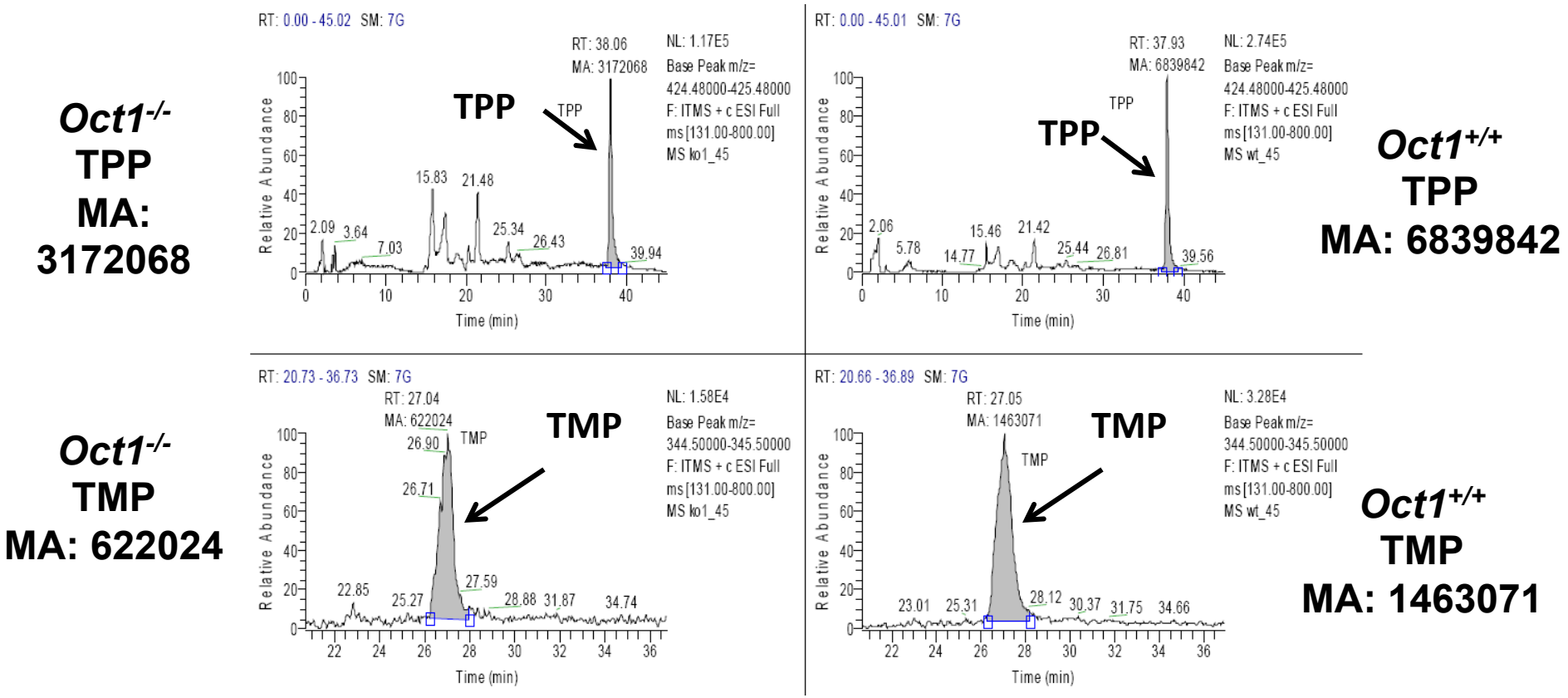
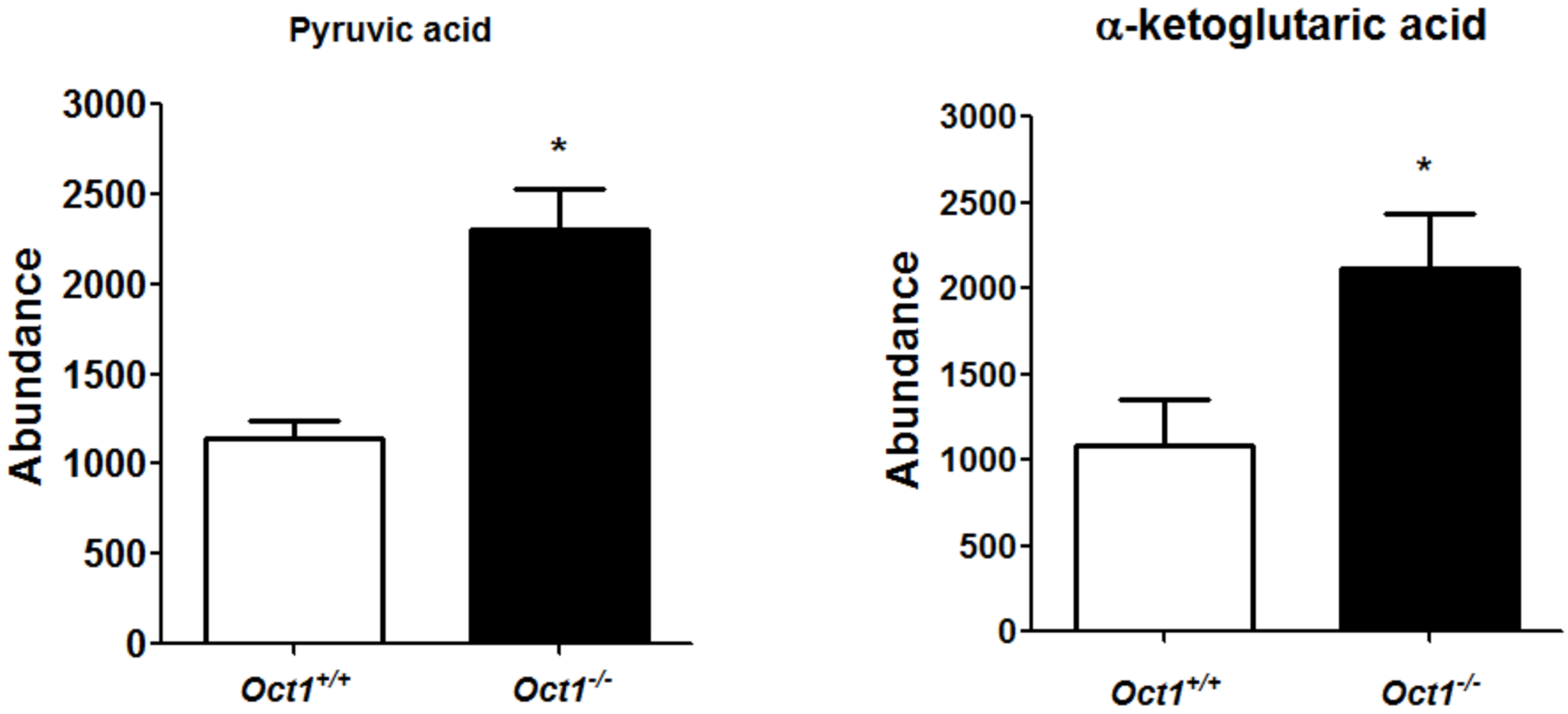


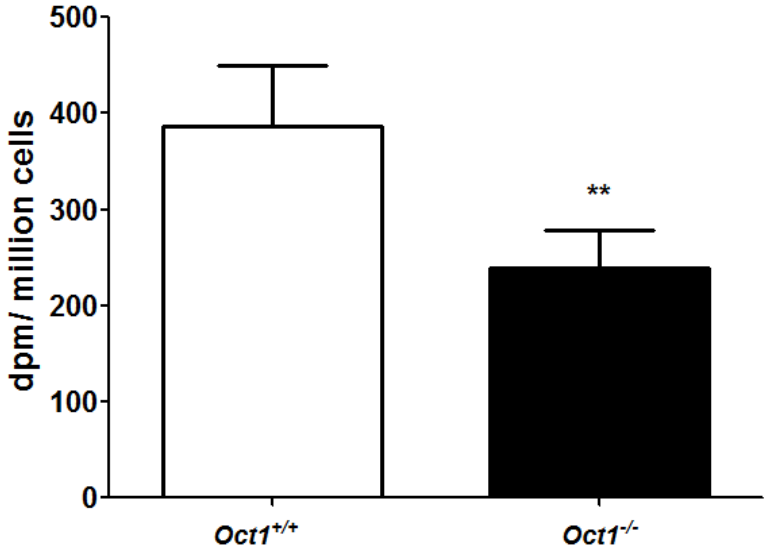
Figure S4. Role of OCT1 in thiamine disposition. *(A)* Quantification of TMP and TPP in mouse liver extracts from *Oct1*$^{+/+}$and *Oct1*$^{-/-}$ mice (n=6/ genotype). Representative LC/MS spectrum picture. MA, measured area.

S4B



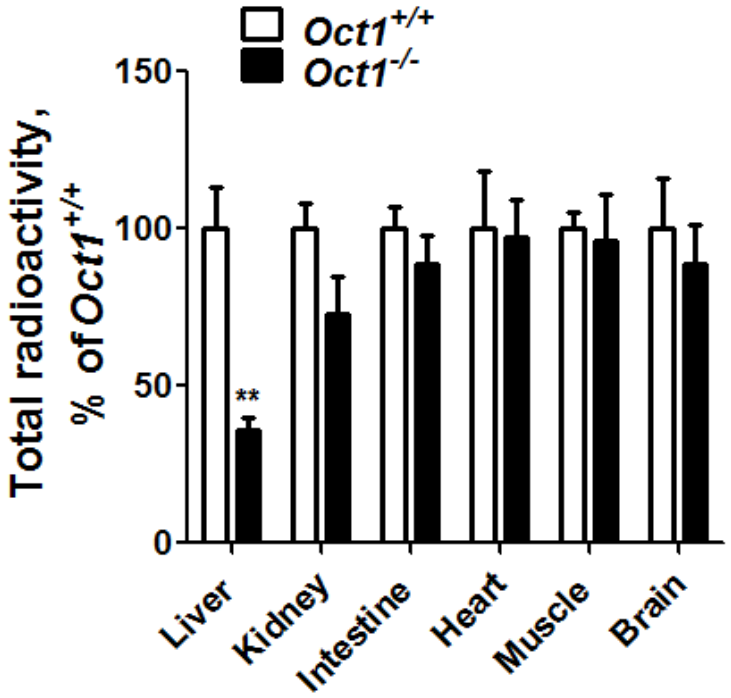
(B) Levels of pyruvic acid and α-ketoglutaric acid in *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice. The major substrates, pyruvic acid and α-ketoglutaric acid, of TPP-associated major enzymes (PDH and OGDH) were measured with LC-MS/MS. The levels of pyruvic acid and α-ketoglutaric acid in the were elevated in the serum of *Oct1*$^{-/-}$ mice, consistent with TPP insufficiency in the livers of *Oct1*$^{-/-}$ mice. 3 gender (male) and age (16- to 20-week) matched mice from each genotype were used in the study.

S4C



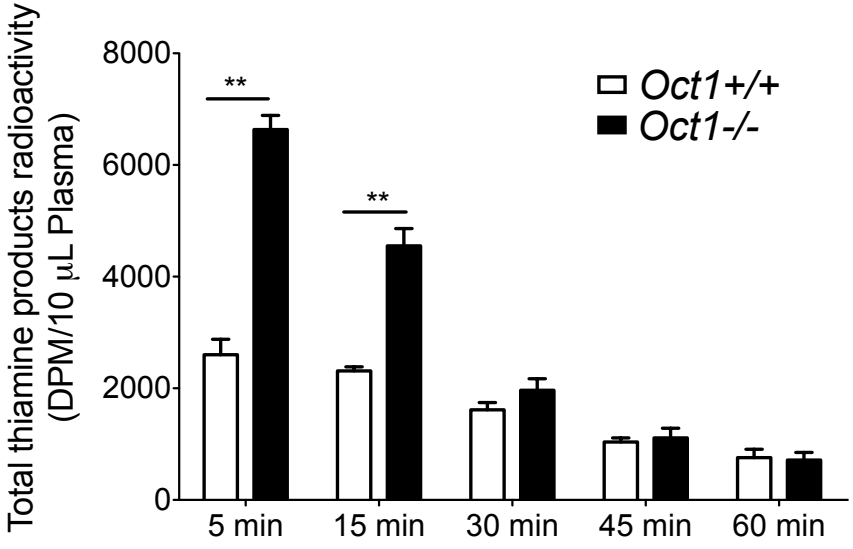
(C) Glycolysis rate in *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ primary hepatocytes. D- [U-^{14}C]-Glucose was added to the DME H21 medium (4g/L, 22.2mM). The [^{14}C]CO_2 gases were collected on the top of the dish. After two hours incubation, the radioactivity of the filter paper associated with [^{14}C]CO_2 was counted. The final production from the glucose oxidation was measured by the release of the [^{14}C] CO_2. The radioactivity from *Oct1*$^{-/-}$ hepatocytes was about 36% less than that from the wildtype hepatocytes. 3 gender (male) and age (20-week)-matched mice from each genotype were used in the study.

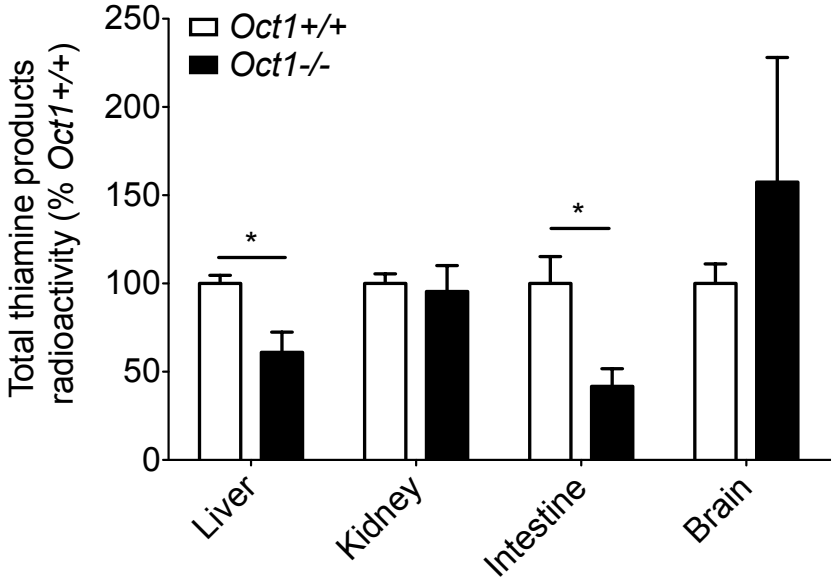
S4D



(D) Total [^{3}H]thiamine radioactivity in various tissues from *Oct1+/+* and *Oct1-/-* mice one hour after administration of an intravenous dose of [^{3}H] thiamine. Data were normalized to WT in the corresponding tissues, which were set at 100%.

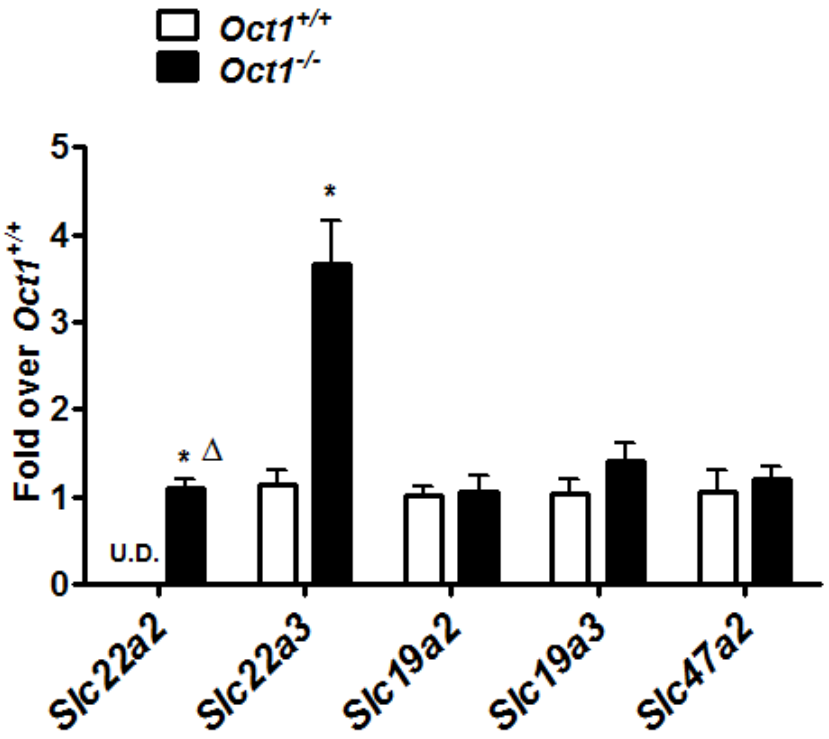
S4E

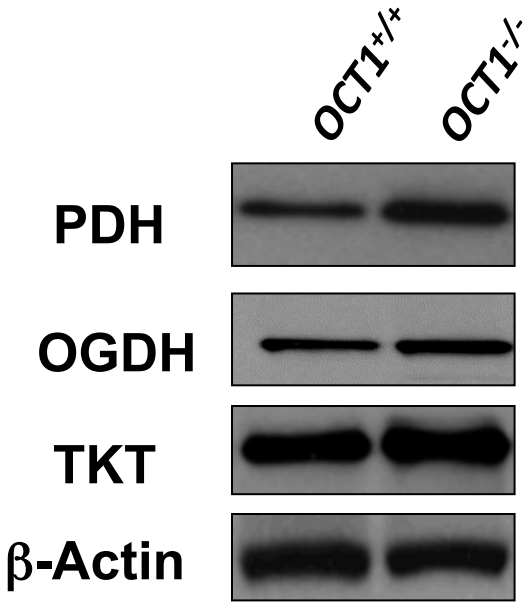




(E) Oct1$^{+/+}$ mice were treated with thiamine (2mg/kg) intraperitoneal (i.p.) injection. Age-matched C57BL/6J mice (*Oct1+/+*, N=3; *Oct1-/-*, N=4) were used in this study. Tissue accumulation of thiamine were performed after 1 hour dosing.

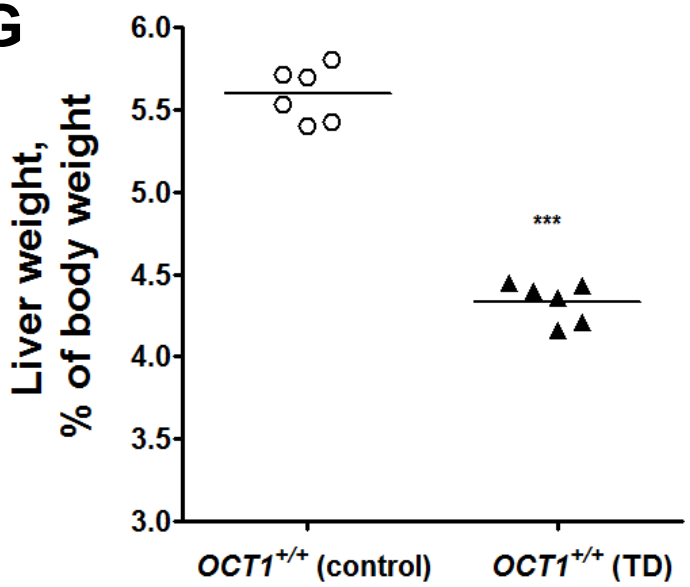
S4F



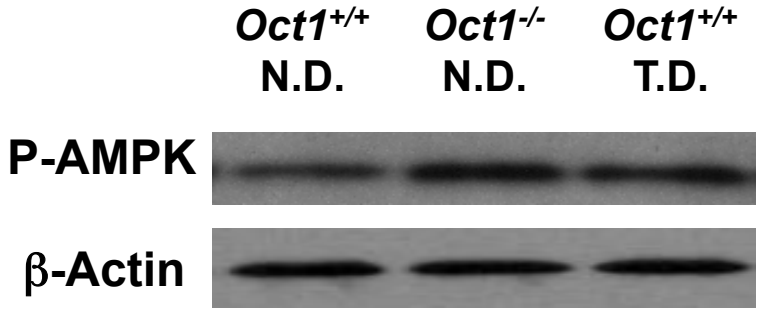


(F) Left: Expression levels of thiamine transporters in the liver of *Oct1*$^{-/-}$ mice. U.D., undetected; Δ, *Oct2* mRNA of KO mice was stimulated to the similar level of Oct3 in WT. *$P<0.05$, compared to the WT mice. *Right:* Regulations of enzymes using TPP as co-factor. PDH, pyruvate dehydrogenase subunit; OGDH, α-ketoglutarate dehydrogenase; TKT, transketolase.

S4G



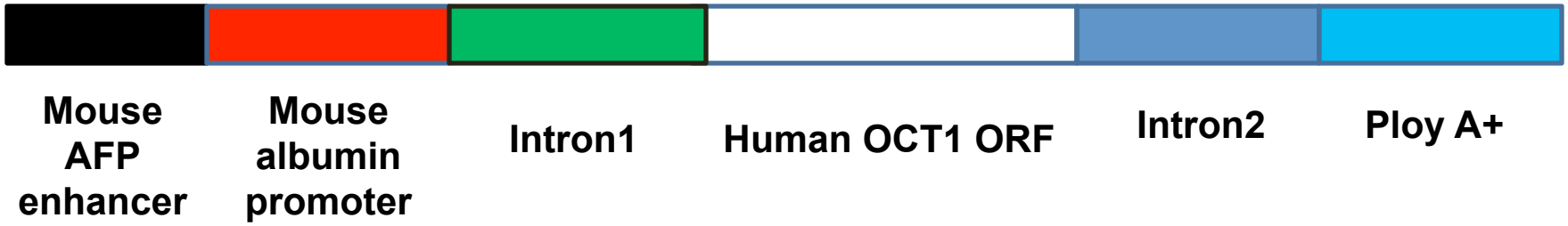
S4H



(G) Effect of dietary thiamine levels on liver weight (normalized to body weight) in pair-fed wildtype mice fed a control and a thiamine deficient (TD) diet (n=6 mice/group, ****P<0.001*). *(H)* AMPK activation in livers from *Oct1*$^{+/+}$ (Normal Diet, N.D.), *Oct1*$^{-/-}$ (Normal Diet, N.D.) and *Oct1*$^{+/+}$ (Thiamine Deficient Diet, TD). Thiamine deficiency resulted in similar AMPK activation in wildtype mouse livers as in *Oct1*$^{-/-}$ mouse livers. 3 gender (male) and age (20-week)-matched mice from each genotype were used in the study.

Figure S5

S5A



S5B

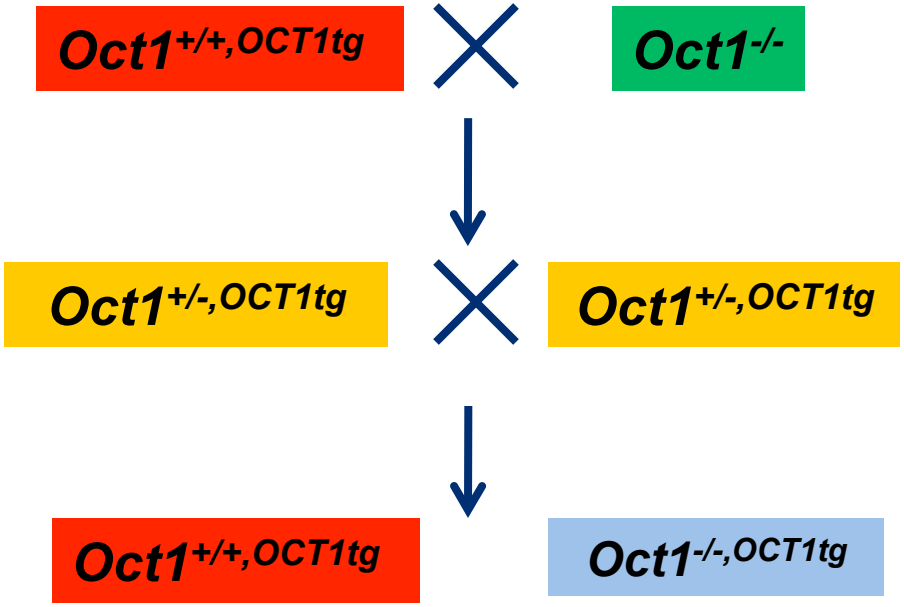
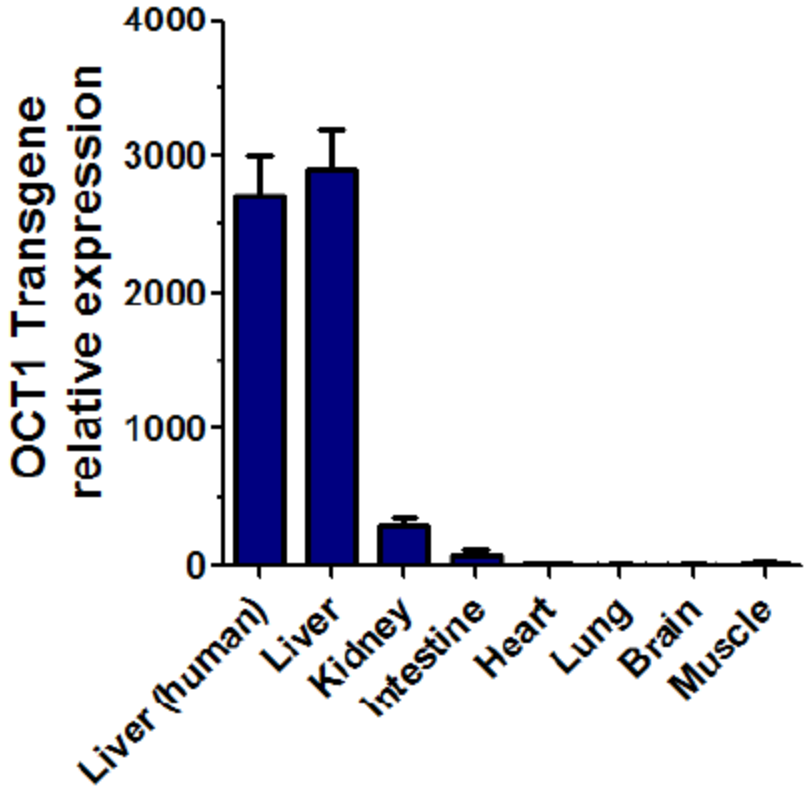


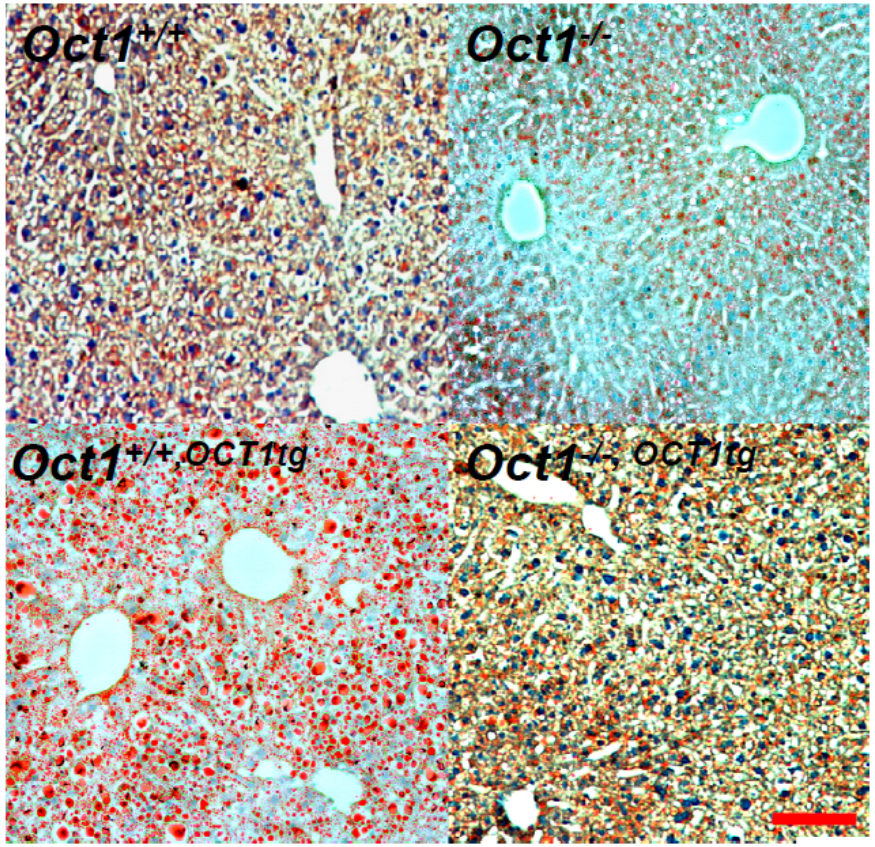
Figure S5. *OCT1* transgenic mice. *(A)* Construct of liver-specific expression human OCT1. *(B)* The breeding strategy for humanized OCT1 mice under *Oct1*$^{-/-}$ background.

