Metabolomics and *in-silico* docking-directed discovery of small-molecule enzyme targets

Tengfei Xu[†,§], Haoduo Zhao[†], Mengjing Wang[†], Agnes Chow[†,§], and Mingliang Fang[*,†,§,#]

† School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

§ Nanyang Environment & Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, Singapore 637141

Lee Kong Chian School of Medicine, Nanyang Technological University, 639798, Singapore

Mingliang Fang: 0000-0002-2204-9783

The supporting information contains 29 pages, 12 figures, 6 tables and 31 references.

Text S1. Sample preparation of *in vitro* activity assay. The protocol for the acyl-coenzyme A extraction from liver tissue was followed by a previous study.[1] Briefly, 100 mg fresh liver was collected from one 2-month-old mouse (C57BL/6 J, purchased from Jackson Laboratory), which was further homogenated with cold methanol-water (v/v, 4:1, 1 mL), incubated on ice for 10 mins and then subsequently subjected to centrifugation for 20,000 × *g* 10 mins at 4°C to obtain the supernatant. The supernatant liver extract was used to dry at 4°C, and then dissolved into 1 mL 50 mM ammonium acetate (pH 6.8) (100 mg/mL, equal to liver weight) for the enzymatic activity assay.

The assay mixtures contain 16 µL 100 mM Tris–HCl (pH 7.0), 25 µL liver extract, 5 µL 10 mM NAD+, 300 ng purified HADH/HSD17B10, 1 µL of DMSO, TPhP and acetyl-CoA (final concentration as 0.5% DMSO, 5 µM TPhP, 10 µM TPhP, 0.5 mM acetyl-CoA and 1 mM acetyl-CoA, respectively). After pre-incubation of the enzyme mixture for 10 mins at 37°C, the reaction was started with the addition of 300 ng HADH/HSD17B10. The mixture was reacted at 37°C for 15 mins, quenched with 100 µL −40°C methanol, centrifuged for 20,000 × *g* 10 mins at 4°C to get the supernatant for relative quantification analysis of the 3-hydroxybutyryl-CoA. Three biological samples were prepared in each treated group.

Text S2. The LC-MS/MS condition for the relatively quantification of acyl-CoAs. The relatively quantification method of acyl-CoAs was modified by a previous study.[2] The triple quadrupole mass spectrometer (Agilent 6460, with an ESI source) coupled with a C18 BEH column (ACQUITY UPLC, 130Å, 1.7 μm, 2.1 mm * 100 mm) was used for the detection of acyl-CoAs, with the mobile phases consisting of A: 10 mM ammonium acetate in water (pH 6.5) and B: acetonitrile. The flow rate was kept at 0.2 mL/min, and the gradient elution started from 20% B, increased to 100% B during 8 mins, maintained 100% B for 2 mins and finally switched to 20% B for another 2 mins. The column temperature was controlled at 30 °C. Samples were analyzed in positive ion mode. The MS/MS parameters were optimized by direct infusion of 10 μM acetyl-CoA (dissolved in 50% acetonitrile) into the instrument. The capillary voltage was set as 3.20 kV, the gas flow and temperature was set as 8 L/min and 300℃, respectively. Multiple reaction monitoring (MRM) function was applied for the simultaneous detection of the acyl-CoAs. The ion transitions for acetyl-CoA, 3-hydroxybutyryl-CoA were 810.1/428.1 and 854.1/347.1, with the collision energies set as 25 eV and 25 eV, respectively.