S5C



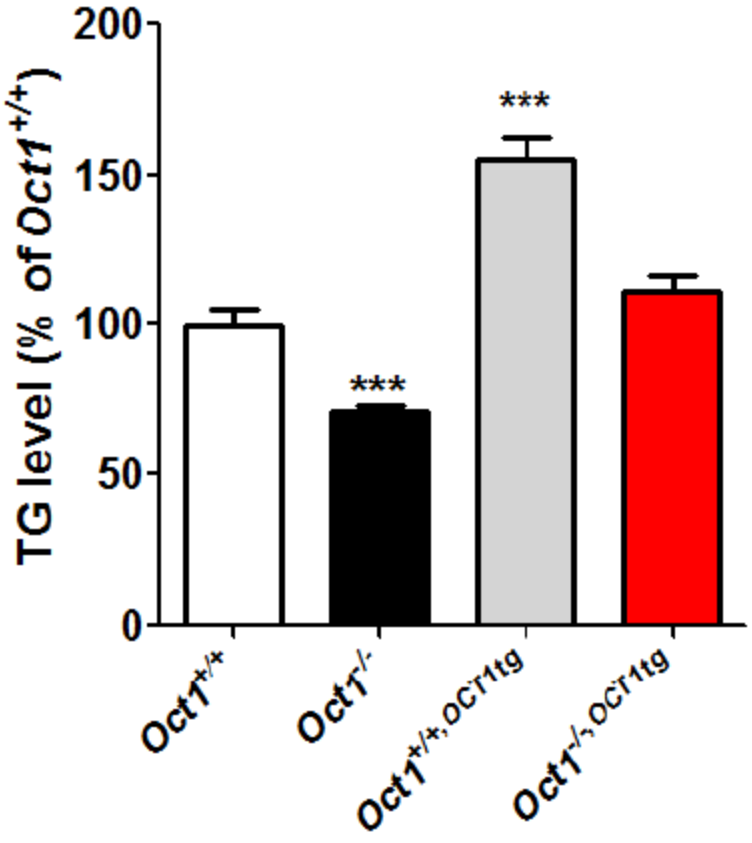
(C) Human *OCT1* mRNA expression levels in various tissues in *Oct1*$^{+/+,\ OCT1tg}$ mice and human liver, relative to OCT1 expression level in mouse lung, set = 1.

S5D



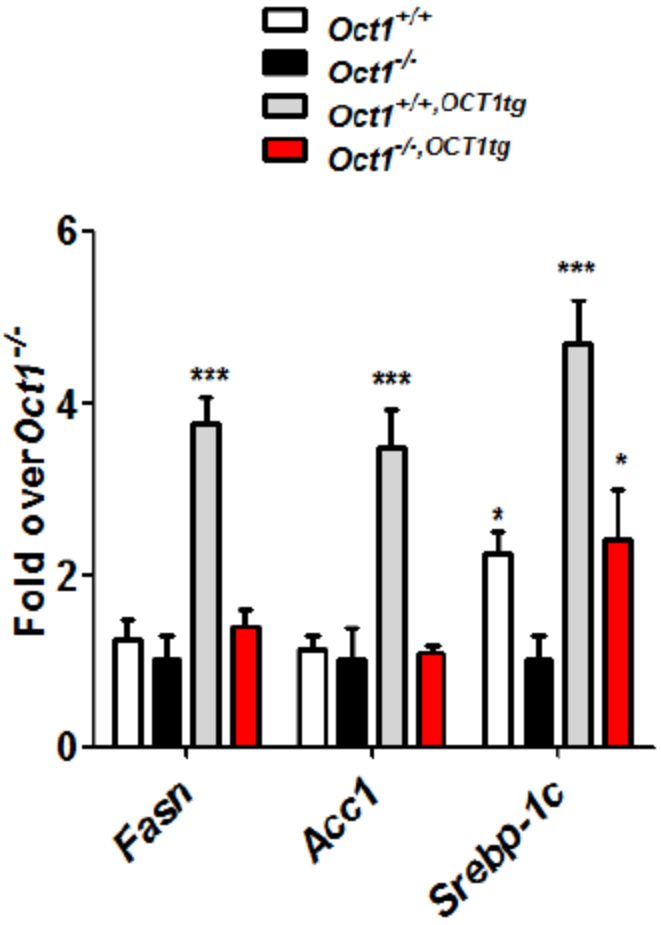
(D) Representative ORO staining of livers sections from *Oct1*$^{+/+}$, *Oct1*$^{-/-}$, *Oct1*$^{+/+,\ OCT1tg}$ and *Oct1*$^{-/-,\ OCT1tg}$ mice fed normal diet. Scale bar, 25 μm.

S5E



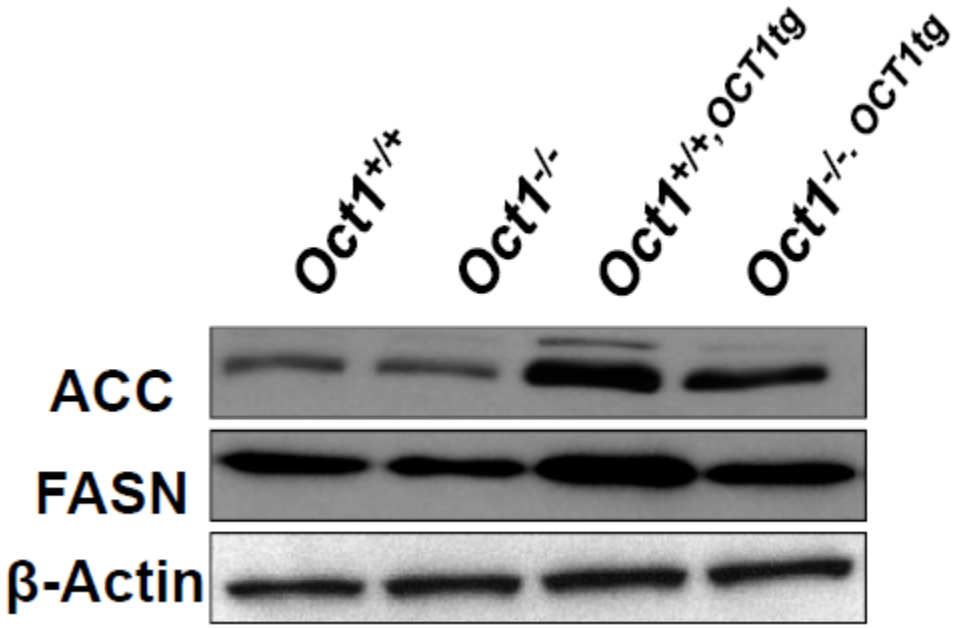
(E) TG levels in livers from *Oct1*$^{+/+}$, *Oct1*$^{-/-}$, *Oct1*$^{+/+}$, ^{OCT1tg} and *Oct1*$^{-/-,}$ ^{OCT1tg} mice.

S5F



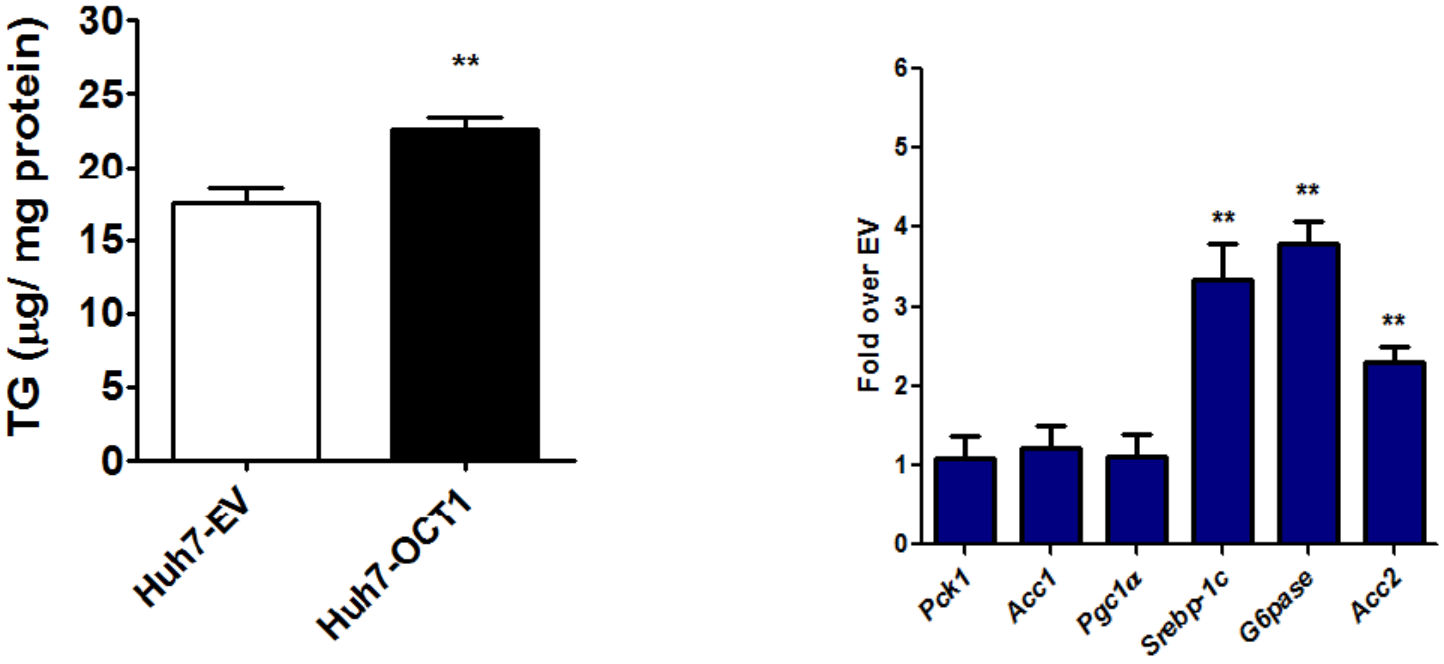
(F) mRNA expression levels of the key lipid metabolism transcription factor *Srebp1-c* and fatty acid synthesis enzymes, *Acc1* and fat acid synthase (*Fasn),* in 4 mouse strains. Expression levels were normalized to *Oct1*$^{-/-}$ mice.

S5G



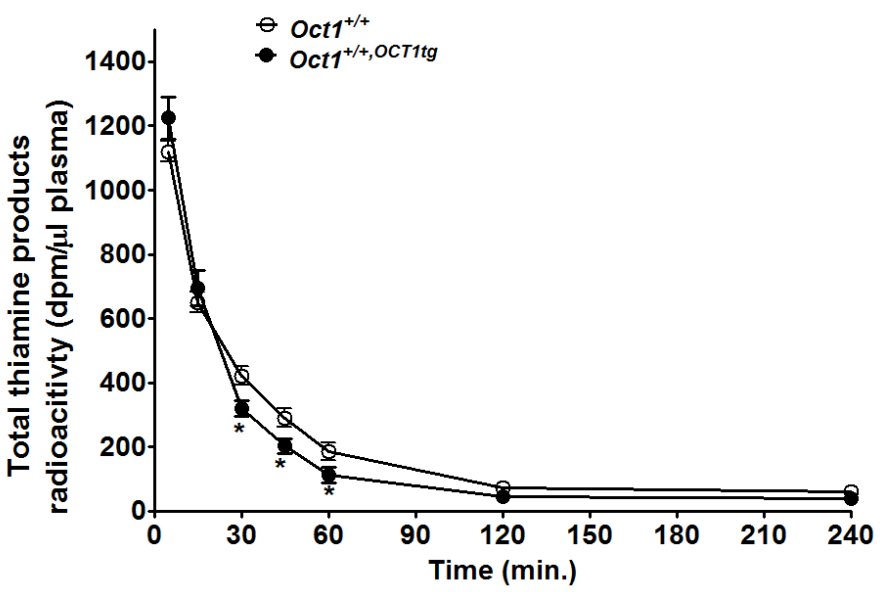
(G) Western blot of ACC and FASN to confirm the protein expression in 4 mouse strains.

S5H

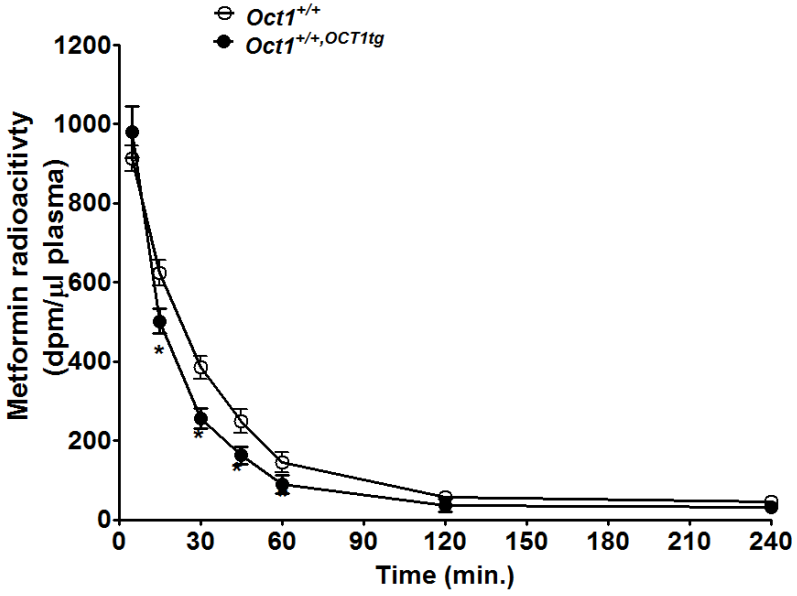


(H) Over-expressing human OCT1 in human hepatocarcinoma cell lines, Huh7. Left: TG levels in pcDNA5-EV and pcDNA5-OCT1 transfected Huh7 cells. All data are mean ± SEM. *$p< 0.05$, **$p <0.01$, ***$p < 0.001$. Right: Critical lipid metabolism and gluconeogenesis gene regulation in human OCT1-transfected Huh7 cells. **$P<0.01$, compared with the expression levels of corresponding genes in EV-transfected Huh7.

S5I

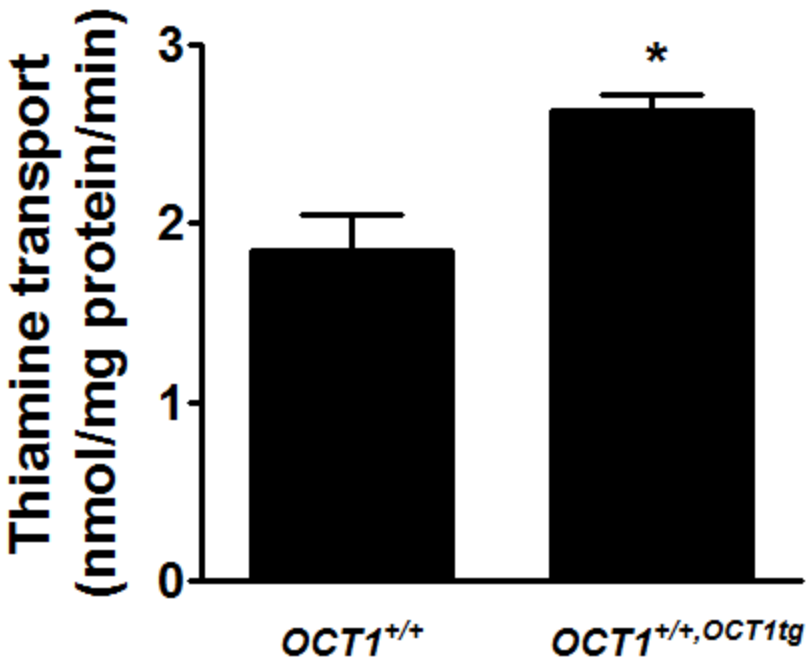


S5J

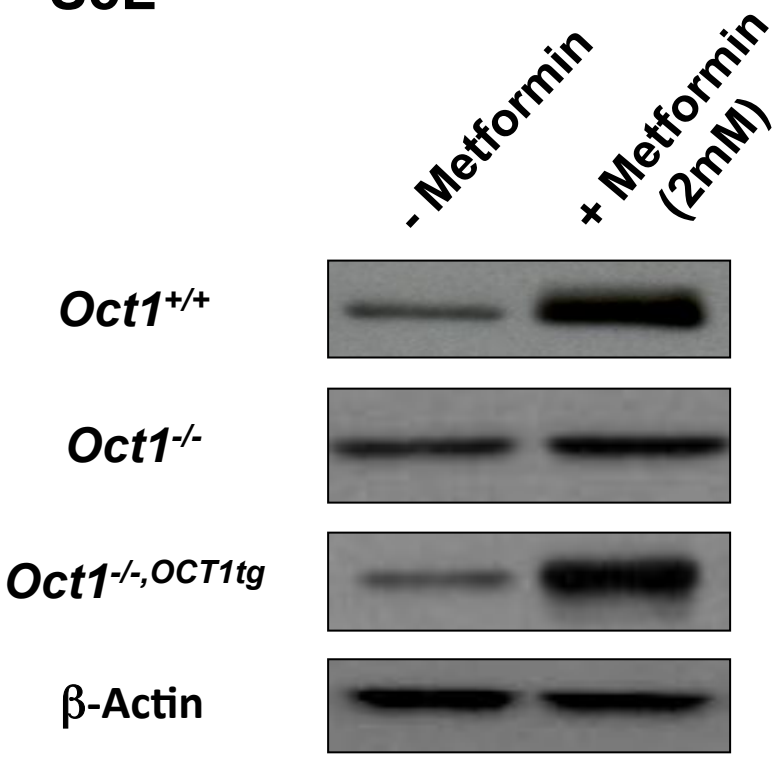


(I and J) Oct1$^{+/+}$ and *Oct1*$^{+/+,OCT1tg}$ mice were given thiamine or metformin intravenously. In *Oct1*$^{+/+,OCT1tg}$ mice, lower plasma levels between 30 min to 60 min or 20 min to 60 min, respectively, were observed in comparison to plasma levels in *Oct1*$^{+/+}$.

S5K

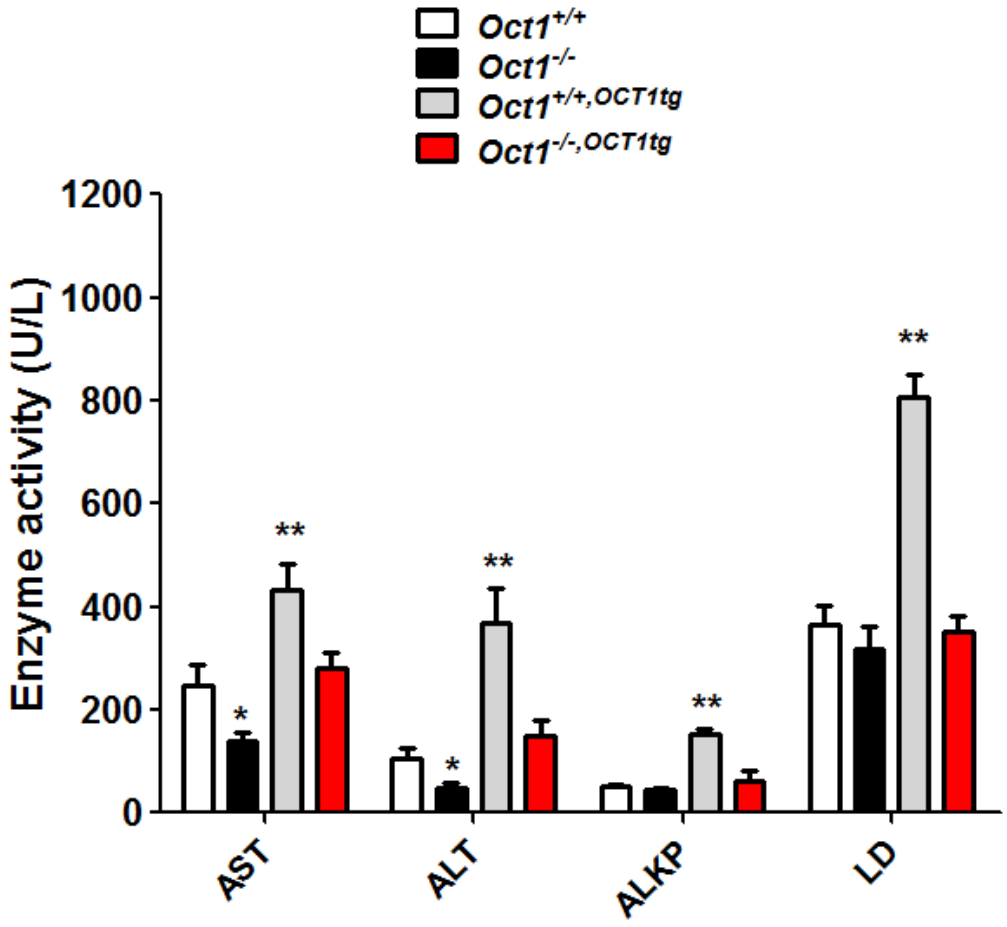


S5L



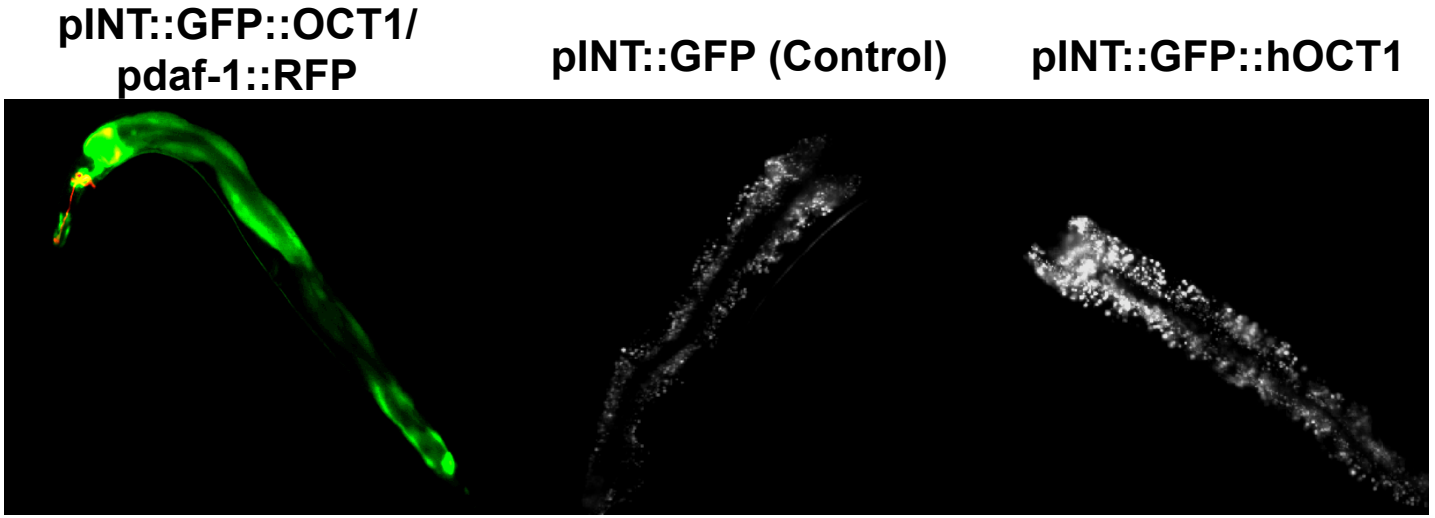
(K) Thiamine transport by *Oct1*$^{+/+}$ and *Oct1*$^{+/+,OCT1tg}$. Human OCT1 transgenic hepatocyte showed more than 32% higher uptake rate than that of *Oct1*$^{+/+}$. *(L)* Expression of human OCT1 in *Oct1*$^{-/-,OCT1tg}$ rescue the AMPK activation in the primary hepatocytes. Primary hepatocytes were cultured in the high glucose DME H21 medium. After fasting 16 hours in DME H21 medium with antibiotics without FBS, cells were treated with or without metformin.

S5M



(M) Plasma levels of liver function enzymes in *Oct1*$^{+/+}$, *Oct1*$^{-/-}$, *Oct1*$^{+/+,\ OCT1tg}$ and *Oct1*$^{-/-,OCT1tg}$ mice. **P<0.05*, ***P<0.01* compared to the *Oct1*$^{+/+}$. n=6/genotype.

S5N



(N) Effect of expression of hOCT1 in the *C. elegans* with intestine-specific expression promoter fused with GFP. Representative images are from L4 WT animals which were co-injected with pINT::GFP::OCT1 and pdaf-1-RFP. Representative images of Nile Red-stained WT and hOCT1-transgenic animals in intestine illustrated as black and white.

Figure S6

S6A

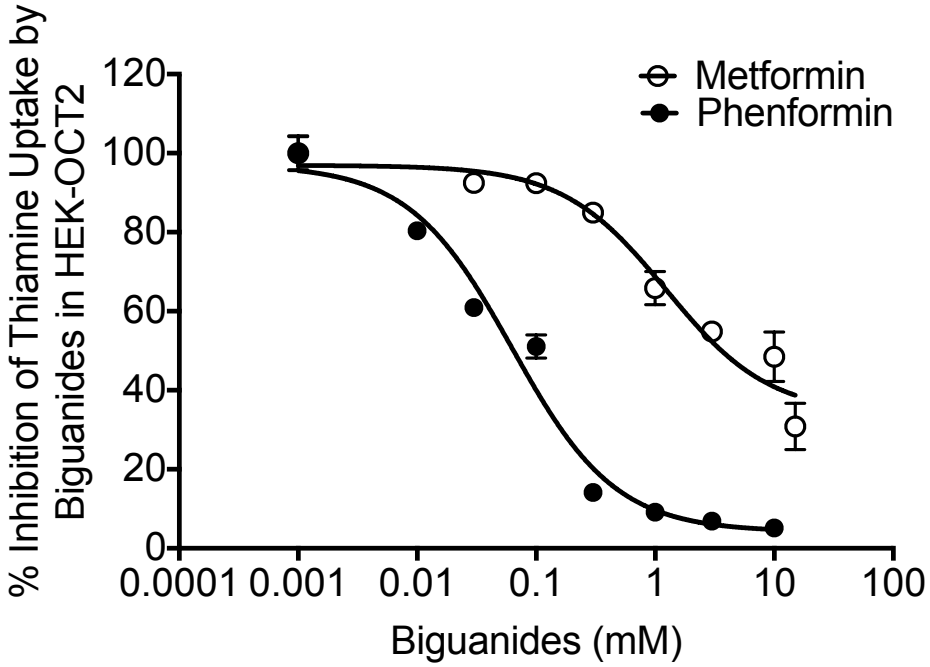
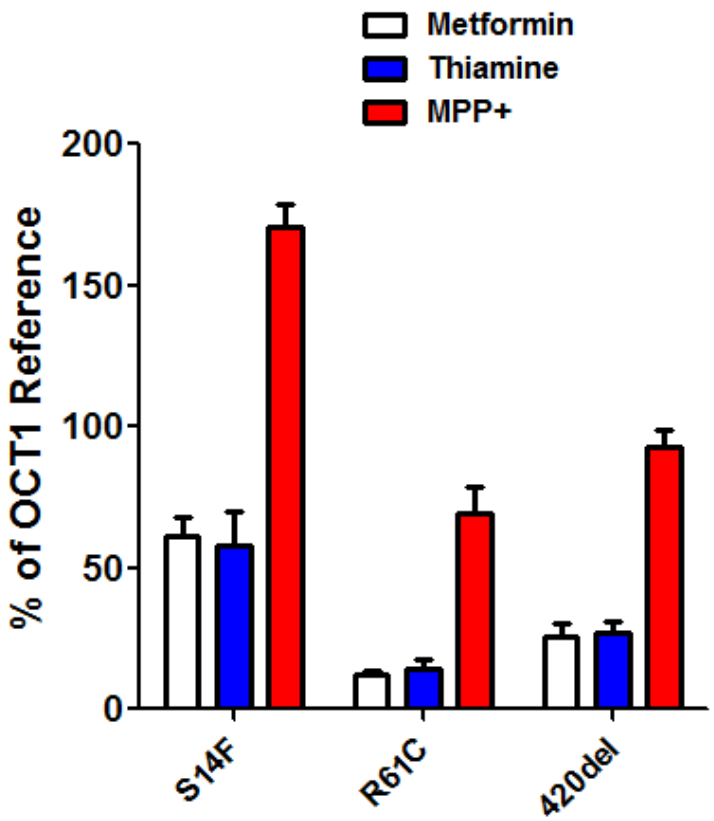
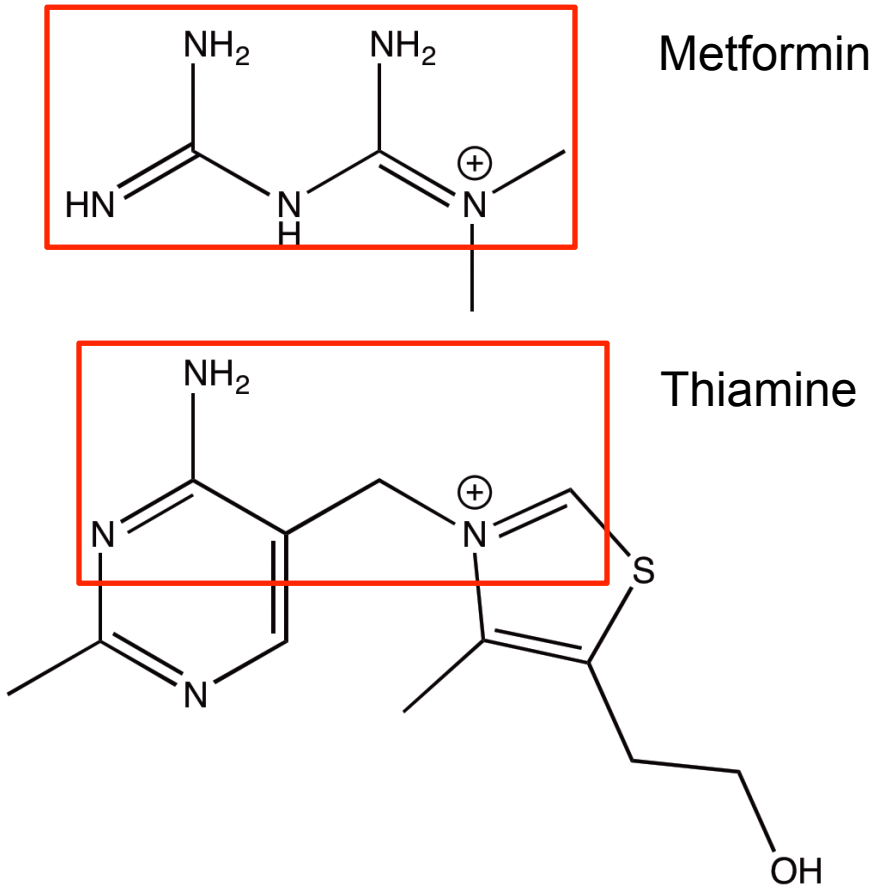


Figure S6. *(A)* **Metformin and phenformin inhibition of OCT2-mediated thiamine uptake in HEK293 transfected cells.** Phenformin is more potent in thiamine uptake by OCT2 transfected cells (IC_{50}=87 μM) compared to metformin (IC_{50}=1.4 mM)

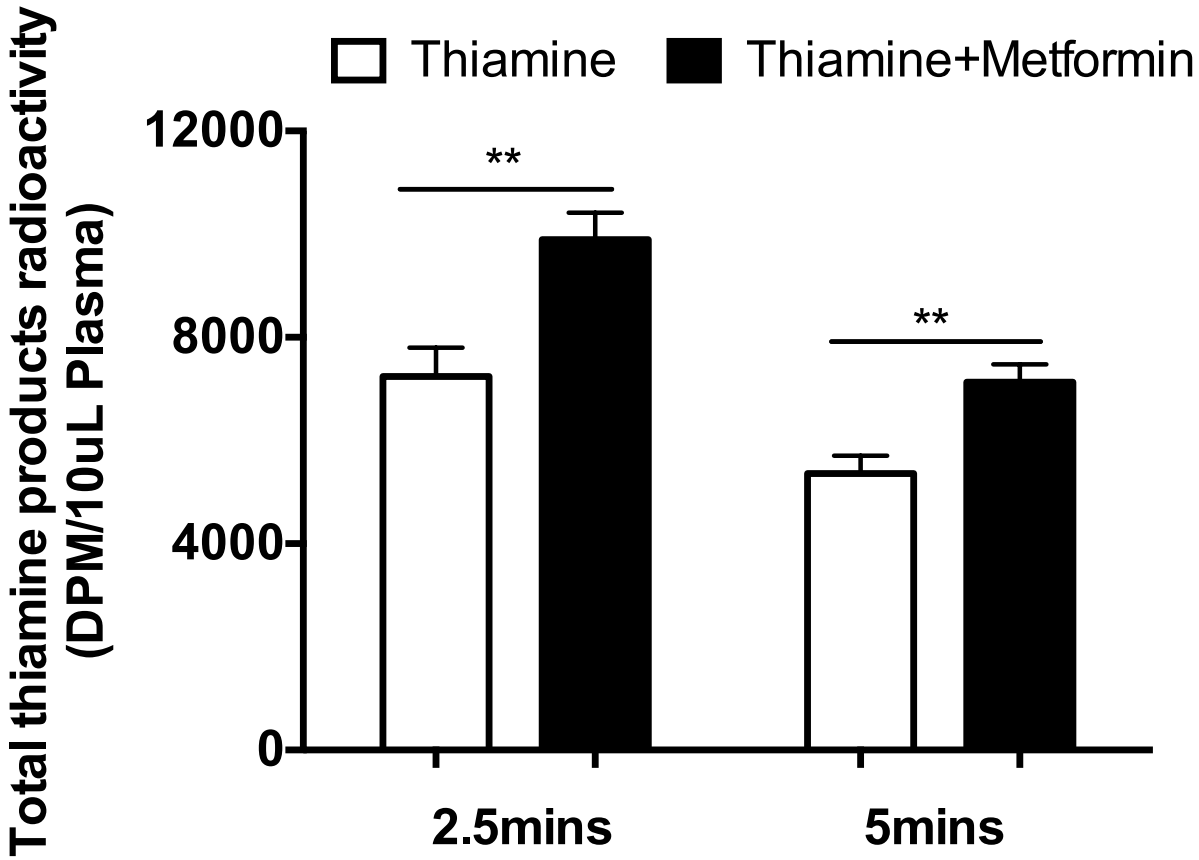
S6B





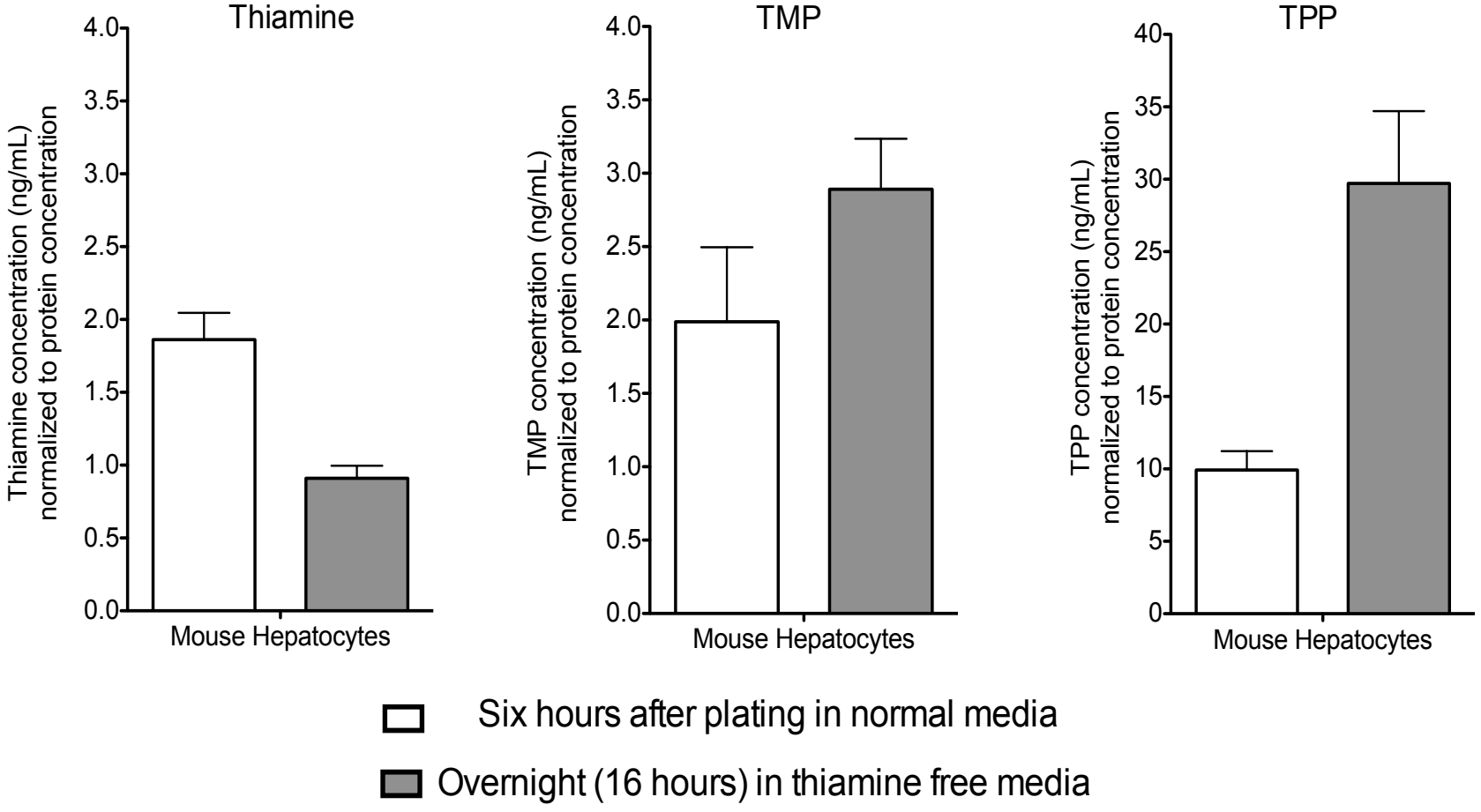
(B) **Interactions of amines with OCT1.** MPP+ uptake pattern among cells expressing OCT1 genetic variants S14F, R61C and 420del. Metformin and thiamine showed different uptake rates from MPP+ with these OCT1 genetic variants. Right panel: Thiamine (lower) and metformin (upper) share structural similarity highlighted in the red frame.

S6C

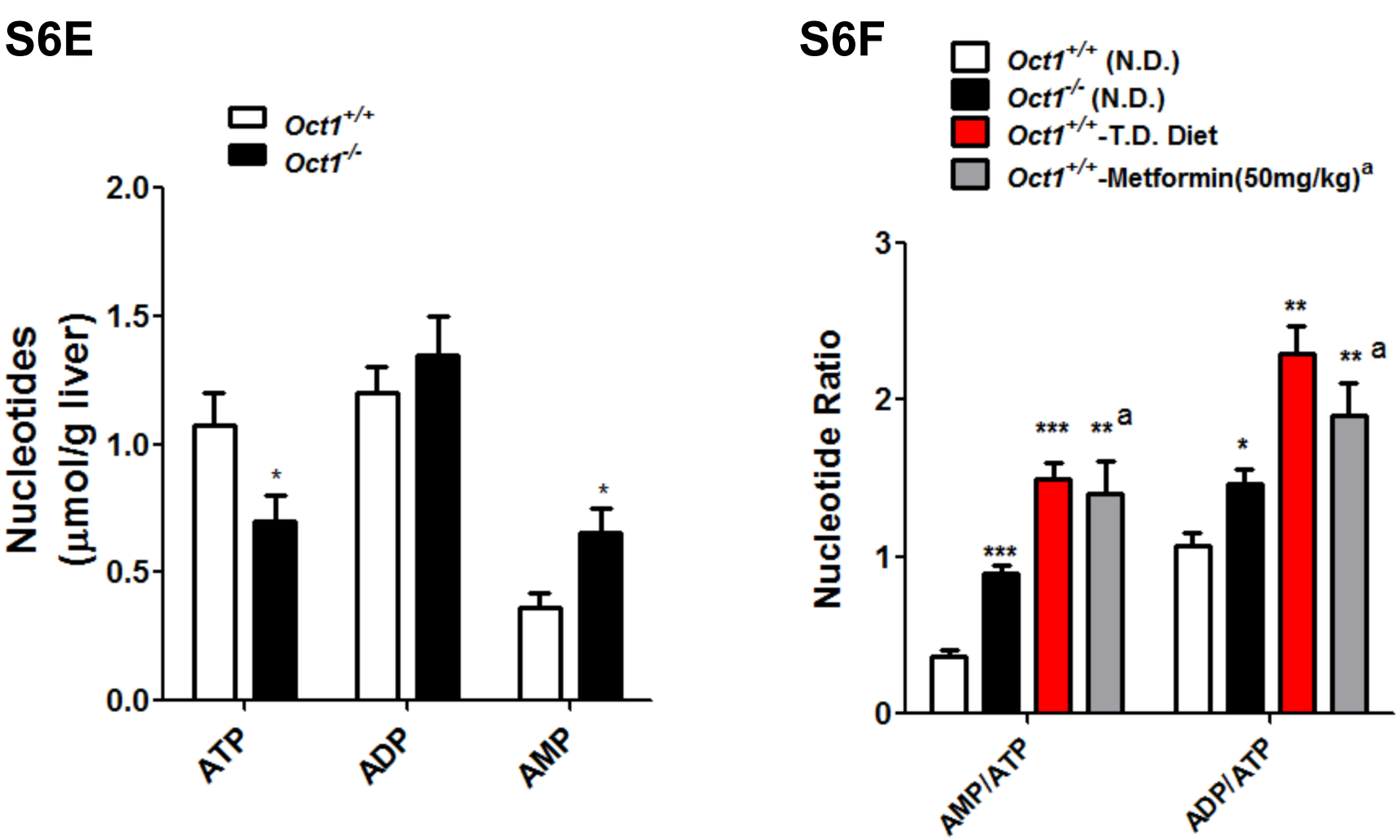


(*C*) Total radioactivity of [^{3}H]thiamine products in plasma obtained from *Oct1*$^{+/+}$ mice treated with [^{3}H]thiamine (2 mg/kg) only or [^{3}H]thiamine (2 mg/kg) plus metformin (50 mg/kg) intravenously, *p< 0.05, **p <0.01, ***p < 0.001, compared to *Oct1*$^{+/+}$ mice (n=6).

S6D

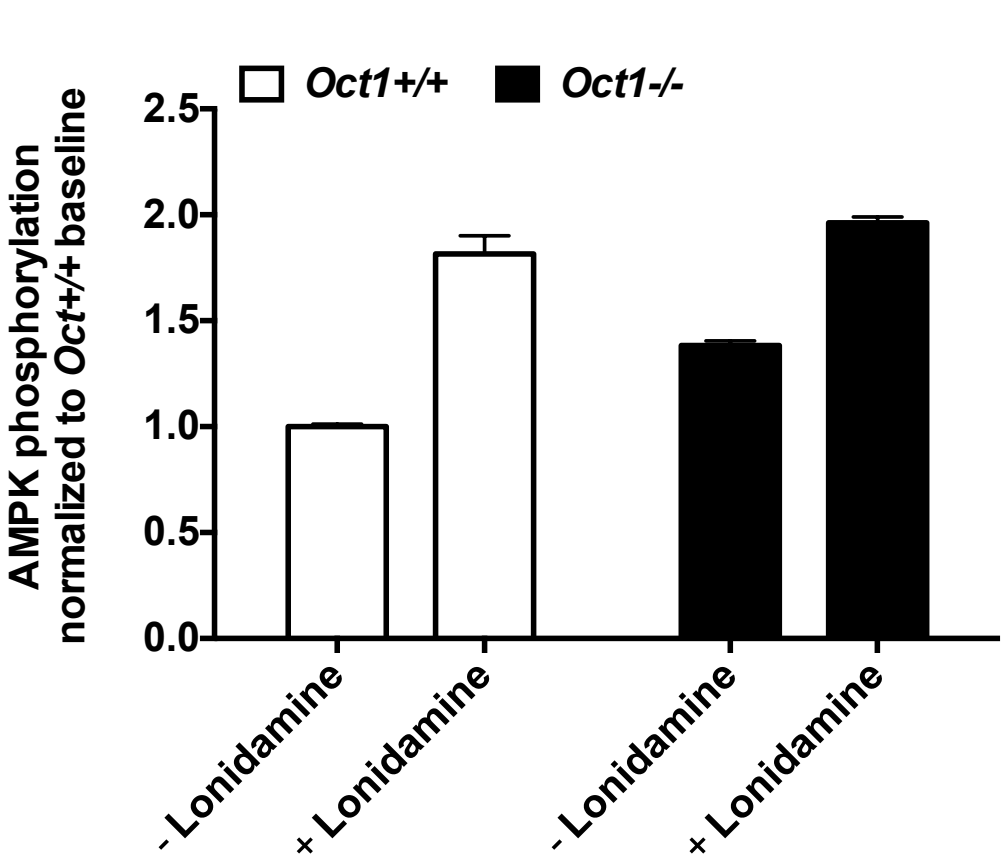


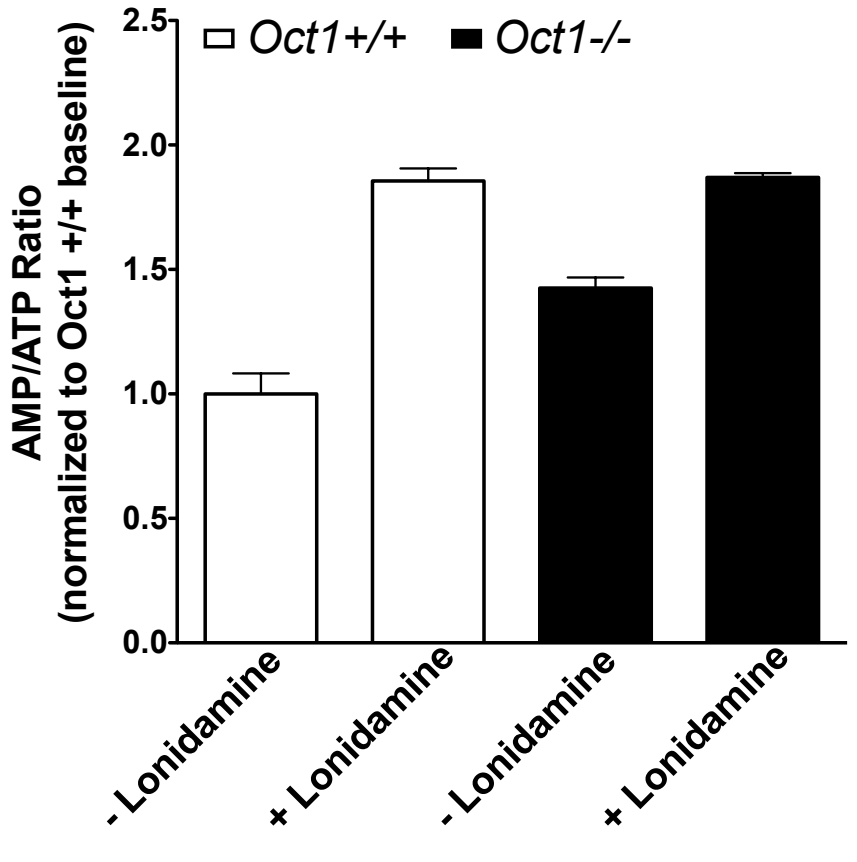
(D) Thiamine, thiamine monophosphate (TMP) and thiamine pyrophosphate (TPP) levels in mouse hepatocytes 16 hours after incubation with thiamine-free media (gray bars). Sixteen hours after thiamine depletion, thiamine levels decreased by ~50%; however, TMP levels and notably TPP levels increased substantially (from 9.9 ± 3.2 ng/ml at baseline to 30 ± 12 ng/ml 16 hours after incubation in thiamine free media).



(E) Adenosine nucleotide levels in *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice livers (n=6/ genotypes). **P<0.05*, ***P<0.01*, compared with adenosine nucleotide levels in wildtype mouse livers. *(F)* AMP/ATP and ADP/ATP ratios in the livers from *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice fed normal diets (N.D.) or thiamine deficiency (TD) diets. The gray bar indicates ratios obtained in a previous study in livers from wildtype mice treated with metformin (Foretz et al).

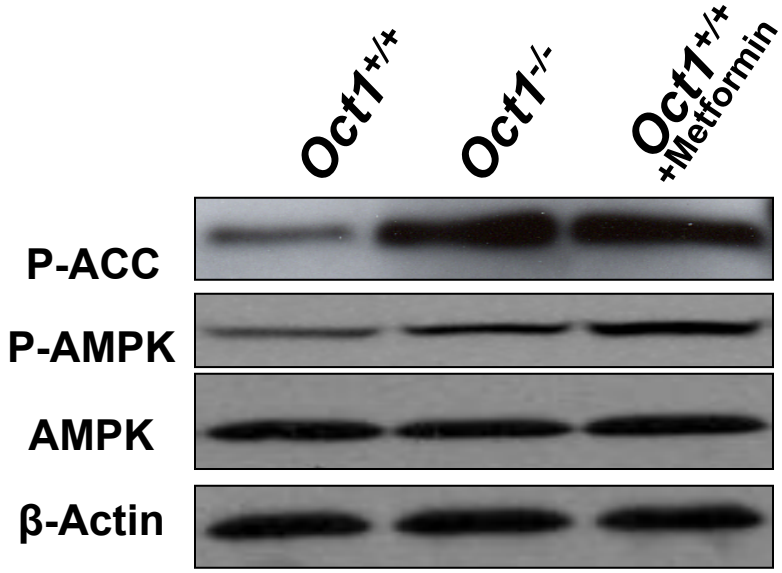
S6G





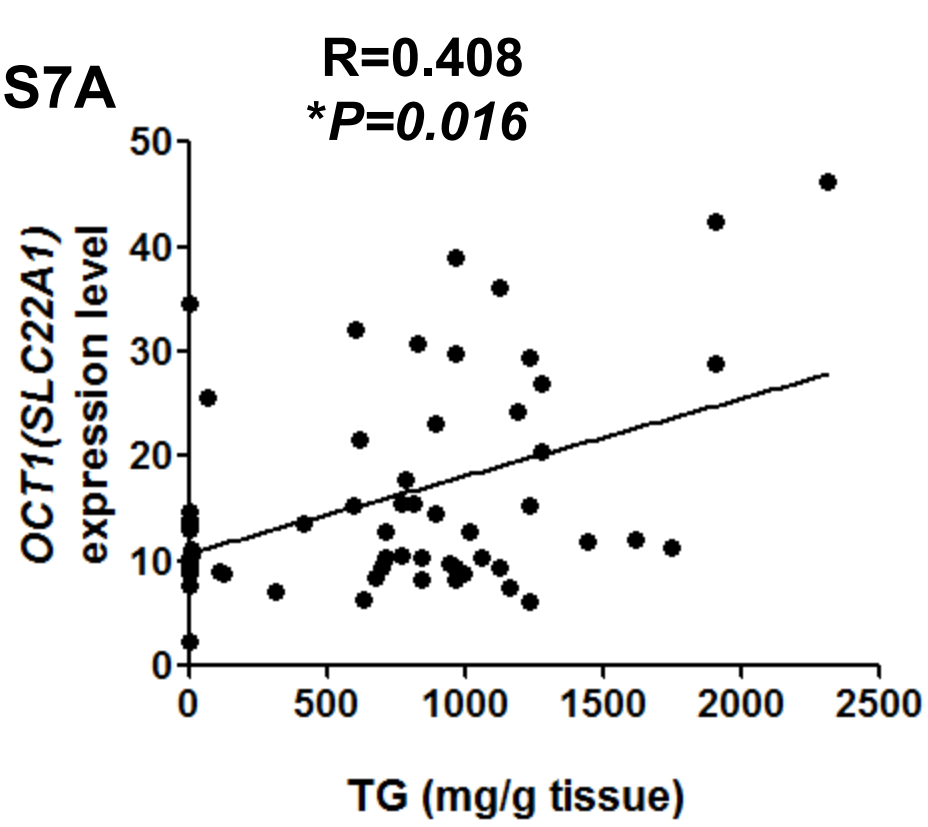
(*G*) The effect of lonidamine on AMPK phosphorylation (using ELISA, see SI Appendix) (Left) and AMP/ATP ratio (Right) in *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ primary hepatocytes.

S6H



(H) Western blot of primary hepatocytes from *Oct1*$^{+/+}$, *Oct1*$^{-/-}$ and *Oct1*$^{+/+}$ mice treated with metformin (2 mM). *Oct1*$^{-/-}$ mouse hepatocytes showed similar levels of P-ACC and P-AMPK to hepatocytes from *Oct1*$^{+/+}$ treated with metformin.

Figure S7



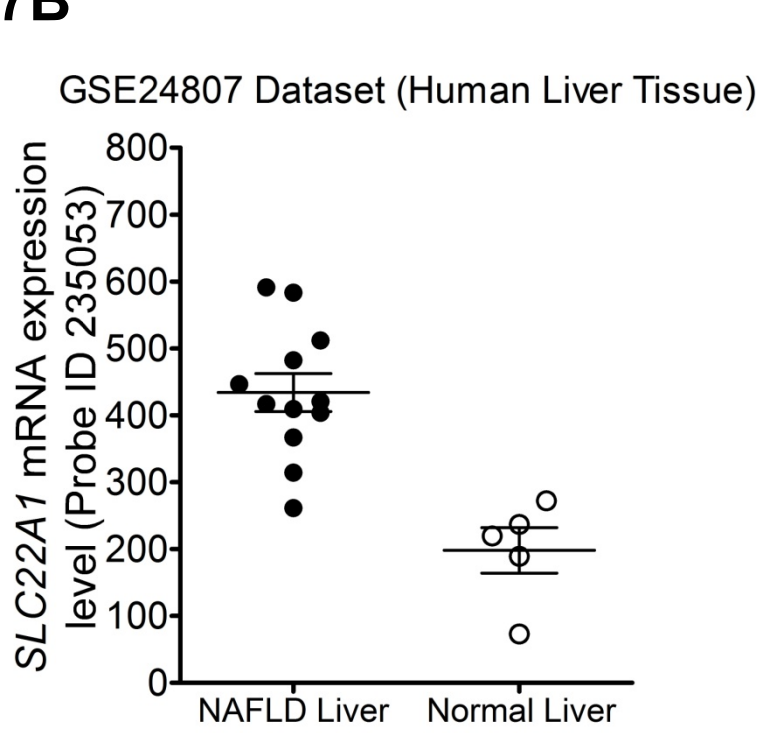


Figure S7. hOCT1 expression level and TG levels in human liver samples. *(A)* A significantly positive correlation is observed between TG levels and expression levels of OCT1 in liver samples from 60 Caucasians. The correlation between TG and *OCT1* expression listed as R=0.408 with a significant *P* value. mRNA expression level was normalized to β-actin. *(B) OCT1* (*SLC22A1*) expression level in human NAFLD and normal liver samples (Liu WS et al., PLoS One 2011) .

Supporting Information (Supplementary Tables)

Table S1. Results of lipidomic studies of serum from wildtype and *Oct1*$^{-/-}$ mice.

	Name	KO mean ± std dev	WT mean ± std dev	mean-WT/ mean-KO	factor_p.values	factor_adjusted. pvalues
5	TG 54:8 M+NH4@9.3	13400 ± 2600	26600 ± 7000	1.98	0.006504911	0.023394169
8	TG 54:6 M+NH4@10.2	2670000 ± 390000	1860000 ± 4e+05	0.696629213	0.005533398	0.020582172
12	TG 52:4 M+NH4@10.6	23500 ± 3300	17100 ± 2200	0.73	0.016184251	0.045724926
53	TG (52:2) [M+NH4]+@11	6340000 ± 110000	4680000 ± 420000	0.74	0.000536819	0.003842923
63	TG (50:1) [M+NH4]+@11	1390000 ± 99000	1170000 ± 140000	0.841726619	0.012267489	0.037487825
70	SM 39:1 M+Na@6.5	22500 ± 2000	34800 ± 1600	1.54	0.000125523	0.001513883
71	SM 38:1 M+Na@6.2	65300 ± 2600	88400 ± 4800	1.35	0.000113217	0.00142296
72	SM 36:1 [M+Na]+@5.5	34700 ± 3300	45300 ± 1600	1.3	0.00174754	0.008878992
77	SM (d18:2/24:0) A M+H@6.6	21400 ± 1200	29800 ± 1400	1.39	0.000111797	0.001420062
79	SM (d18:2/23:0) B M+H@6.5	342000 ± 18000	436000 ± 21000	1.28	0.000443445	0.003296459
80	SM (d18:1/24:0) [M+H]+@7.6	980000 ± 32000	1260000 ± 61000	1.29	0.000134588	0.001591065
81	SM (d18:1/23:0) A M+H@7.2	369000 ± 14000	458000 ± 23000	1.24	0.000537494	0.003842923
82	SM (d18:1/22:0) [M+H]+@6.9	1560000 ± 58000	2050000 ± 95000	1.31	0.000103423	0.00140019
83	SM (d18:1/21:0) M+H@6.6	149000 ± 7700	223000 ± 9400	1.5	1.90433E-05	0.000561653
84	SM (d18:1/20:0) M+H@6.2	325000 ± 15000	461000 ± 25000	1.42	7.09912E-05	0.001145453
87	SM (d16:1/20:1) [M+H]+@5	292000 ± 15000	395000 ± 17000	1.35	0.000107148	0.00140019
88	SM (d16:1/20:0) M+H@5.5	348000 ± 17000	470000 ± 23000	1.35	0.0001231	0.00149981
90	Plasmenyl-PE 40:5 M+H@6.1	149000 ± 9100	179000 ± 10000	1.2	0.00451483	0.01761669
92	Plasmenyl-PE 38:4 M+H@6.2	137000 ± 7700	157000 ± 9300	1.15	0.015555294	0.044165077
93	Plasmenyl-PE 38:4 M+H@6	43700 ± 2300	24600 ± 1400	0.56	6.32E-06	0.000342987
94	Plasmenyl-PE 38:4 M+H@5.9	16800 ± 730	14200 ± 460	0.84	0.000749936	0.004840125
95	Plasmenyl-PE 36:4@5.5	161000 ± 5800	141000 ± 7700	0.88	0.007729525	0.02655153
98	Plasmenyl-PC 38:4 [M+H]+@6	83800 ± 3700	96400 ± 4200	1.15	0.004322689	0.017204302
99	Plasmenyl-PC (34:1) M+H@5.5	226000 ± 9100	277000 ± 15000	1.22	0.001083733	0.006208874
102	PE 40:6 M+H@5.5	63900 ± 2200	58700 ± 2000	0.92	0.013190139	0.039114312
105	PE 37:3 M+H@4.8	2e+06 ± 80000	2740000 ± 130000	1.37	6.60617E-05	0.001080516
106	PE 37:3 M+H@4.6	3350000 ± 150000	5270000 ± 250000	1.57	8.93E-06	0.000375054
107	PE 36:2 M+H@4.8	315000 ± 15000	395000 ± 19000	1.25	0.000576576	0.004025919
108	PE 36:1 M+H@6.5	17200 ± 850	14900 ± 1000	0.86	0.013425963	0.039558413
109	PE 36:1 M+H@5.3	169000 ± 7600	206000 ± 9500	1.22	0.000954457	0.005634925
114	PC 42:6 M+H@6	51500 ± 2200	39100 ± 1900	0.76	0.000159084	0.001715953
118	PC 40:6 M+Na@5.4	111000 ± 7600	77800 ± 3900	0.7	0.000154406	0.001715953
123	PC 38:8@4.5	283000 ± 20000	388000 ± 25000	1.37	0.000589743	0.00407025
124	PC 38:5 M+H@5.3	4290000 ± 250000	3700000 ± 180000	0.86	0.008016742	0.027193155
134	PC 38:2 M+H@6.4	429000 ± 16000	386000 ± 16000	0.9	0.009473258	0.031074368
136	PC 37:3 M+H@5.5	84300 ± 3000	74100 ± 3900	0.88	0.006918042	0.024698779
138	PC 36:5 M+H@5.4	51700 ± 3400	75200 ± 11000	1.45	0.002623443	0.011820345
145	PC 36:3 A M+H@5.2	5400000 ± 330000	6290000 ± 3e+05	1.16	0.007706095	0.02655153
149	PC 36:1 M+H@6.3	3850000 ± 190000	4440000 ± 210000	1.15	0.005745468	0.021173113
152	PC 34:0 M+H@6.2	1310000 ± 46000	1560000 ± 80000	1.19	0.00161588	0.008444839
153	PC 32:2@4.6	21400 ± 2000	37700 ± 6500	1.76	0.001520374	0.008104135
154	PC 32:2@4.5	360000 ± 10000	712000 ± 39000	1.98	6.18E-07	0.000166027
156	PC 32:1 M+H@5	1710000 ± 77000	2520000 ± 120000	1.48	2.16467E-05	0.000587412
166	PC (36:6)@4.3	60000 ± 3900	110000 ± 3500	1.84	2.80E-06	0.00029434
167	PC (34:2) [M+H]+@5.5	199000 ± 17000	259000 ± 20000	1.3	0.003594909	0.015008117
170	LPC (20:2)@1.9	37700 ± 3700	31600 ± 1400	0.84	0.017662078	0.049043073
174	LPC (18:2)@1.3	4120000 ± 190000	4880000 ± 280000	1.18	0.003947821	0.016142802
175	LPC (18:2)@1.2	2280000 ± 240000	3210000 ± 190000	1.41	0.001344925	0.007299273
177	LPC (18:1)@1.7	3440000 ± 180000	3910000 ± 2e+05	1.14	0.013591782	0.039775951
178	LPC (18:1)@1.6	788000 ± 95000	1070000 ± 64000	1.36	0.003644372	0.015161604
183	DG 36:3 M+Na@6.6	265000 ± 7800	227000 ± 9500	0.86	0.000899616	0.00542496
184	DG 36:2 [M+Na]+@7.1	123000 ± 2800	90700 ± 2600	0.74	3.11E-06	0.00029434
187	Ceramide d40:1 [M+Na]+@7.8	38600 ± 1100	44800 ± 2900	1.16	0.005993105	0.021883079

188 CE 22:6 M+Na@10.1	42400 ± 4600	58200 ± 2200	1.37	0.001355288	0.007322235
189 CE 18:2 M+Na@10.6	44700 ± 960	76800 ± 5900	1.72	8.20E-06	0.000375054

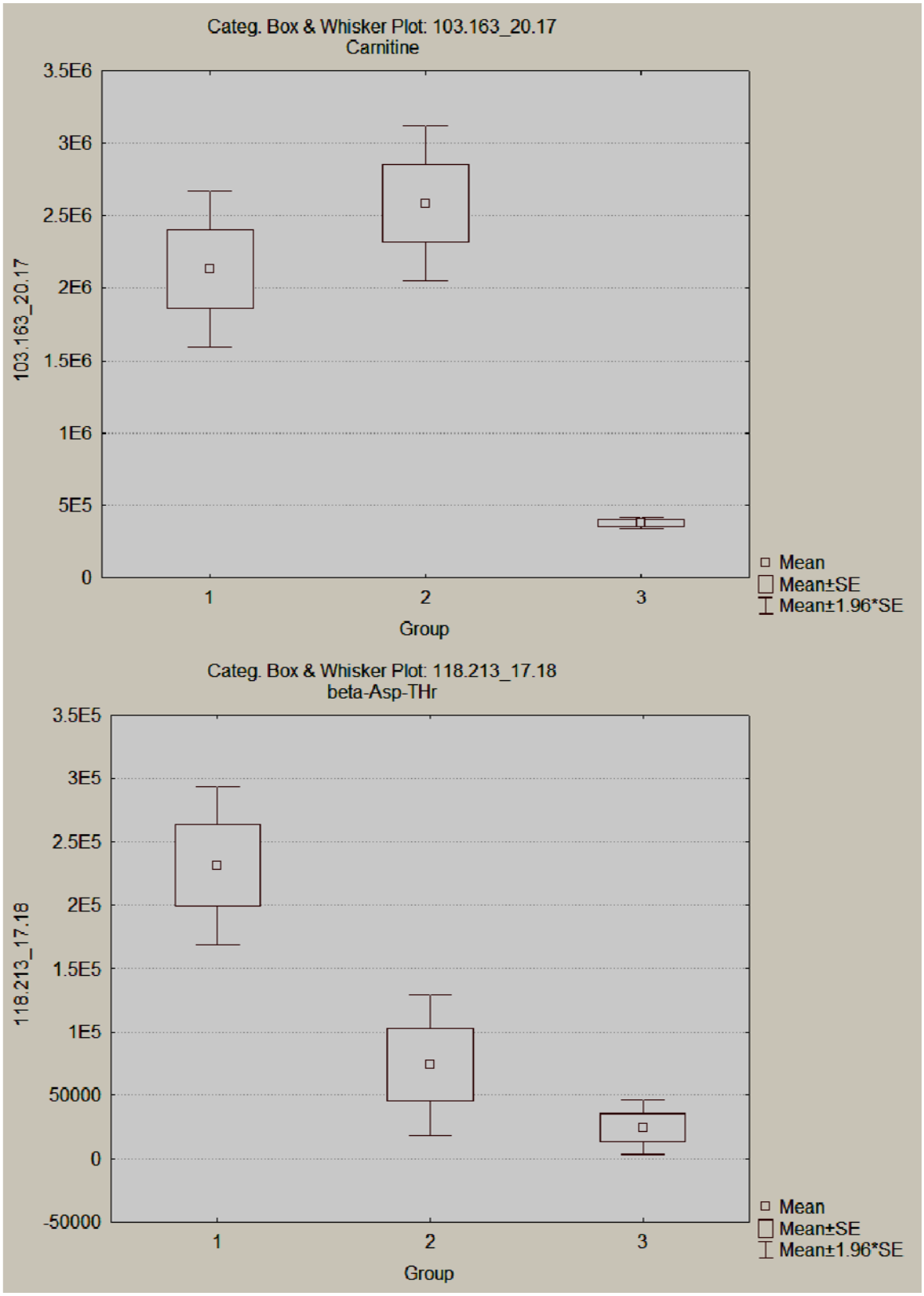
Lipidomics of serums from *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mice. All data are expressed as Mean ± SEM, **P*<*0.05*, compared with OCT1. Mice were fed with normal diet for 20 weeks (n=3/genotypes). The data were listed which had a **P* <0.05, compared with wildtype mice.

Table S2. *Oct1*$^{+/+}$ and *Oct1*$^{-/-}$ mouse liver tissue extraction metabolomics HILIC profiling report: Group 1: Wildtype mice, Group 2: Oct1-/- mice, Group 3: Blank

Period	1
Experiment	1
Min. Retention Time	1.80 min
Max. Retention Time	29.00 min
Subtraction Offset	10 scans
Subtraction Mult. Factor	1.3
Noise Threshold	3
Min. Spectral Peak Width	0.80 amu
Min. RT Peak Width	5 scans
Max. RT Peak Width	1000 scans
Retention Time Tolerance	0.50 min
Mass Tolerance	0.80 amu
Use Global Exclusion List	False
Max. Number of Peaks	5000
Use Raw Data Area	Tue

Samples:

Sample Name	Group	Use	Scale Factor	RT Correction
Blank	3	Yes	1.00	None
Blank	3	Yes	1.00	None
Blank	3	Yes	1.00	None
ko1	2	Yes	1.00	None
ko2	2	Yes	1.00	None
ko3	2	Yes	1.00	None
ko4	2	Yes	1.00	None
Ko5	2	Yes	1.00	None
Ko6	2	Yes	1.00	None
wt1	1	Yes	1.00	None
wt2	1	Yes	1.00	None
wt3	1	Yes	1.00	None
wt4	1	Yes	1.00	None
wt5	1	Yes	1.00	None
wt6	1	Yes	1.00	None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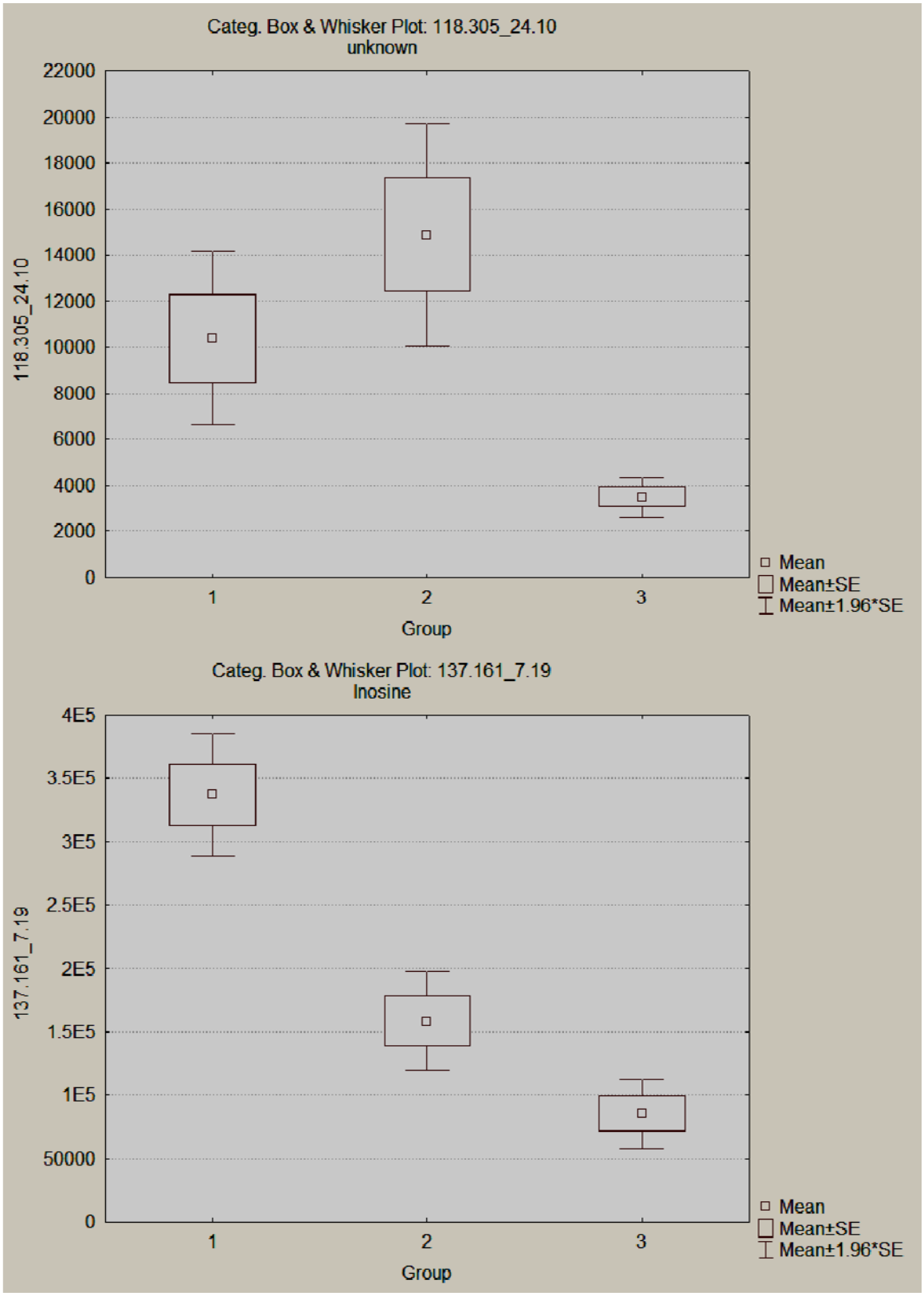
2E6
1.5E6
1E6
5E5
0
103.163_20.17
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE
Categ. Box & Whisker Plot: 118.213_17.18
beta-Asp-THr
3.5E5
3E5
2.5E5
2E5
1.5E5
1E5
50000
0
-50000
118.213_17.18
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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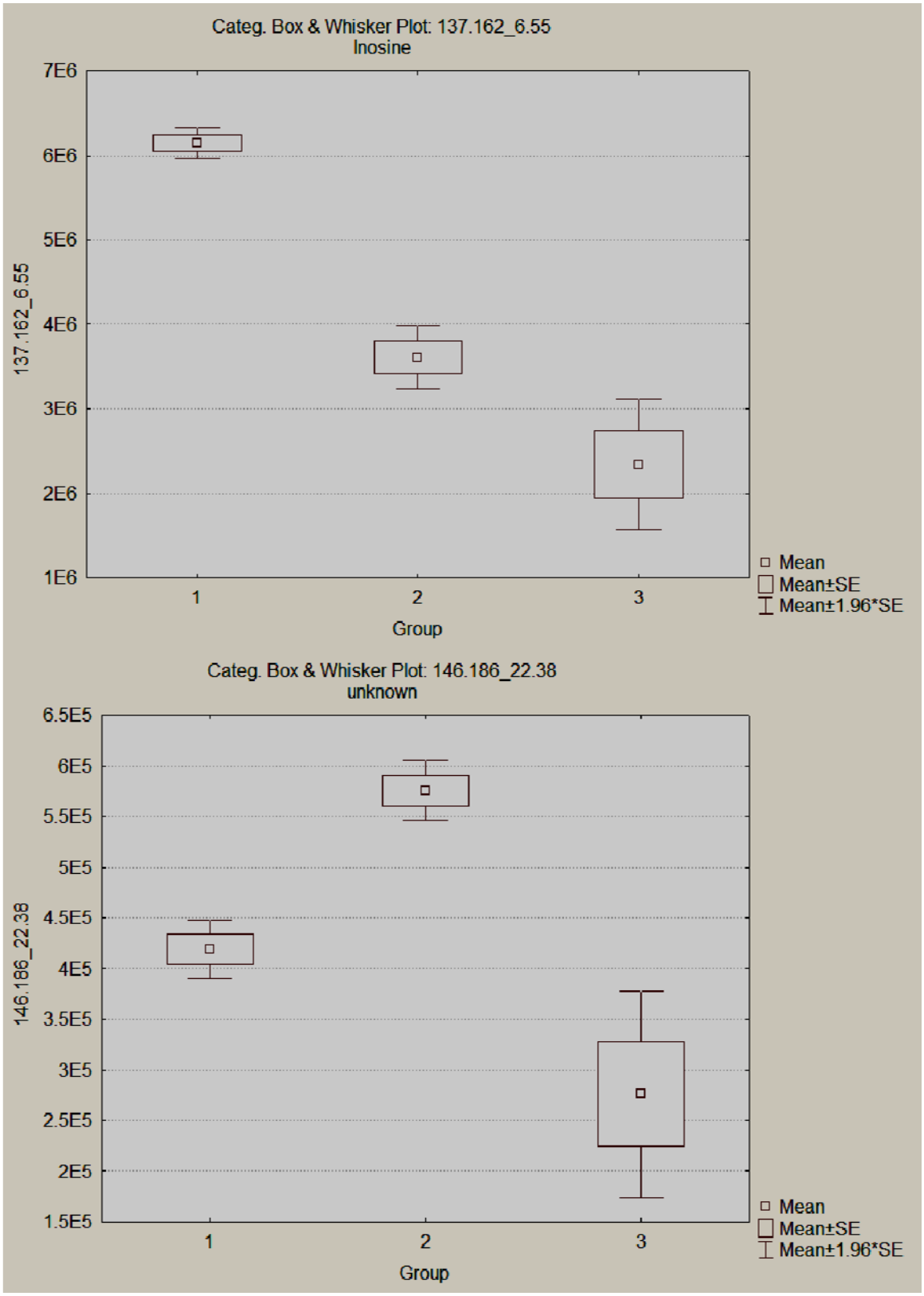
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE
Categ. Box & Whisker Plot: 137.161_7.19
Inosine
137.161_7.19
4E5
3.5E5
3E5
2.5E5
2E5
1.5E5
1E5
50000
0
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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Inosine
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6E6
5E6
4E6
3E6
2E6
1E6
137.162_6.55
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE
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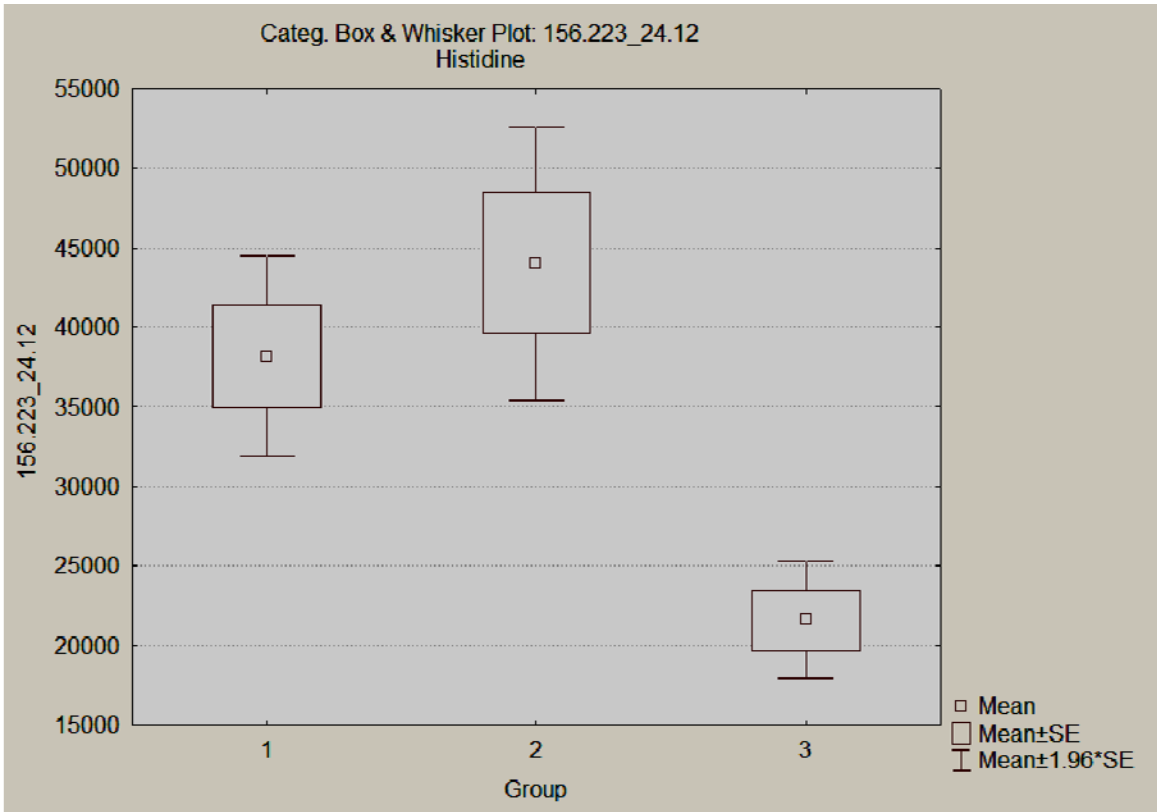
unknown
6.5E5
6E5
5.5E5
5E5
4.5E5
4E5
3.5E5
3E5
2.5E5
2E5
1.5E5
146.186_22.38
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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156.223_24.12
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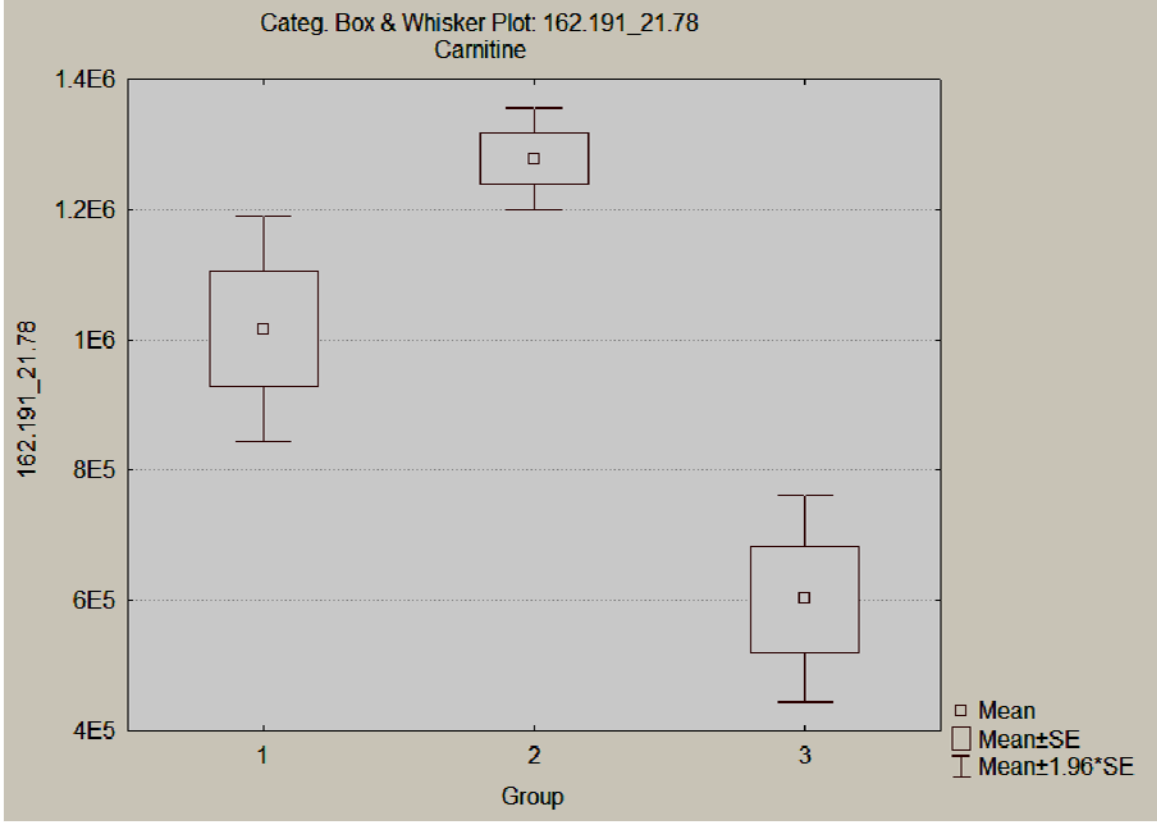
15000
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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Carnitine
162.191_21.78
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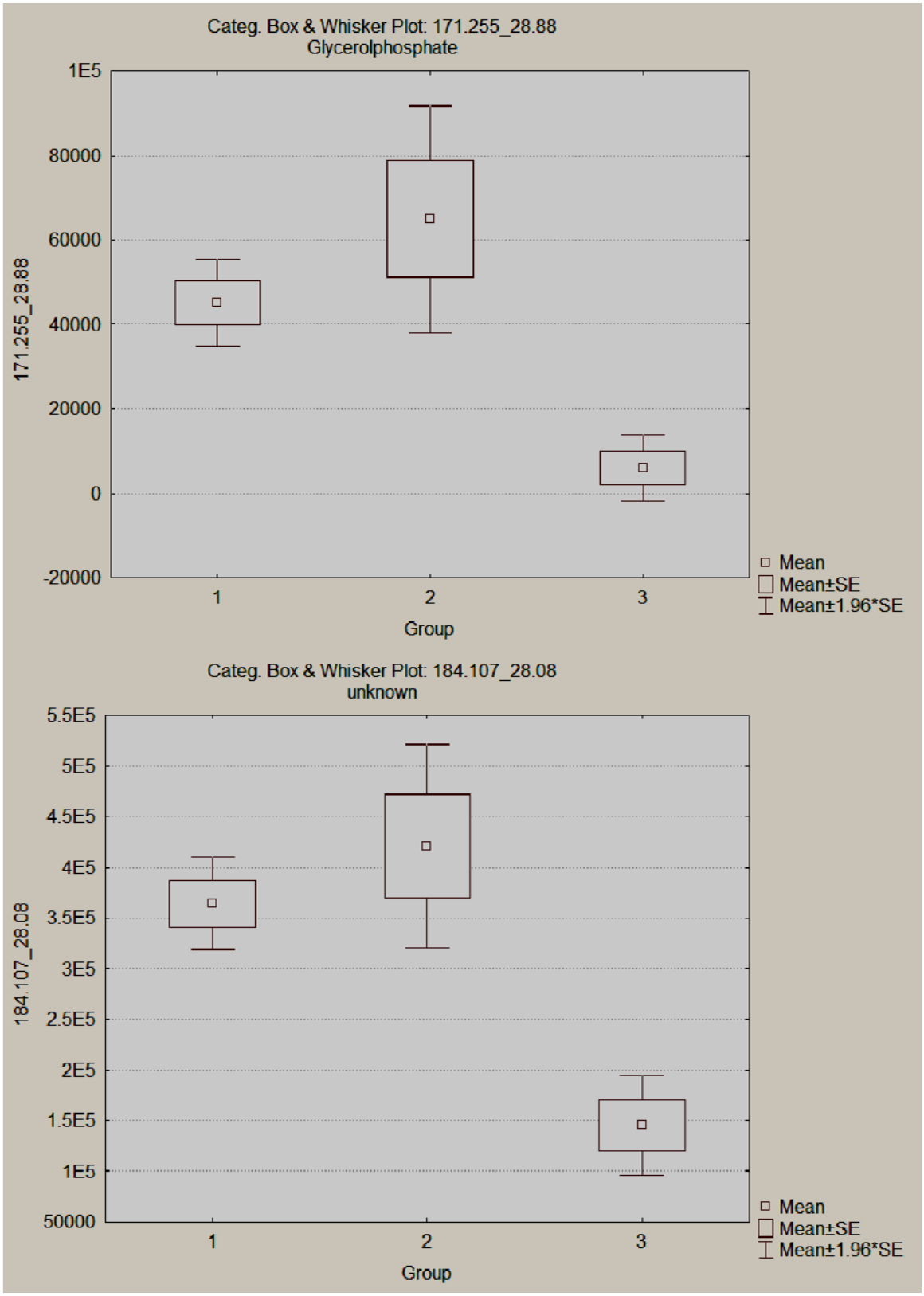
1.2E6
1E6
8E5
6E5
4E5
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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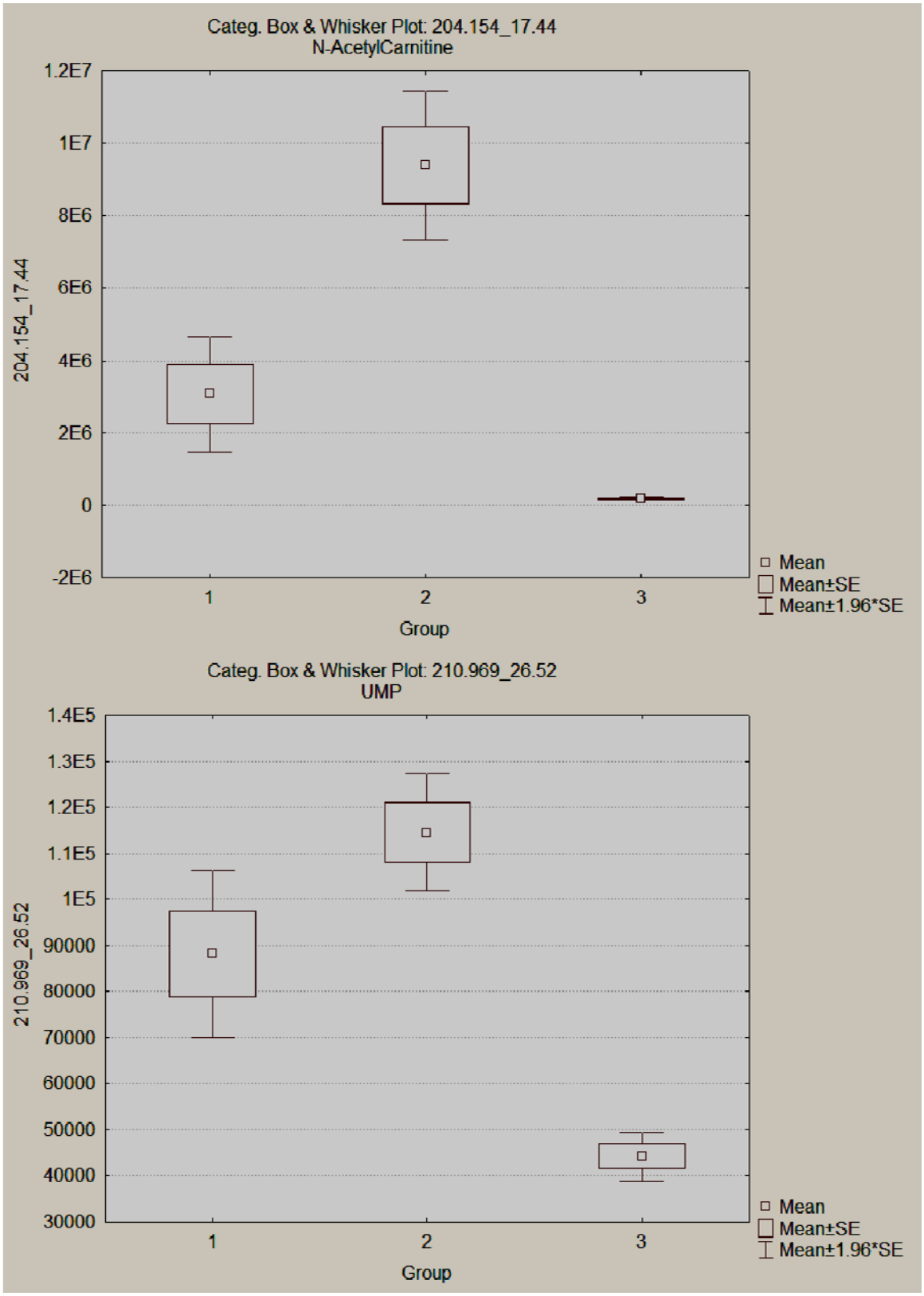
80000
60000
40000
20000
0
-20000
171.255_28.88
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE
Categ. Box & Whisker Plot: 184.107_28.08
unknown
5.5E5
5E5
4.5E5
4E5
3.5E5
3E5
2.5E5
2E5
1.5E5
1E5
50000
184.107_28.08
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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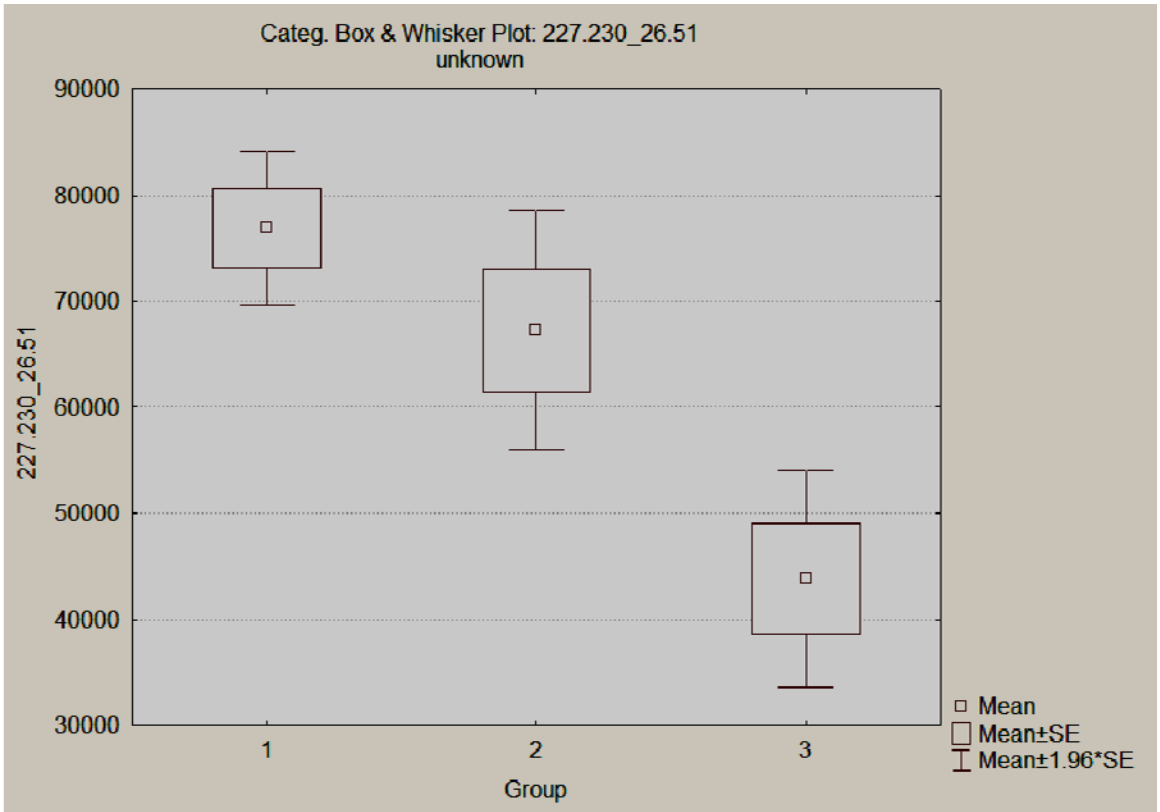
1E7
8E6
6E6
4E6
2E6
0
-2E6
204.154_17.44
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE
Categ. Box & Whisker Plot: 210.969_26.52
UMP
1.4E5
1.3E5
1.2E5
1.1E5
1E5
90000
80000
70000
60000
50000
40000
30000
210.969_26.52
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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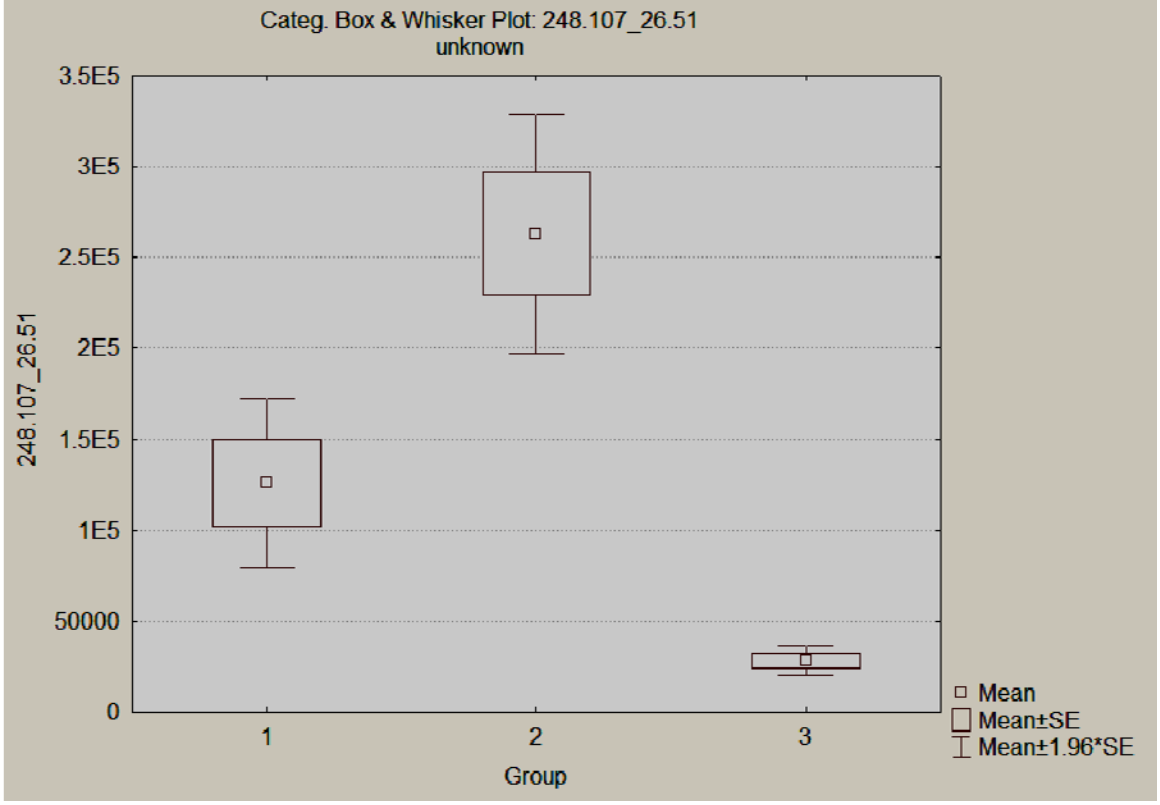
90000
80000
70000
60000
50000
40000
30000
227.230_26.51
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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3E5
2.5E5
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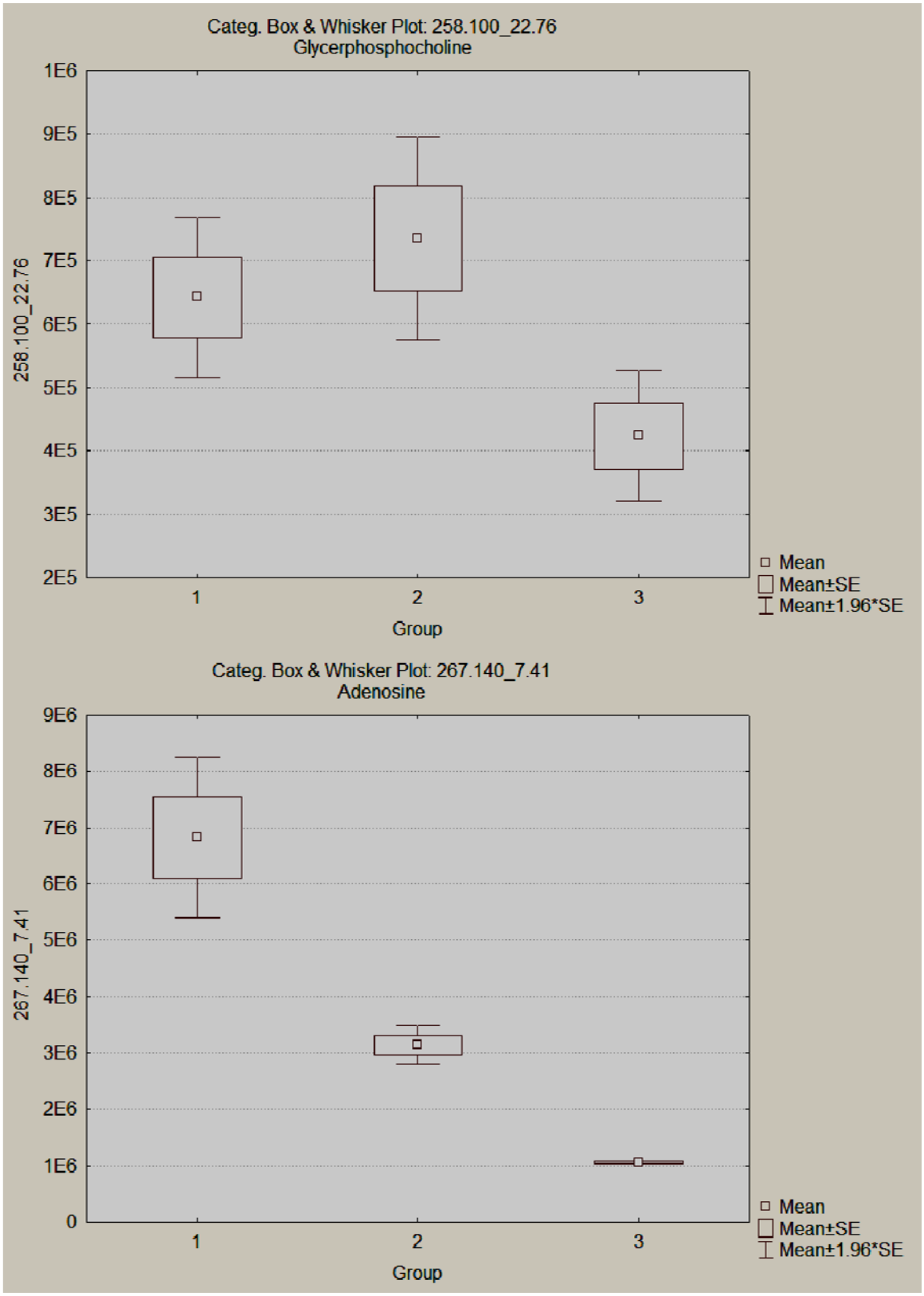
1E5
50000
0
248.107_26.51
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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9E5
8E5
7E5
6E5
5E5
4E5
3E5
2E5
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE
Categ. Box & Whisker Plot: 267.140_7.41
Adenosine
267.140_7.41
9E6
8E6
7E6
6E6
5E6
4E6
3E6
2E6
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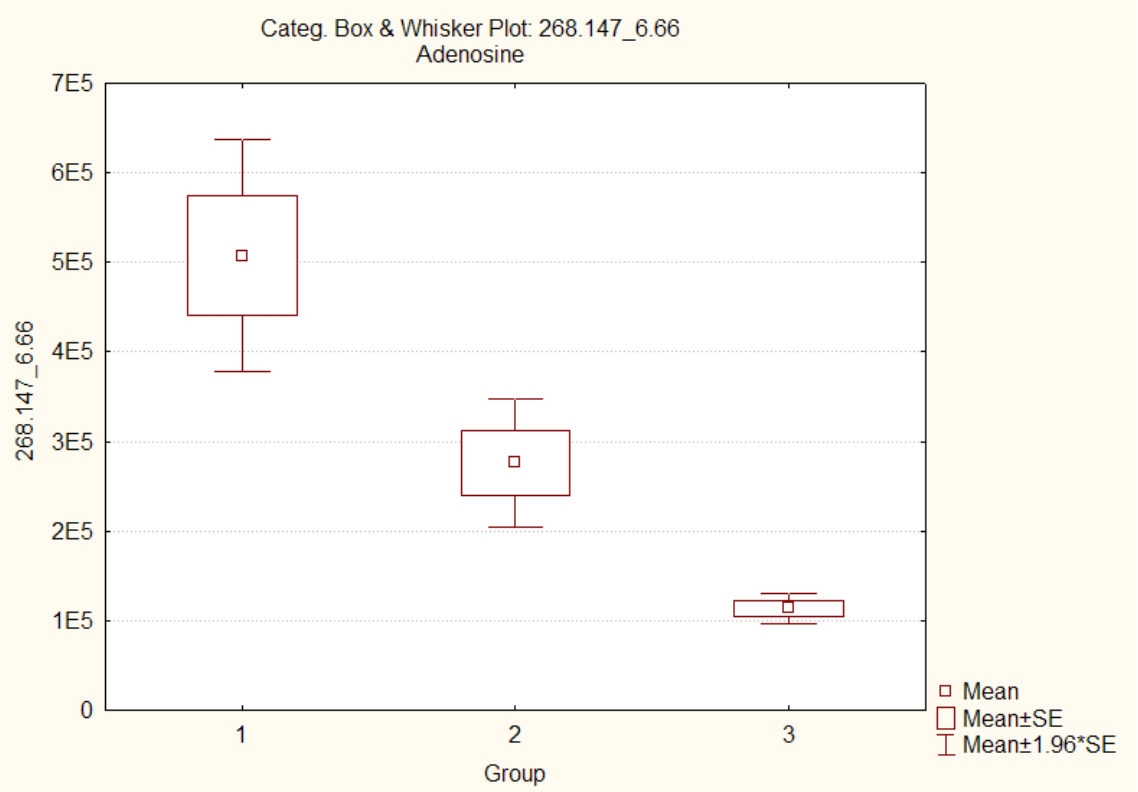
3
Group
Mean
Mean±SE
Mean±1.96*SE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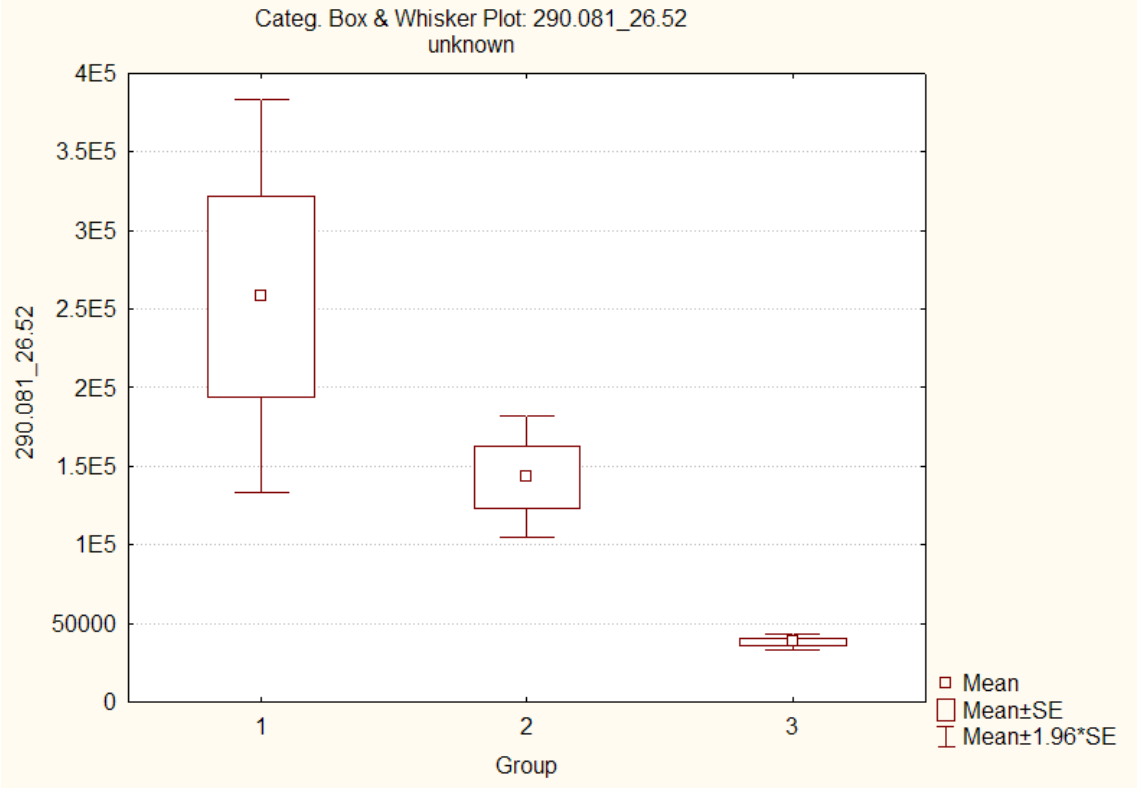
Adenosine
7E5
6E5
5E5
4E5
3E5
2E5
1E5
0
268.147_6.66
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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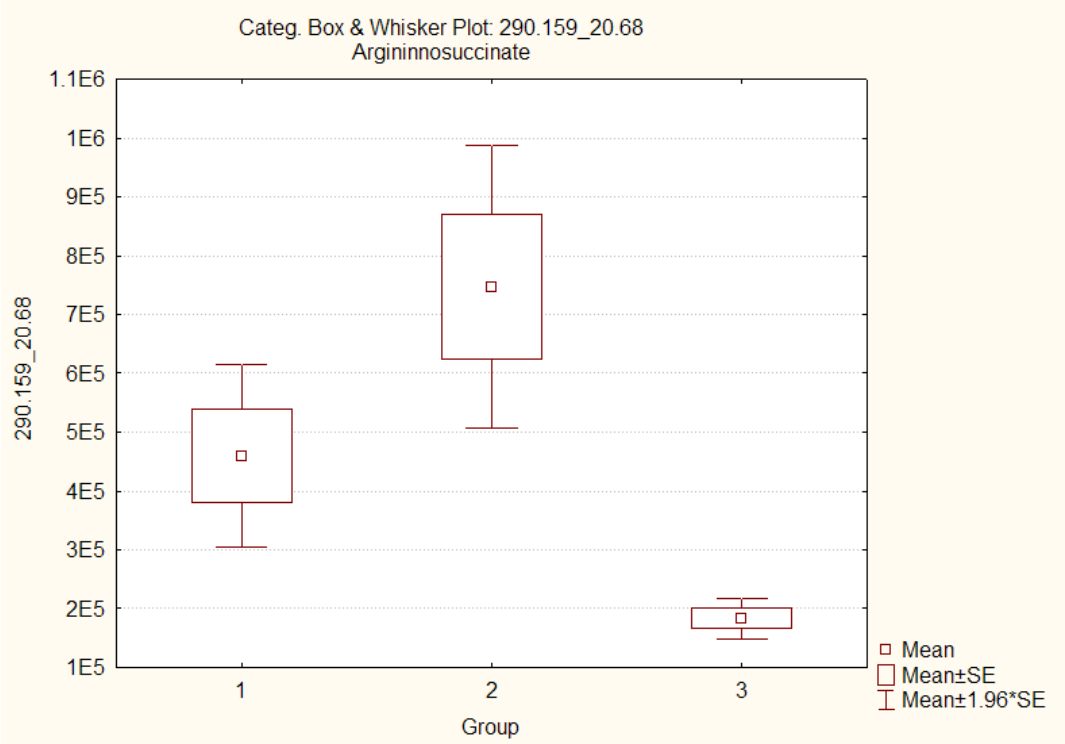
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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Argininnosuccinate
290.159_20.68
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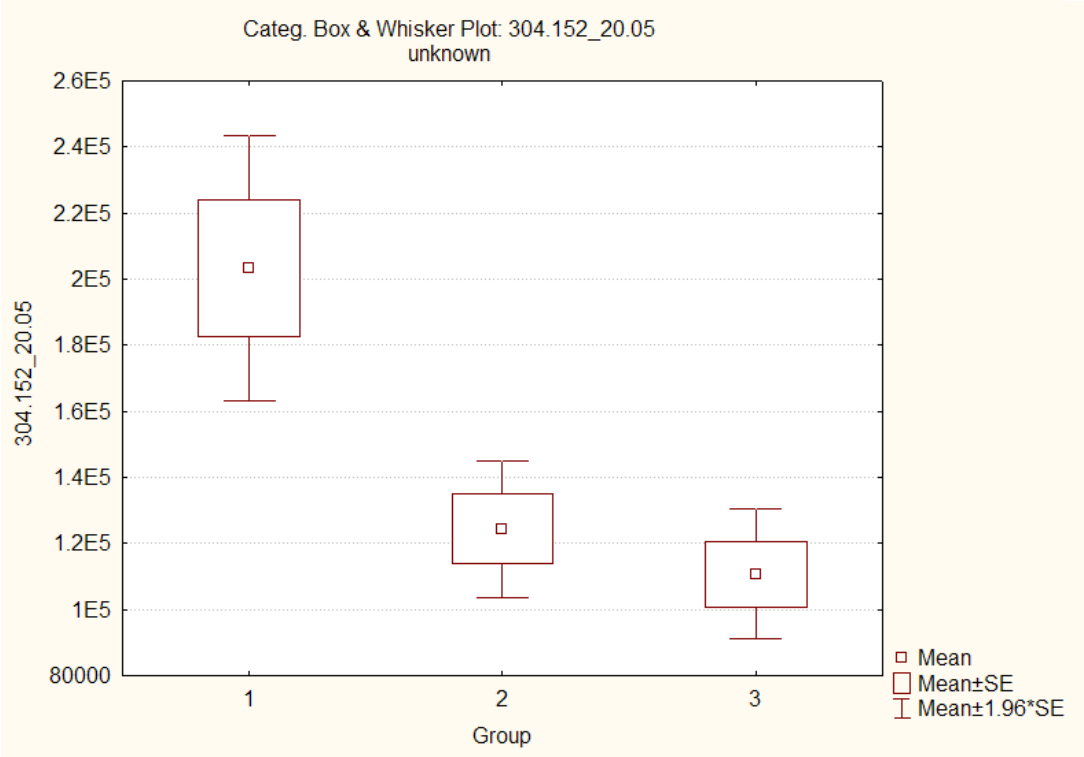
1E6
9E5
8E5
7E5
6E5
5E5
4E5
3E5
2E5
1E5
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE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304.152_20.05
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2.4E5
2.2E5
2E5
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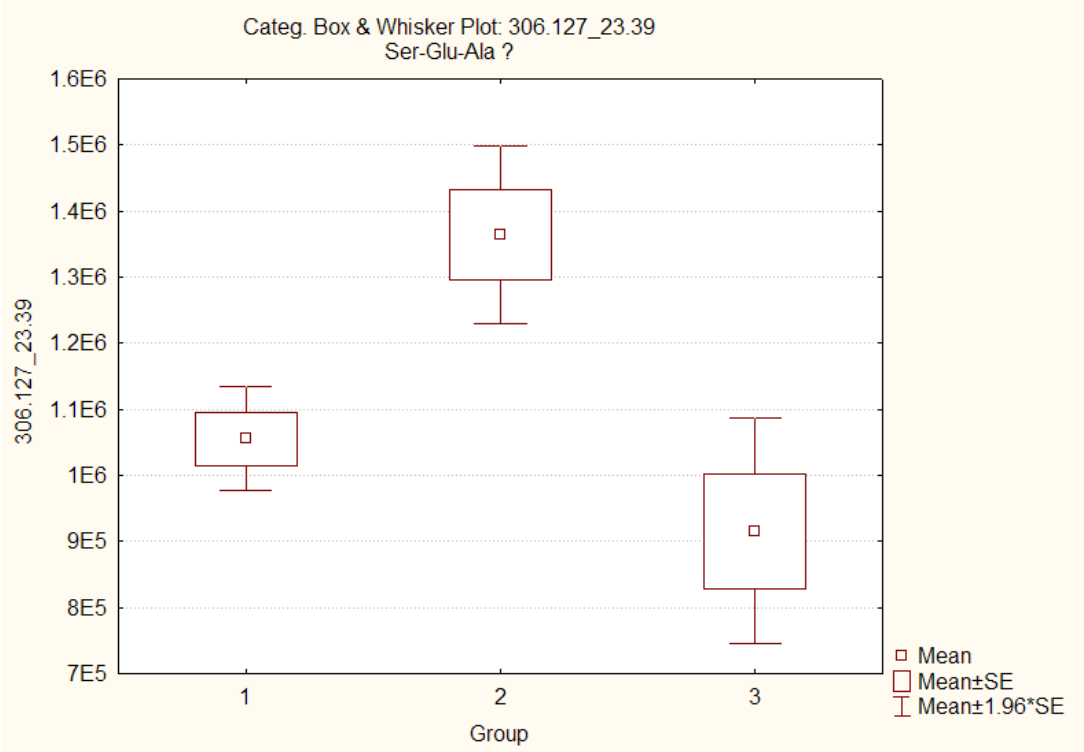
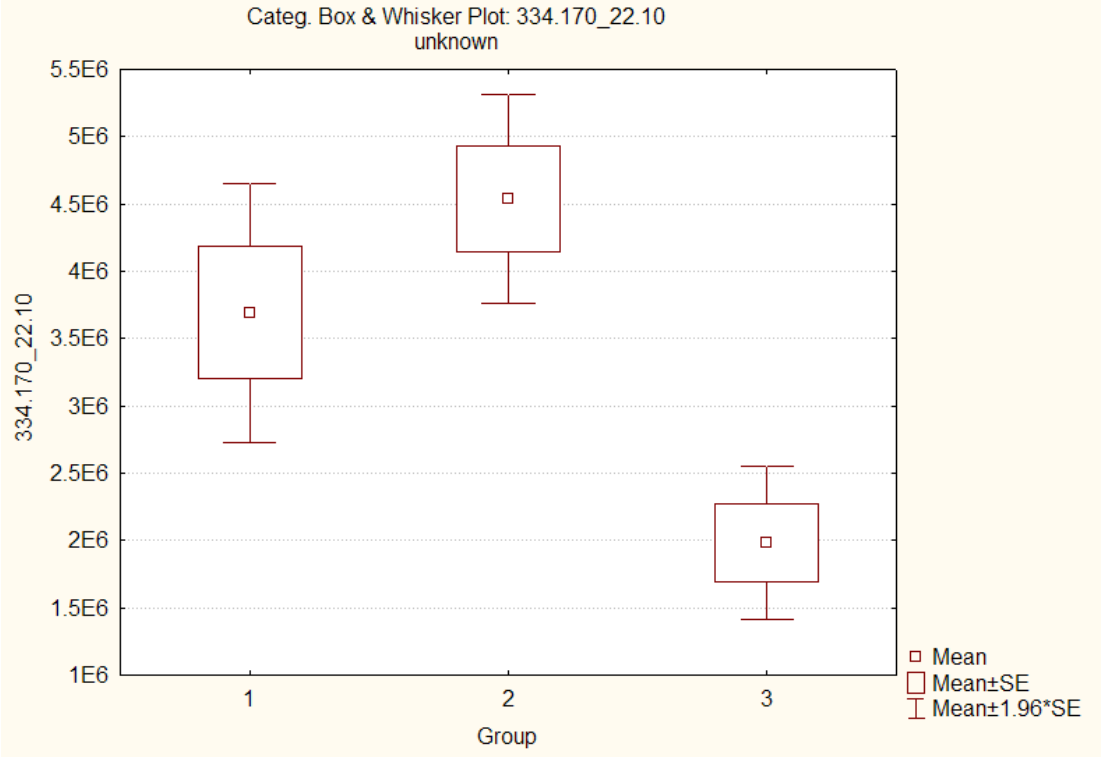
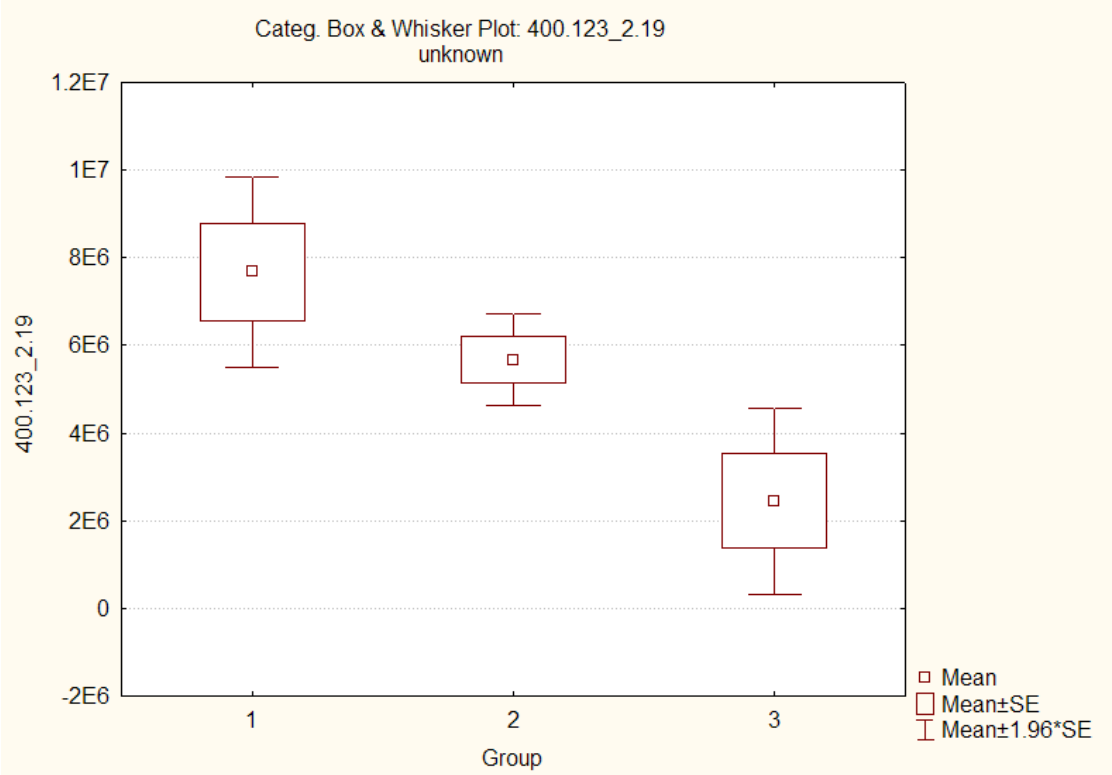
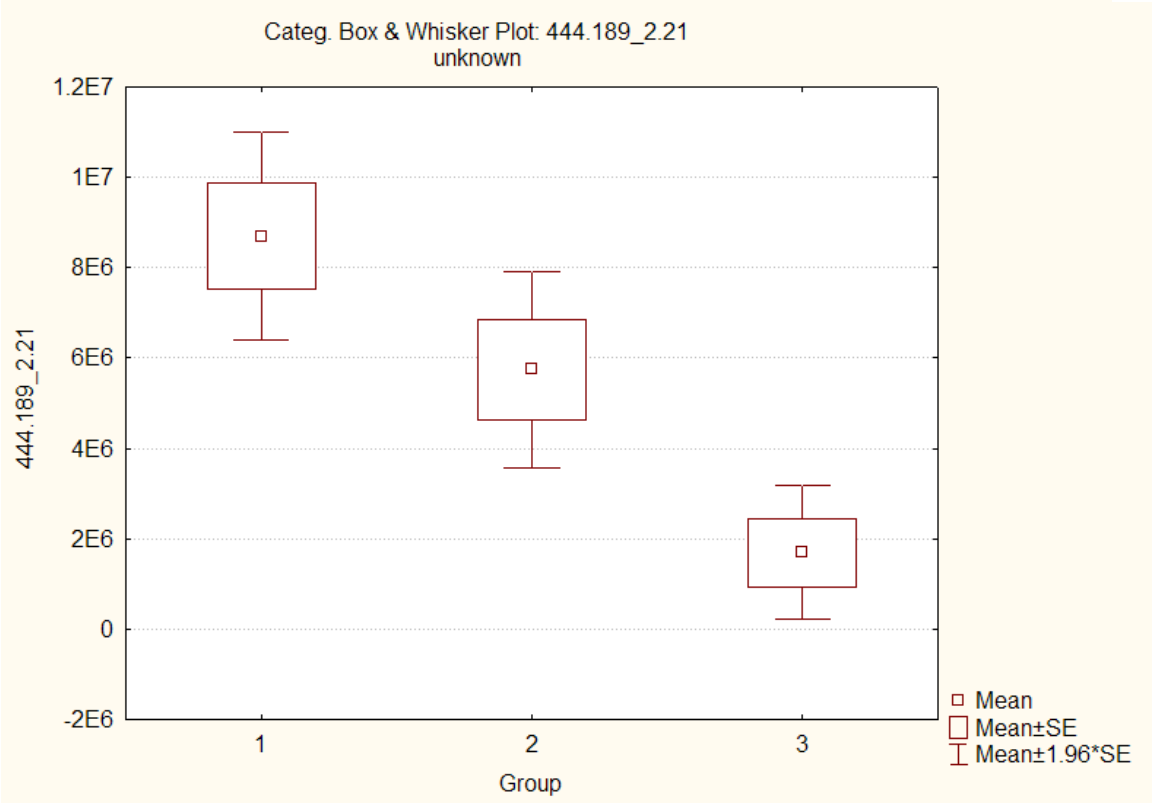
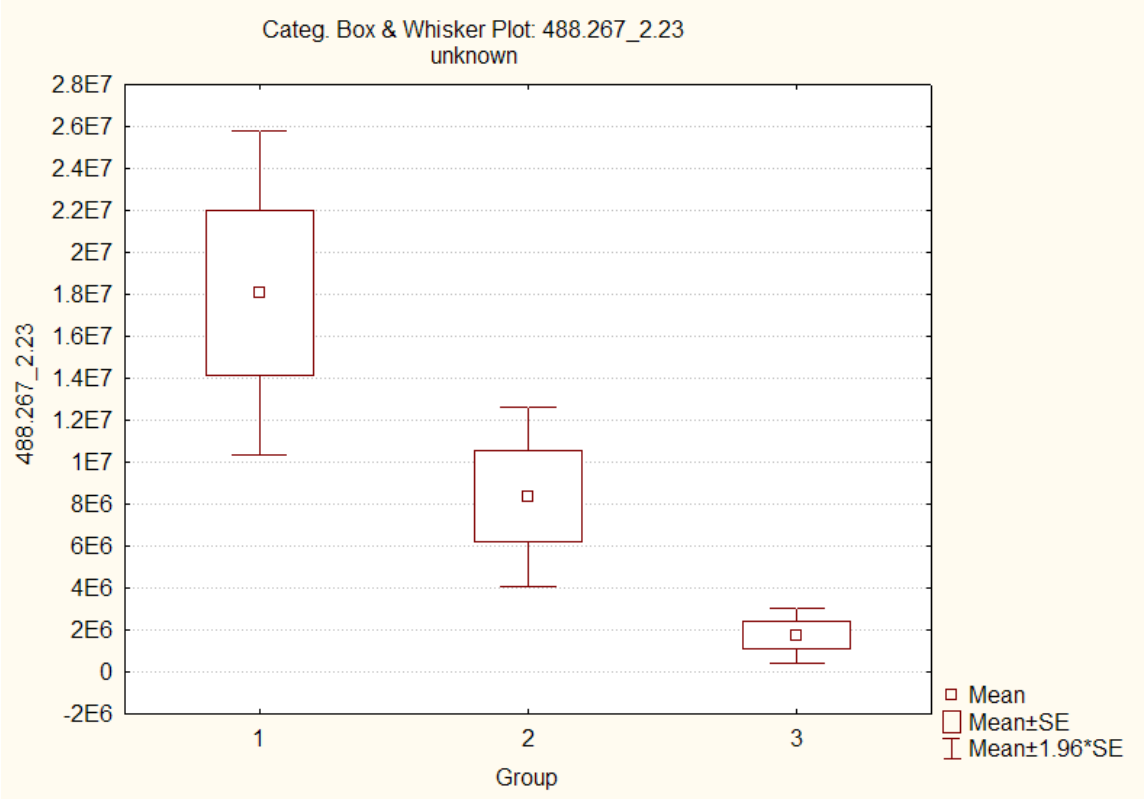
1.6E5
1.4E5
1.2E5
1E5
80000
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE

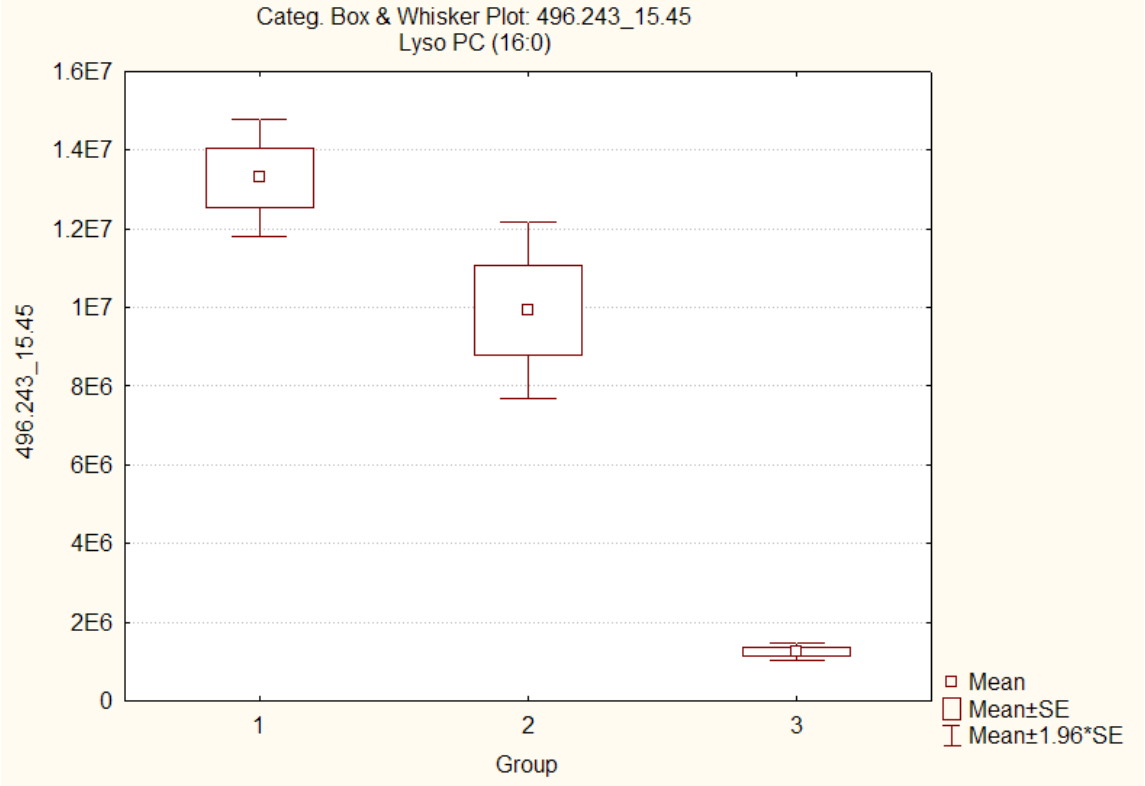

Categ. Box & Whisker Plot: 306.127_23.39
Ser-Glu-Ala ?
306.127_23.39
1.6E6
1.5E6
1.4E6
1.3E6
1.2E6
1.1E6
1E6
9E5
8E5
7E5
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE

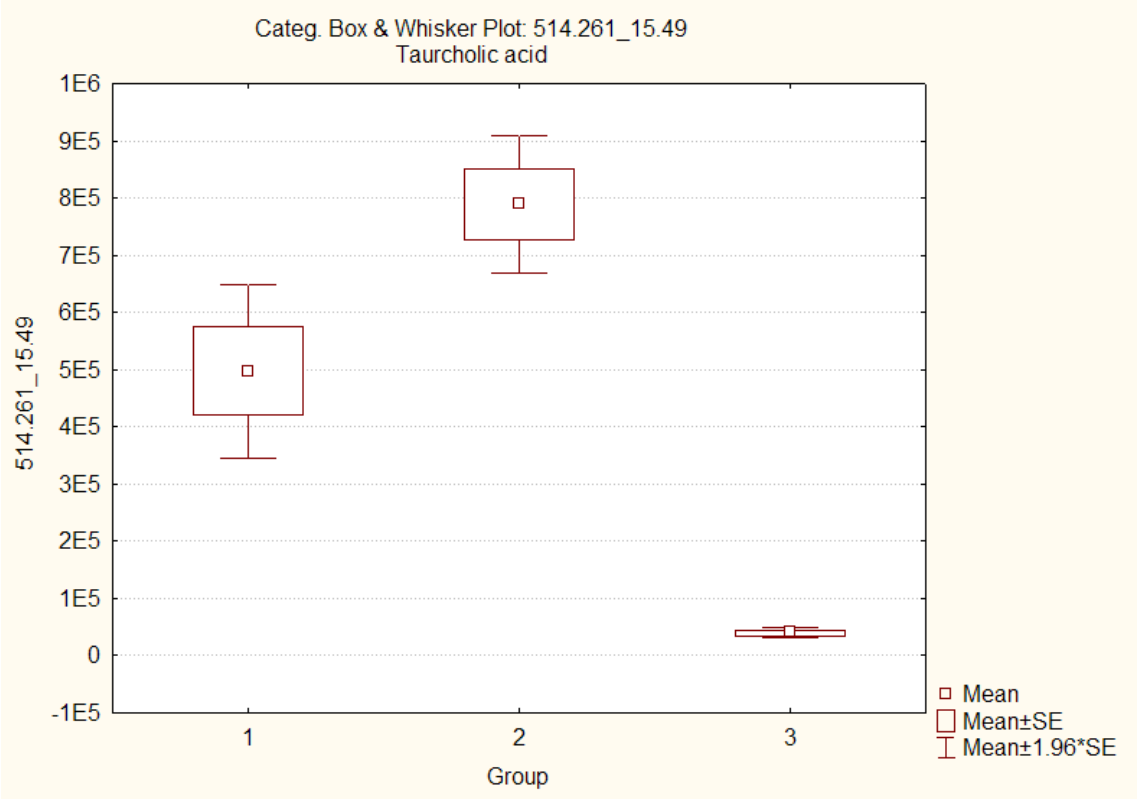

Categ. Box & Whisker Plot: 334.170_22.10
unknown
334.170_22.10
5.5E6
5E6
4.5E6
4E6
3.5E6
3E6
2.5E6
2E6
1.5E6
1E6
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE

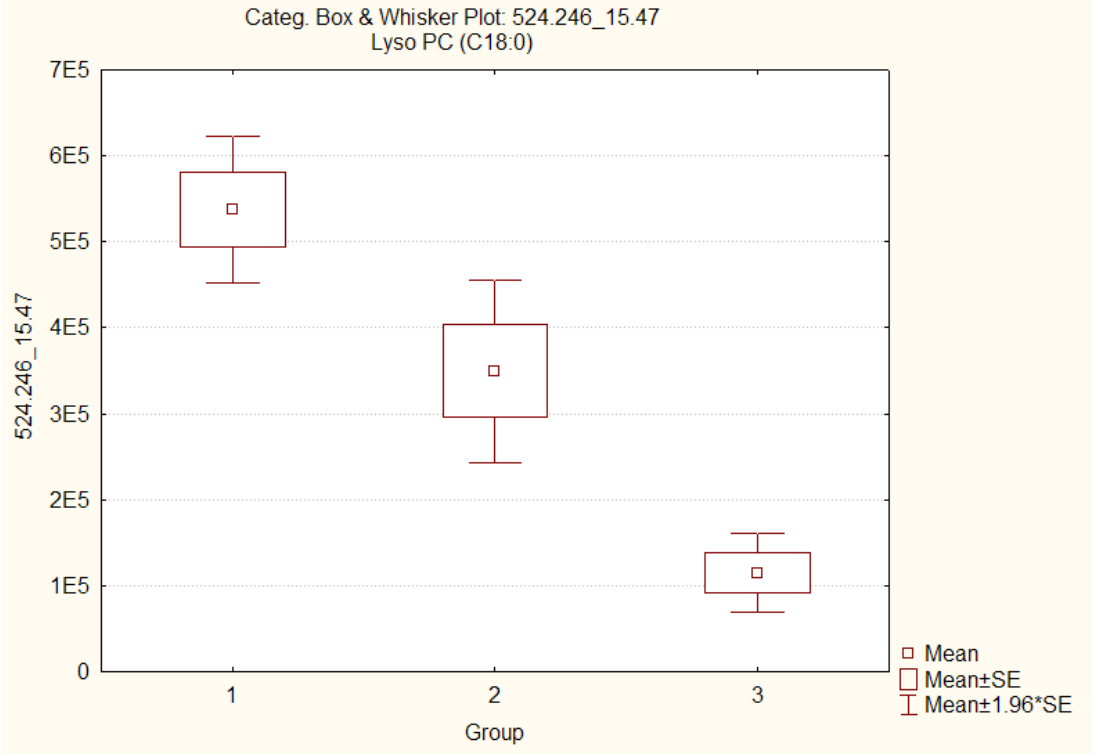

Categ. Box & Whisker Plot: 400.123_2.19
unknown
1.2E7
1E7
8E6
6E6
4E6
2E6
0
-2E6
400.123_2.19
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE

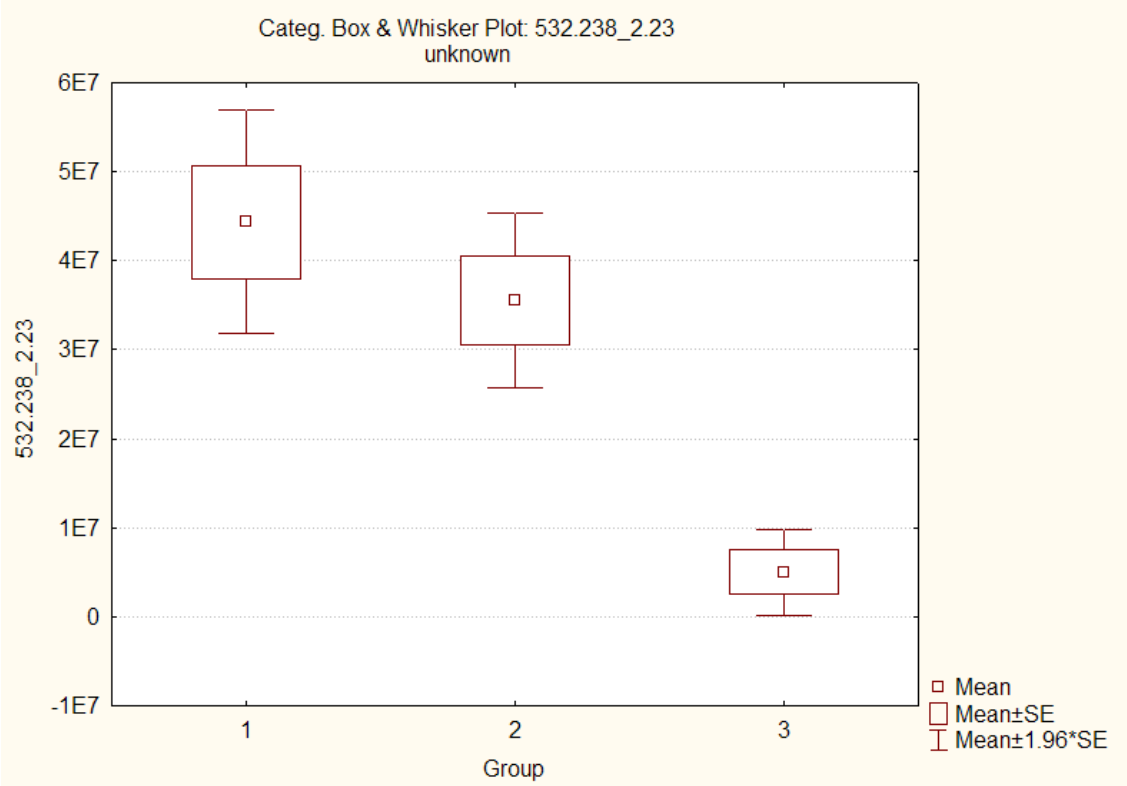

Categ. Box & Whisker Plot: 444.189_2.21
unknown
1.2E7
1E7
8E6
6E6
4E6
2E6
0
-2E6
444.189_2.21
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE


Categ. Box & Whisker Plot: 488.267_2.23
unknown
2.8E7
2.6E7
2.4E7
2.2E7
2E7
1.8E7
1.6E7
1.4E7
1.2E7
1E7
8E6
6E6
4E6
2E6
0
-2E6
488.267_2.23
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE


Categ. Box & Whisker Plot: 496.243_15.45
Lyso PC (16:0)
1.6E7
1.4E7
1.2E7
1E7
8E6
6E6
4E6
2E6
0
496.243_15.45
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE


Categ. Box & Whisker Plot: 514.261_15.49
Taurcholic acid
1E6
9E5
8E5
7E5
6E5
5E5
4E5
3E5
2E5
1E5
0
-1E5
514.261_15.49
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE


Categ. Box & Whisker Plot: 524.246_15.47
Lyso PC (C18:0)
7E5
6E5
5E5
4E5
3E5
2E5
1E5
0
524.246_15.47
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE


Categ. Box & Whisker Plot: 532.238_2.23
unknown
6E7
5E7
4E7
3E7
2E7
1E7
0
-1E7
532.238_2.23
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE

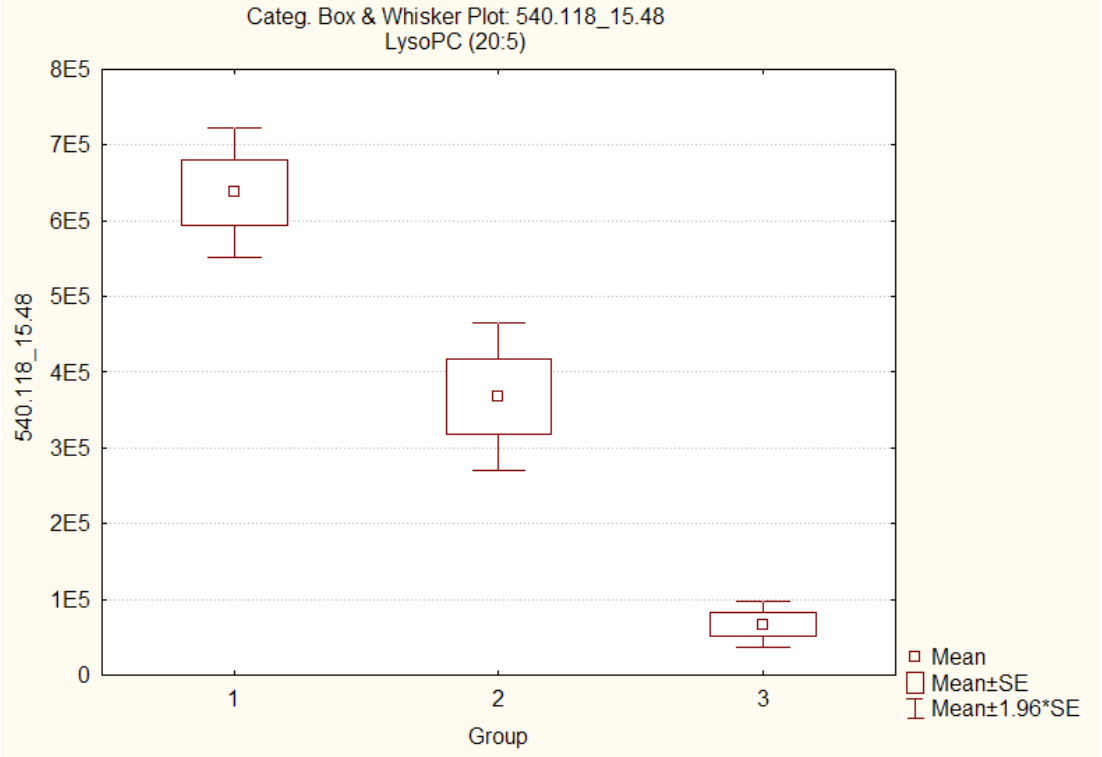

Categ. Box & Whisker Plot: 540.118_15.48
LysoPC (20:5)
8E5
7E5
6E5
5E5
4E5
3E5
2E5
1E5
0
540.118_15.48
1
2
3
Group
Mean
Mean±SE
Mean±1.96*SE

Table S3. Report on HILIC-LC/MS profiling annotation of HEK293-hOCT1 versus HEK293-empty vector cells

Samples:
Group 1: HEK293-Empty Vector
Group 2: HEK293-human OCT1(HEK293-hOCT1)

Sample Name	Group
1ch.wiff (sample 1)	1
1h.wiff (sample 1)	2
2ch.wiff (sample 1)	1
2h.wiff (sample 1)	2
3ch.wiff (sample 1)	1
3h.wiff (sample 1)	2
4ch.wiff (sample 1)	1
4h.wiff (sample 1)	2
5ch.wiff (sample 1)	1
5h.wiff (sample 1)	2
6ch.wiff (sample 1)	1
6h.wiff (sample 1)	2

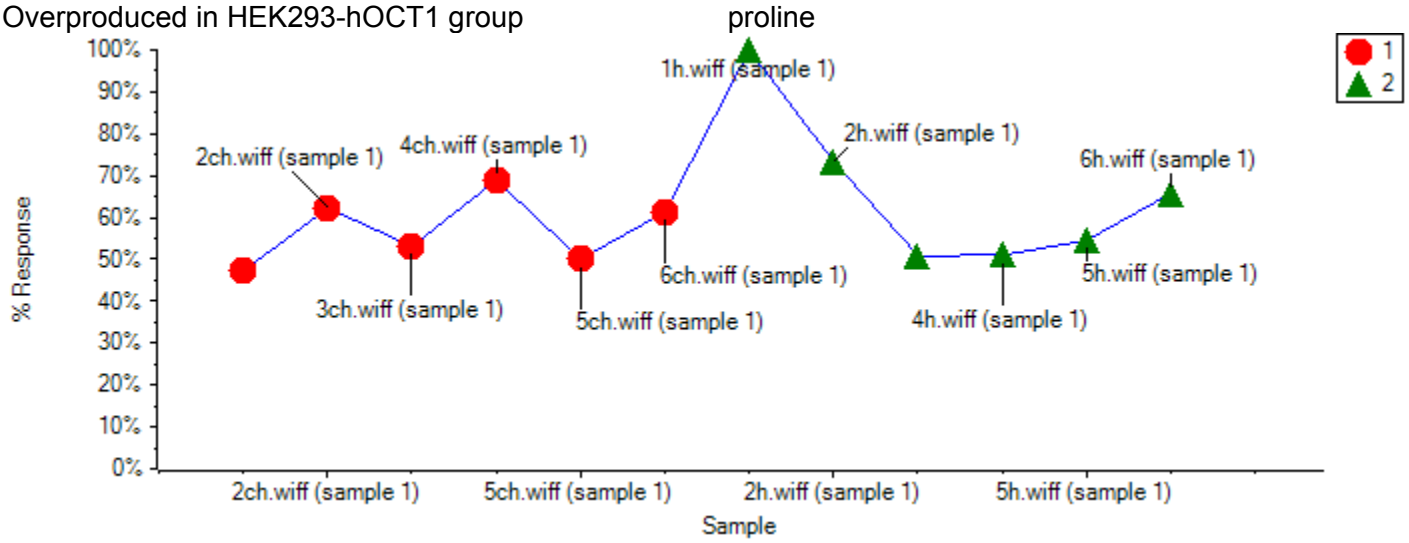
List of metabolites overproduced in HEK293-hOCT1 group

Peak Name	m/z	Ret. Time (min)	Comment
116.2/19.1 (94)	116.186	19.1	proline
120.2/21.0 (108)	120.169	21.0	threonine
122.1/15.2 (109)	122.117	15.2	Aminobutantriol
144.1/15.2 (255)	144.076	15.2	Amino-oxo-proline
177.1/14.3 (470)	177.070	14.3	2,3-Dimethyl-3-hydroxyglutarate
218.1/17.0 (805)	218.137	17.0	propionyl carnitine
226.9/19.4 (840)	226.950	19.4	unknown
232.2/16.2 (874)	232.160	16.2	unknown
235.0/19.3 (898)	235.035	19.3	5,6-dihydroxy-1H-indole-2-carboxylic acid
246.2/15.7 (1010)	246.164	15.7	2-Methylbutyroylcarnitine
258.1/22.9 (1094)	258.059	22.9	5-Methylcytidine
265.0/15.2 (1117)	265.022	15.2	thiamine
277.1/14.4 (1294)	277.092	14.4	6-Ribitylamino-5-aminouracil
304.3/2.3 (1427)	304.314	2.3	N-Acylsphingosine
308.0/26.6 (1508)	308.026	26.6	Glutathione
333.1/14.3 (1641)	333.086	14.3	Gln-Asp-Ala
339.3/8.9 (1686)	339.282	8.9	Cetoleic acid
351.0/14.4 (1724)	351.049	14.4	Gln-Asp-Ala ammonia adduct
399.1/2.2 (2119)	399.055	2.2	2-Acetyl-1-oleoyl-sn-glycerol
400.3/14.0 (2123)	400.267	14.0	unknown
520.2/2.2 (2609)	520.201	2.2	unknown lipid
535.8/2.2 (2645)	535.778	2.2	unknown lipid
543.0/16.3 (2672)	543.032	16.3	LysoPE ammonia adduct

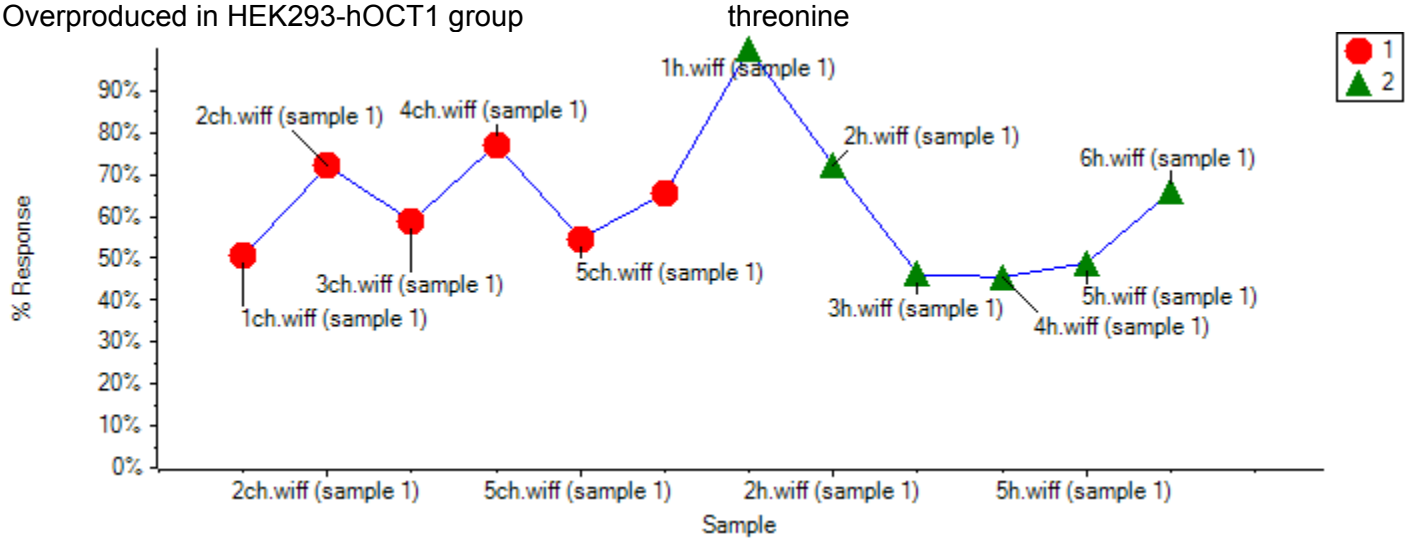
Peaks and their responses

1: HEK293-Empty Vector (●)
2: HEK293-human OCT1(HEK293-hOCT1)(▲)

Peak: 116.2/19.1 (94)
Overproduced in HEK293-hOCT1 group



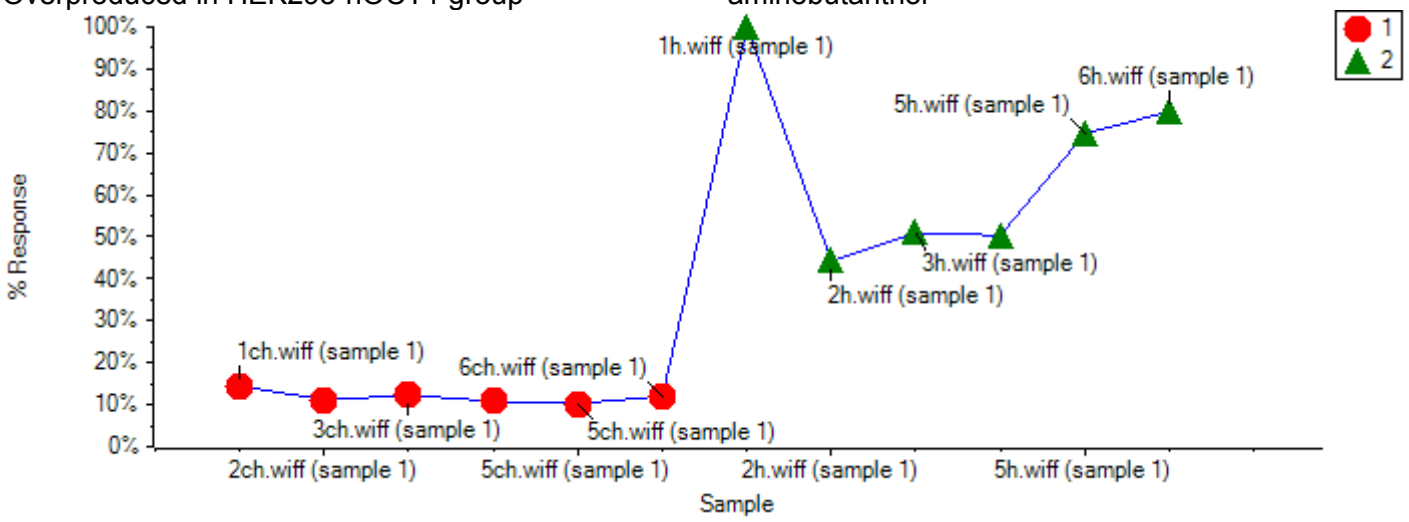
Peak: 120.2/21.0 (108)
Overproduced in HEK293-hOCT1 group



Peak: 122.1/15.2 (109)

Overproduced in HEK293-hOCT1 group

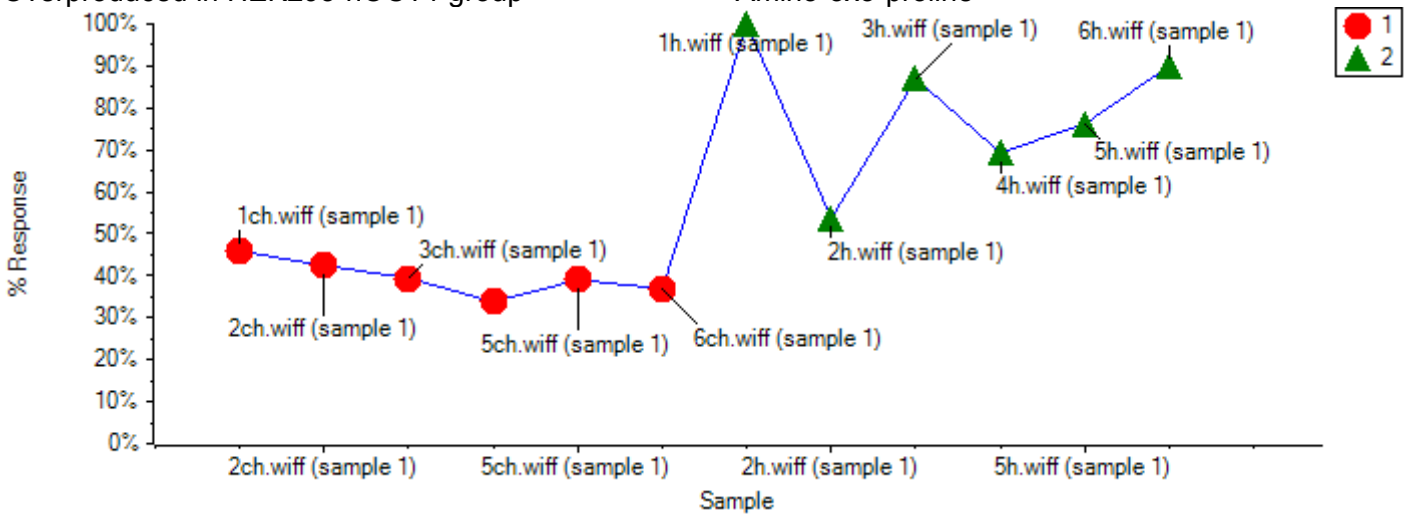
aminobutantriol



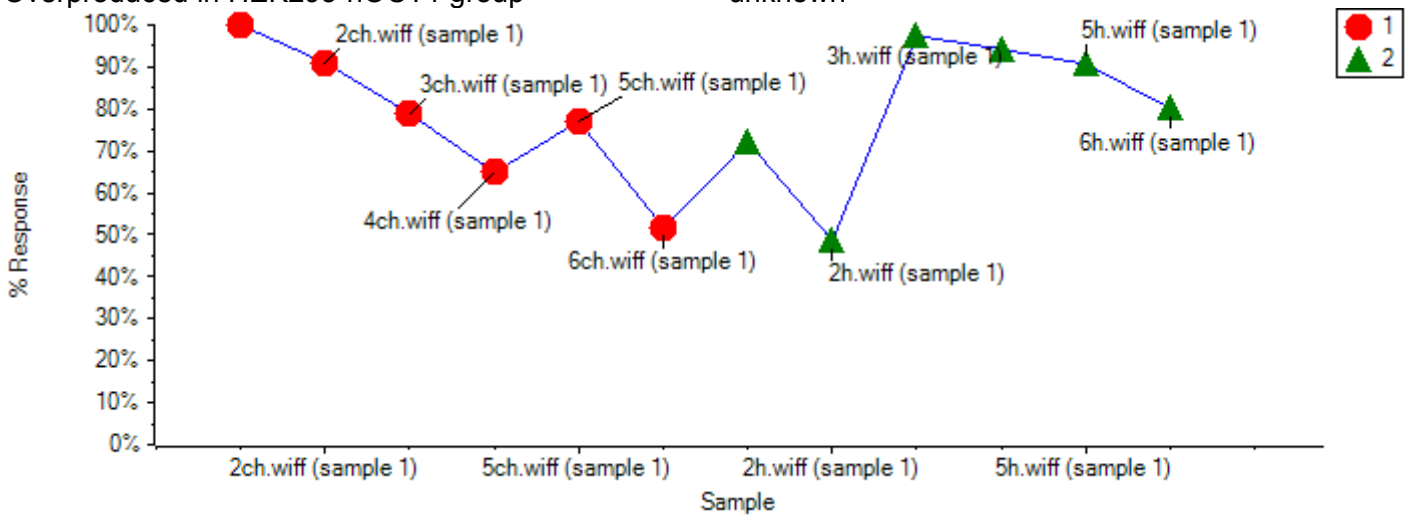
Peak: 144.1/15.2 (255)

Overproduced in HEK293-hOCT1 group

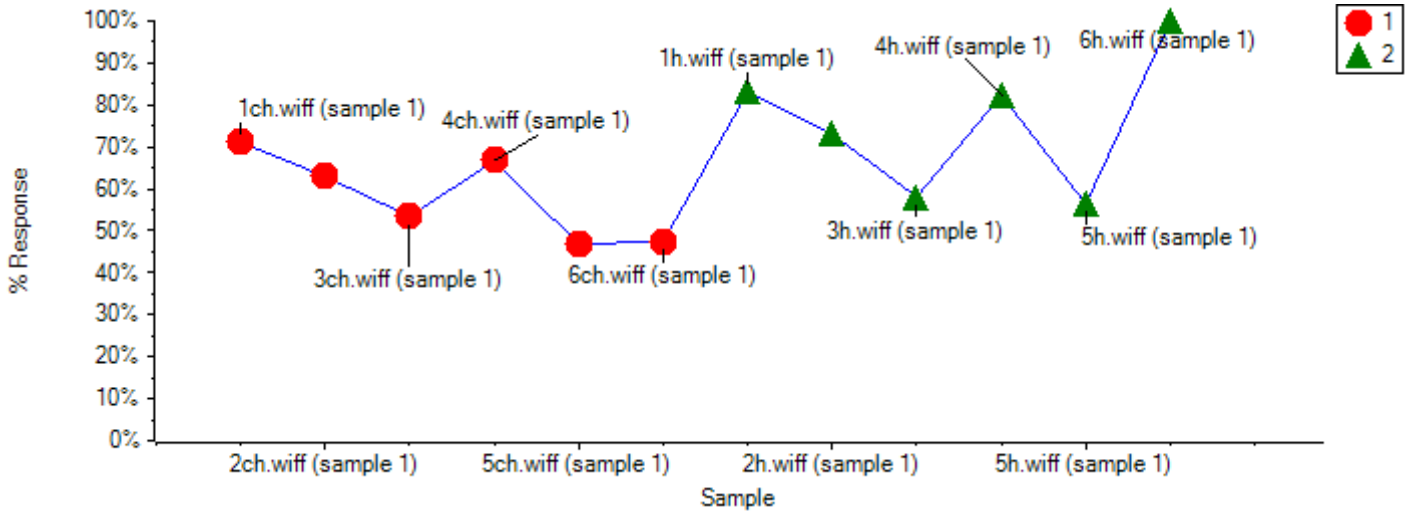
Amino-oxo-proline



Peak: 177.1/14.3 (470)
Overproduced in HEK293-hOCT1 group unknown

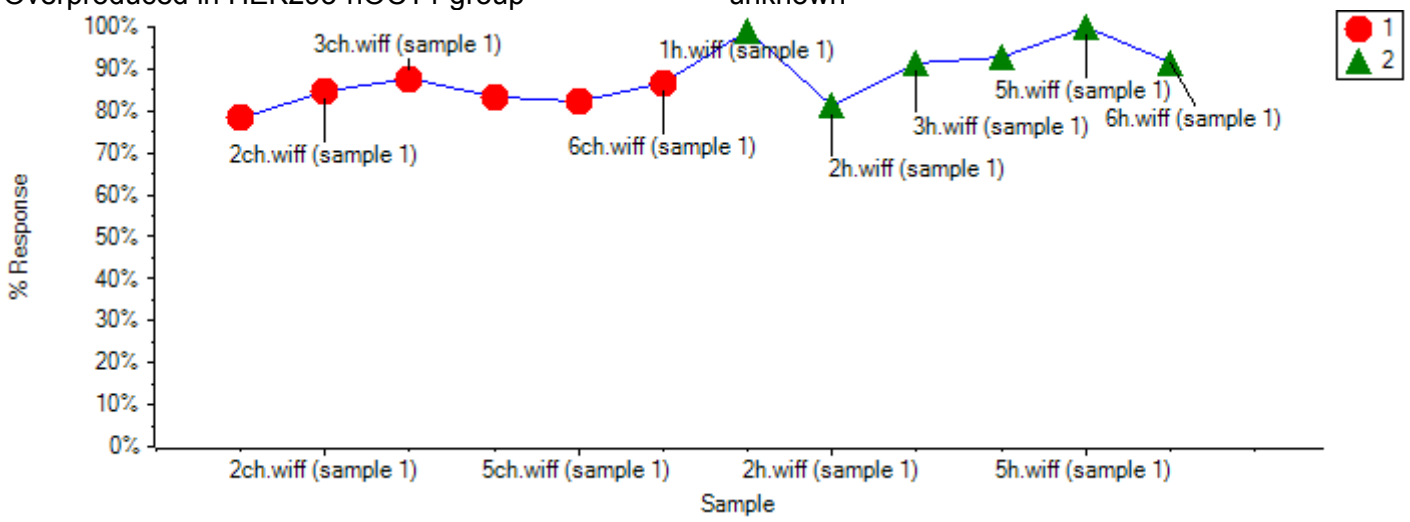


Peak: 218.1/17.0 (805)
Overproduced in HEK293-hOCT1 group propionyl carnitine



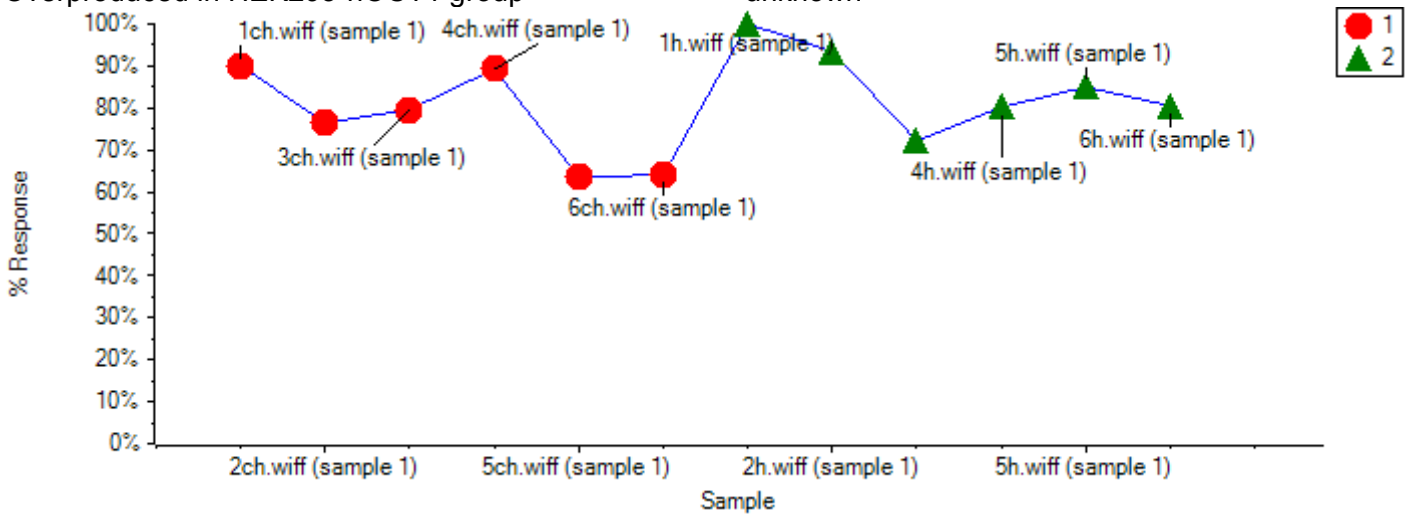
Peak: 226.9/19.4 (840)

Overproduced in HEK293-hOCT1 group unknown



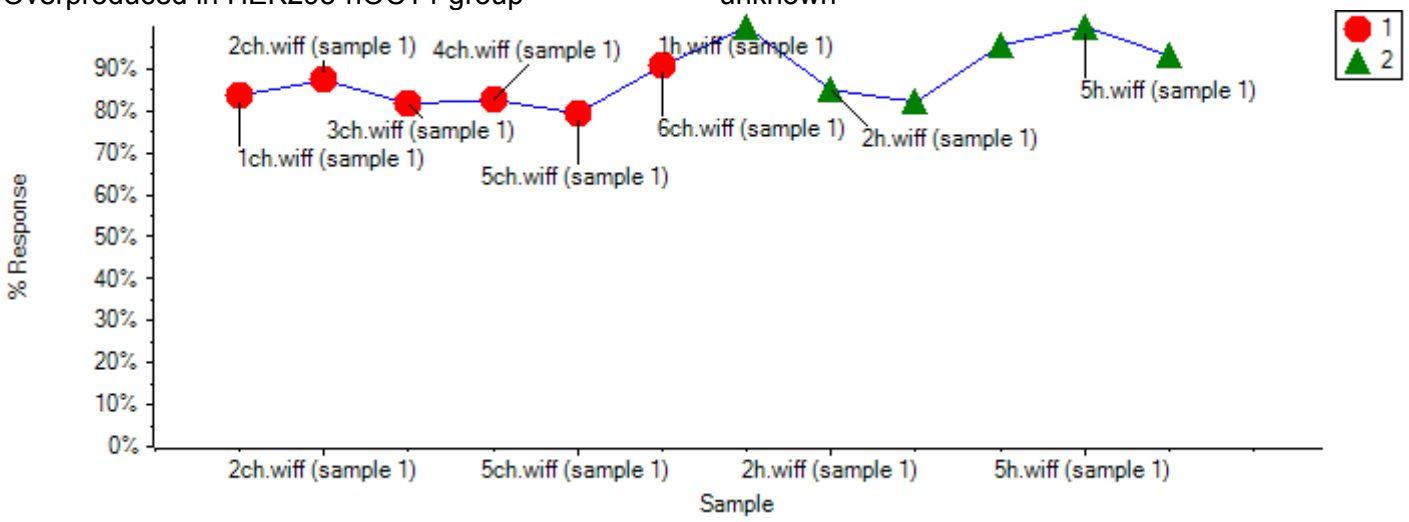
Peak: 232.2/16.2 (874)

Overproduced in HEK293-hOCT1 group unknown



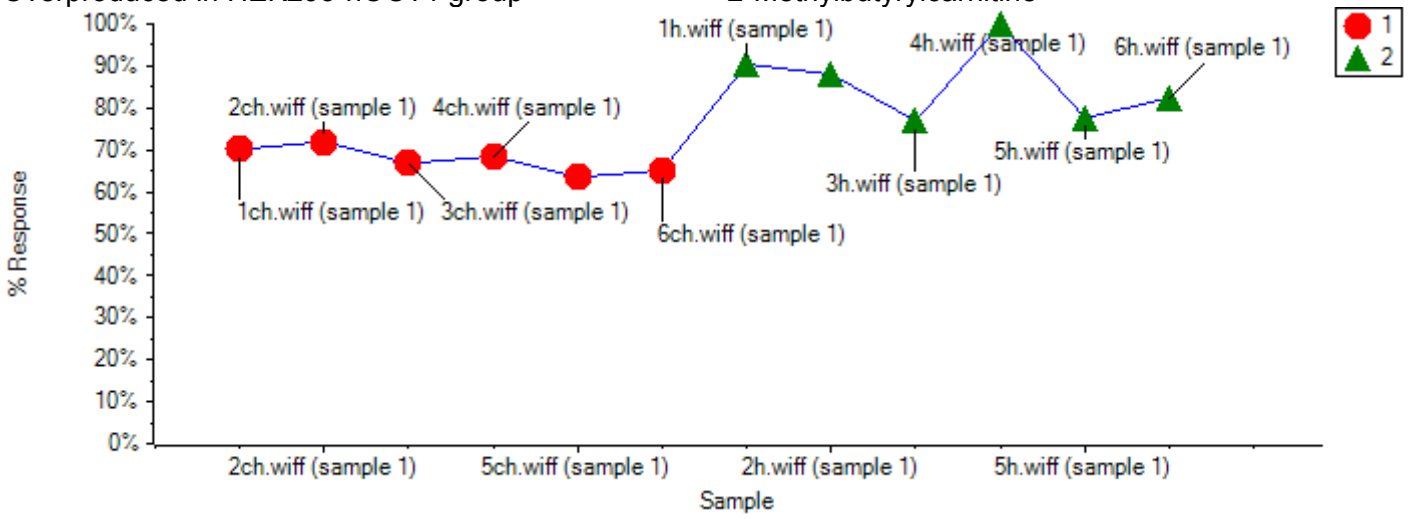
Peak: 235.0/19.3 (898)

Overproduced in HEK293-hOCT1 group unknown



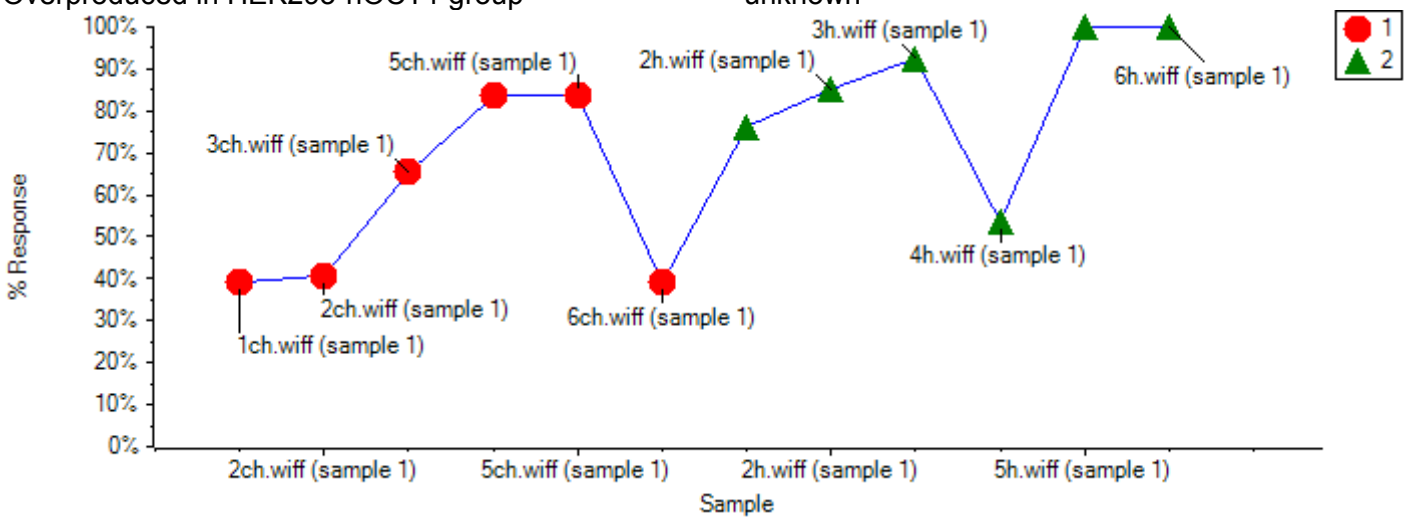
Peak: 246.2/15.7 (1010)

Overproduced in HEK293-hOCT1 group 2-Methylbutyrylcarnitine



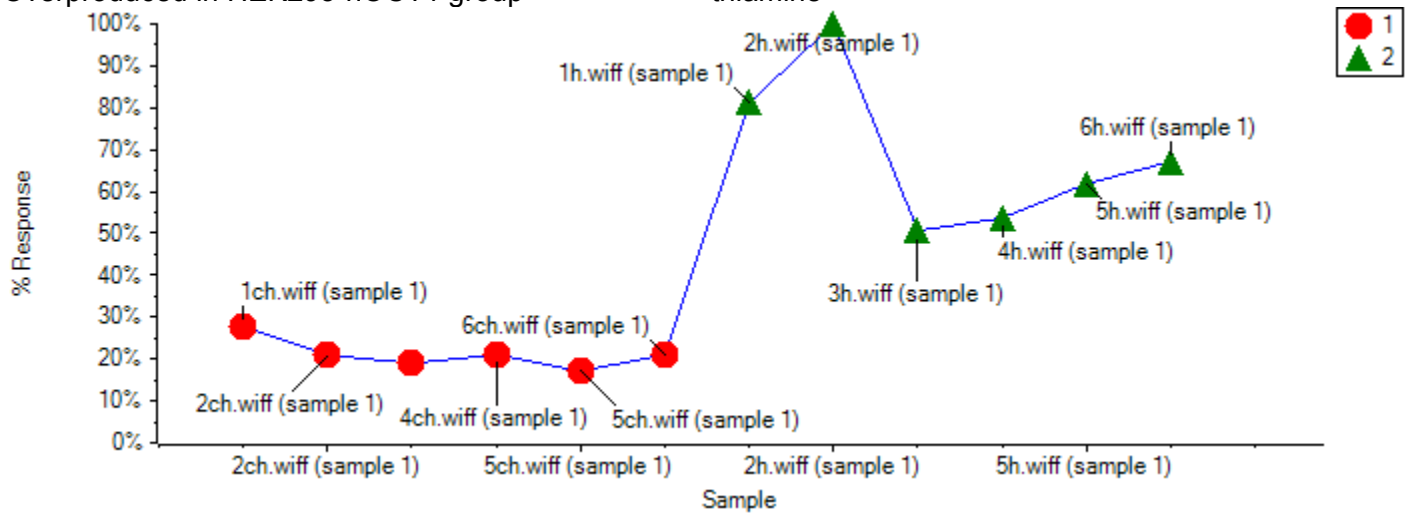
Peak: 258.1/22.9 (1094)

Overproduced in HEK293-hOCT1 group unknown



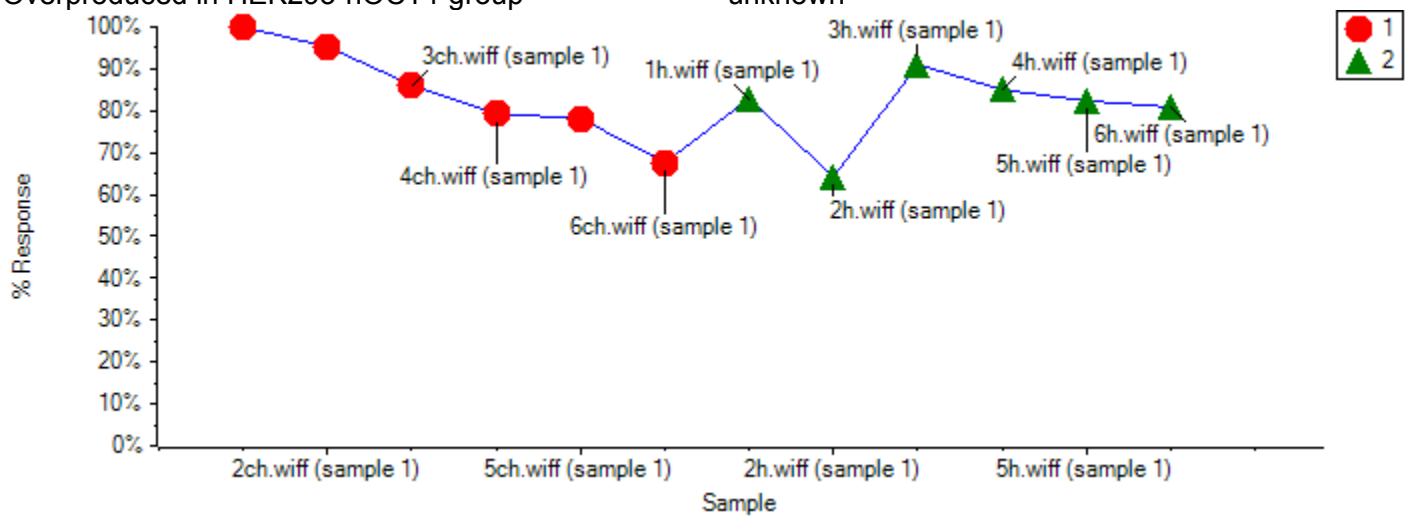
Peak: 265.0/15.2 (1117)

Overproduced in HEK293-hOCT1 group thiamine



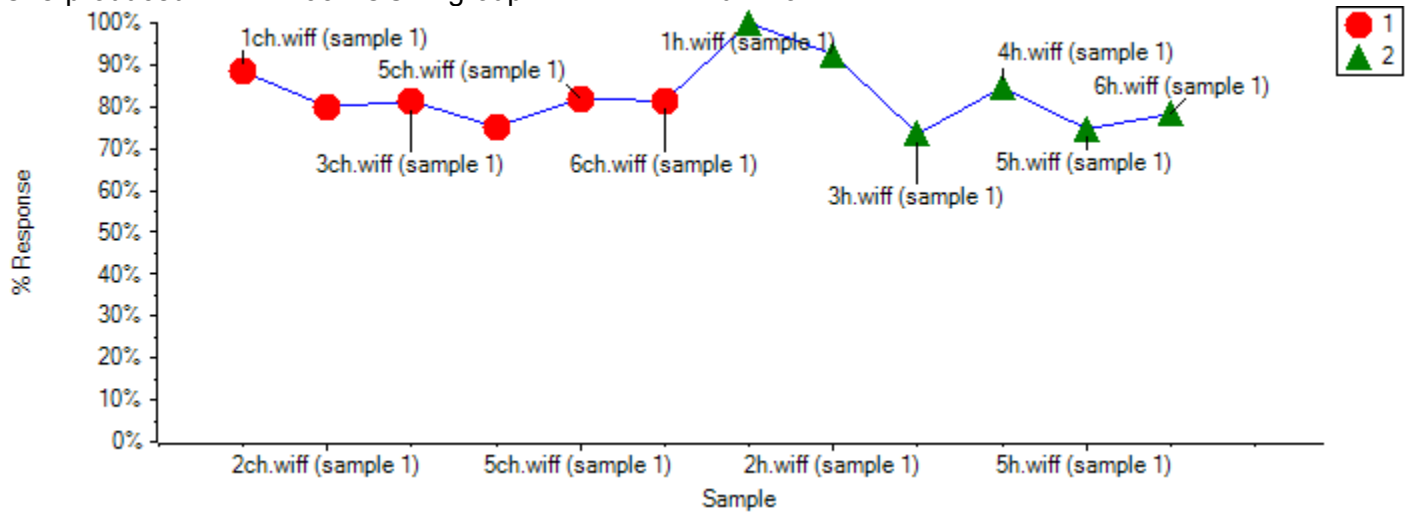
Peak: 277.1/14.4 (1294)

Overproduced in HEK293-hOCT1 group unknown



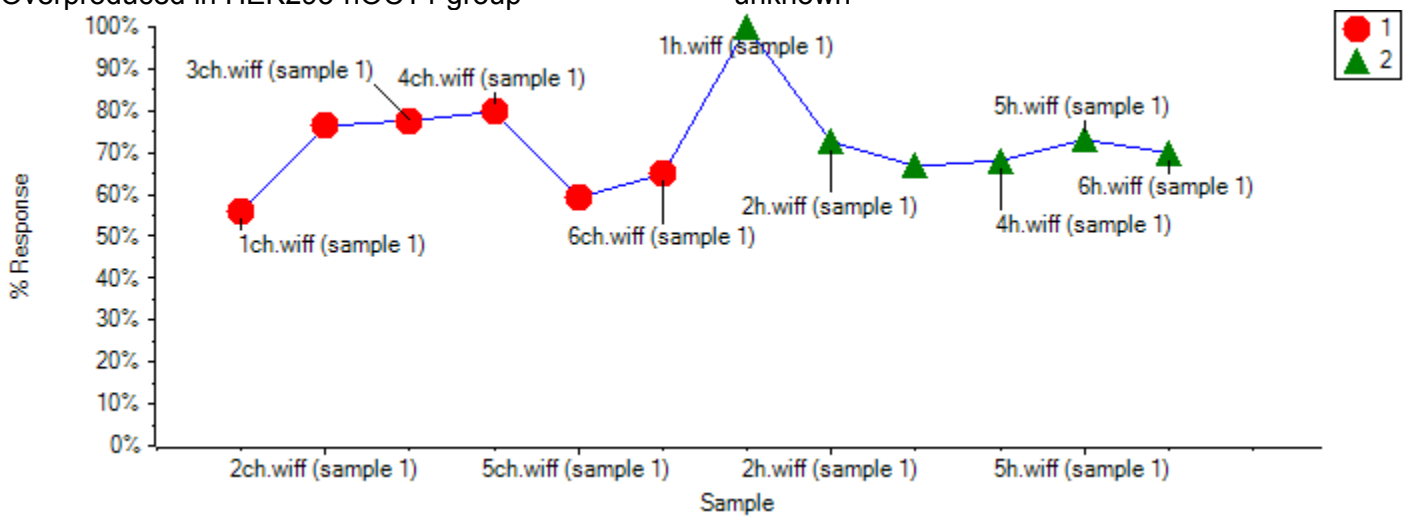
Peak: 304.3/2.3 (1427)

Overproduced in HEK293-hOCT1 group unknown



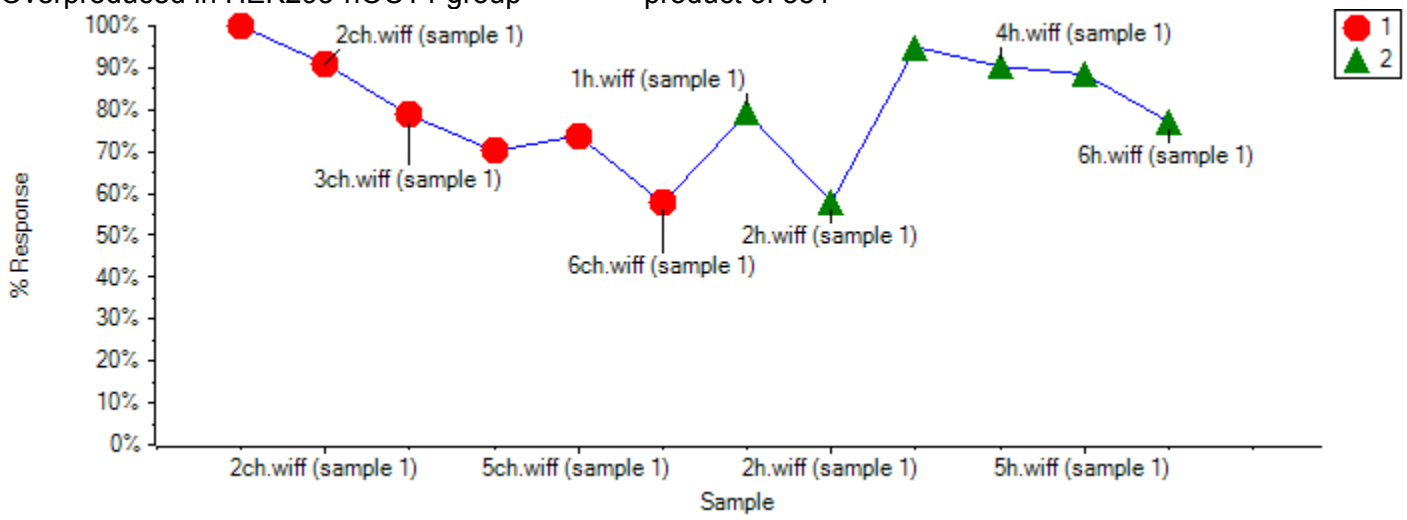
Peak: 308.0/26.6 (1508)

Overproduced in HEK293-hOCT1 group unknown



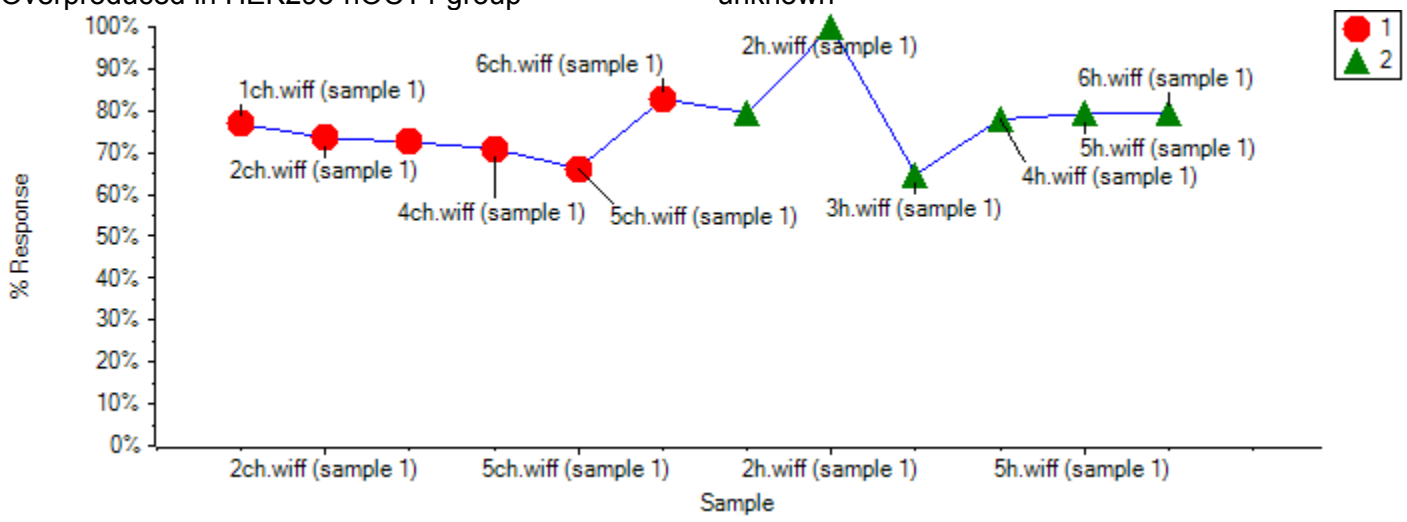
Peak: 333.1/14.3 (1641)

Overproduced in HEK293-hOCT1 group product of 351



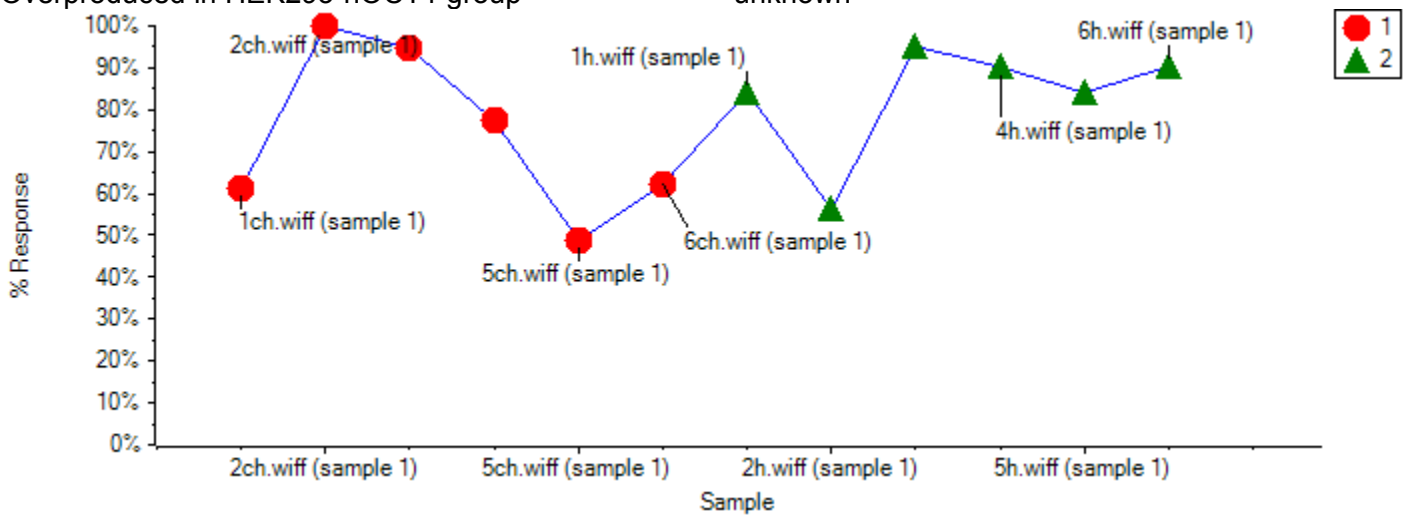
Peak: 339.3/8.9 (1686)

Overproduced in HEK293-hOCT1 group unknown



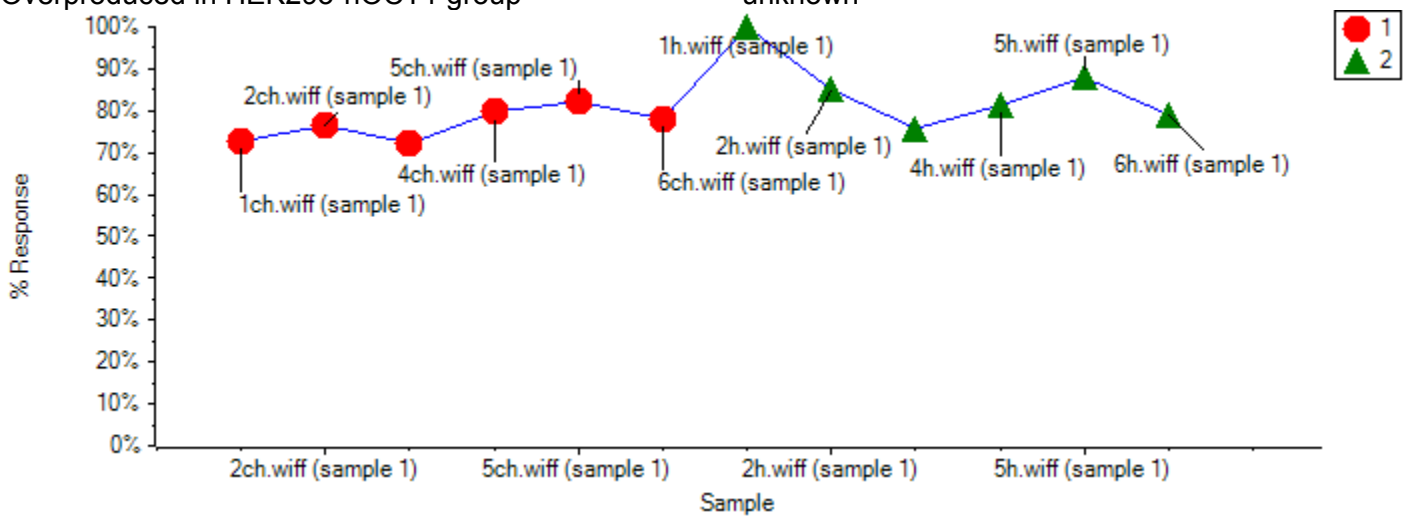
Peak: 351.0/14.4 (1724)

Overproduced in HEK293-hOCT1 group unknown



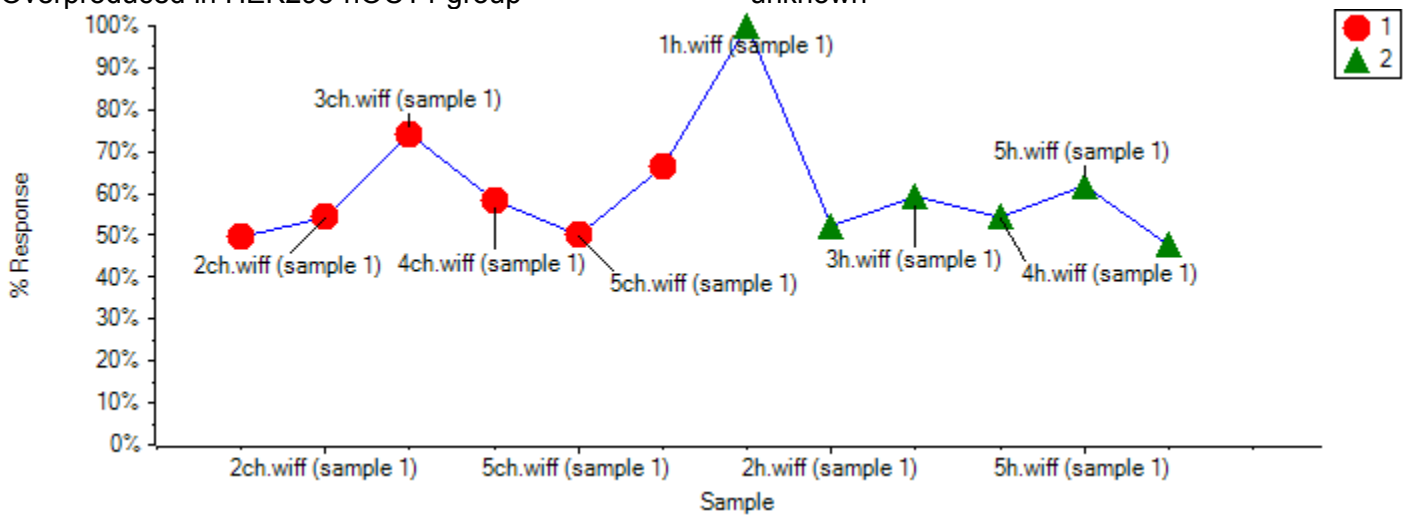
Peak: 399.1/2.2 (2119)

Overproduced in HEK293-hOCT1 group unknown



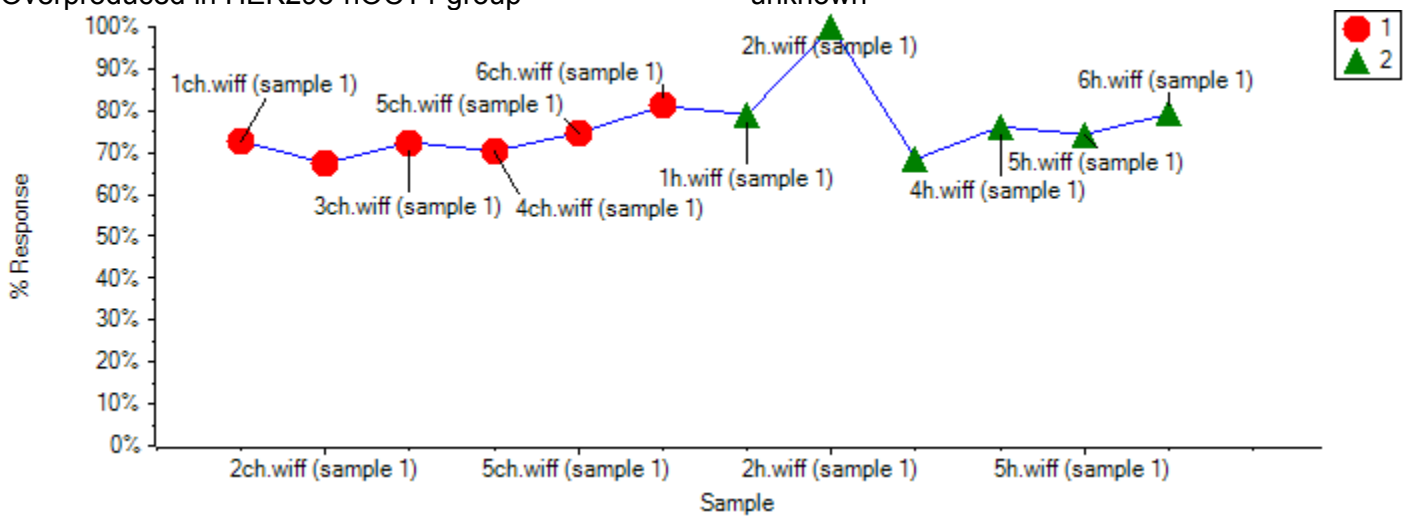
Peak: 400.3/14.0 (2123)

Overproduced in HEK293-hOCT1 group unknown



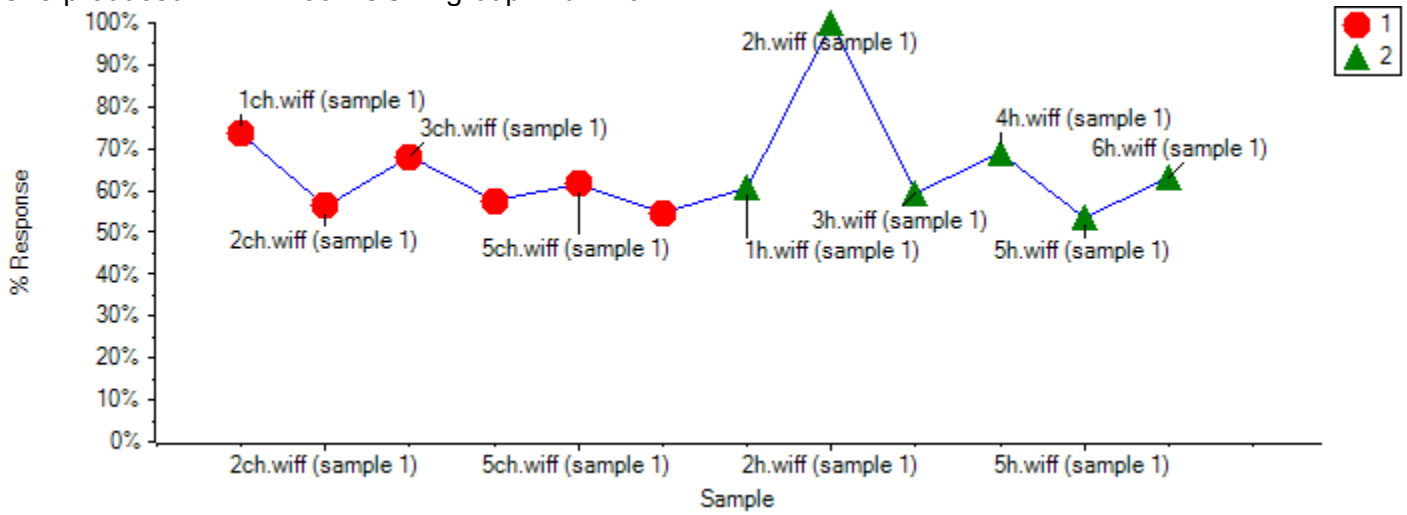
Peak: 520.2/2.2 (2609)

Overproduced in HEK293-hOCT1 group unknown



Peak: 535.8/2.2 (2645)

Overproduced in HEK293-hOCT1 group unknown



Peak: 543.0/16.3 (2672)

Overproduced in HEK293-hOCT1 group unknown

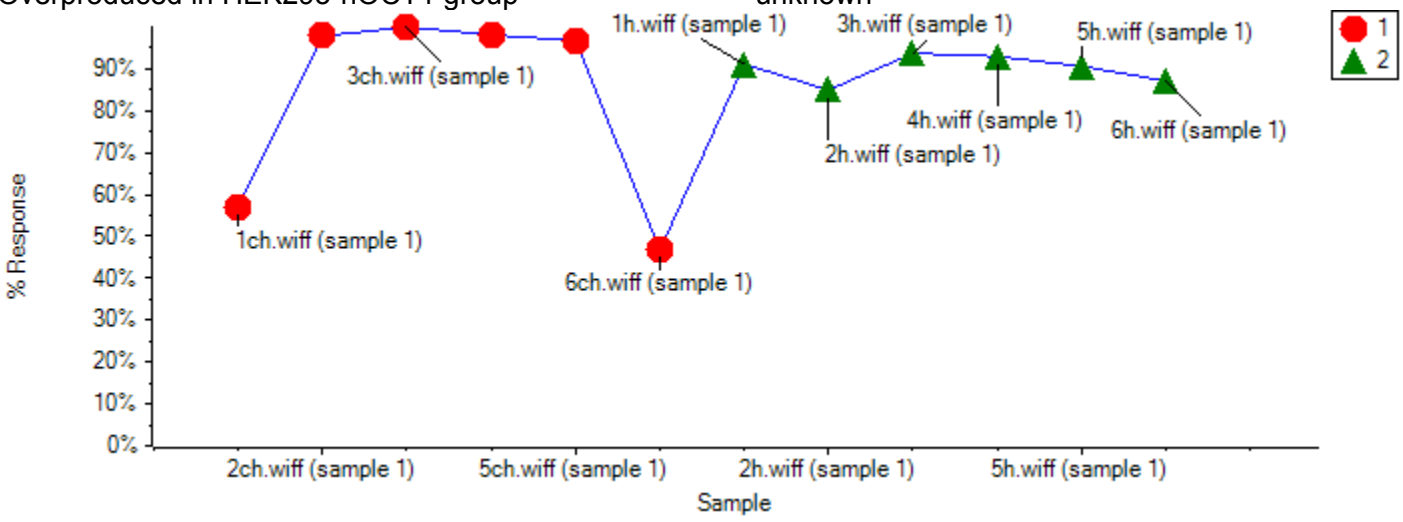


Table S4. The kinetics parameters of thiamine transport by human OCT1, OCT2, MATE1 and mouse Oct1 and Slc19a2 in transfected cells.

Kinetic parameter	OCT1	Oct1	Slc19a2	OCT2	MATE1
V_{max} (nmol/mg protein/min)	2.77 ± 0.14	5.80 ± 0.37**	0.76 ± 0.092**	4.56 ± 0.33**	2.94 ± 0.27**
K_m(mM)	0.78 ± 0.064	0.49 ± 0.035**	0.17 ± 0.021**	0.75 ± 0.045	0.83 ± 0.11
Vmax/Km	3.64	12.08	4.47	6.08	3.54

Studies were performed in HEK293 cells stably-transfected with the following transporters. Each uptake experiment was repeated at least 3 times with triplicate samples each time. All data are expressed as Mean ± SEM, ***P<0.01*, compared with OCT1.

Table S5. Parameters obtained from MRI studies and following liver surgery.

	Oct1$^{+/+}$	*Oct1*$^{-/-}$	*Oct1*$^{+/+,OCT1tg}$	*Oct1*$^{-/-,OCT1tg}$
Volume (cm^3)	2.05 ± 0.13	1.61 ± 0.18**	3.21 ± 0.35**	2.13± 0.21
% liver fat	37.3± 6.2 %	28.5 ± 2.6%***	52.2 ± 5.1%***	40.6 ± 2.8%
Liver weight (g)	2.76 ± 0.28	2.19 ± 0.14**	4.53 ± 0.35**	2.84 ± 0.19
% BW	5.21 ± 0.50%	4.13 ± 0.29%**	8.95 ± 1.2%**	5.54 ± 0.71%

All data are expressed as Mean ± SEM, ***P<0.01*, compared with OCT1. Mice were fed with high fat diet for 16 weeks (n=3/genotypes).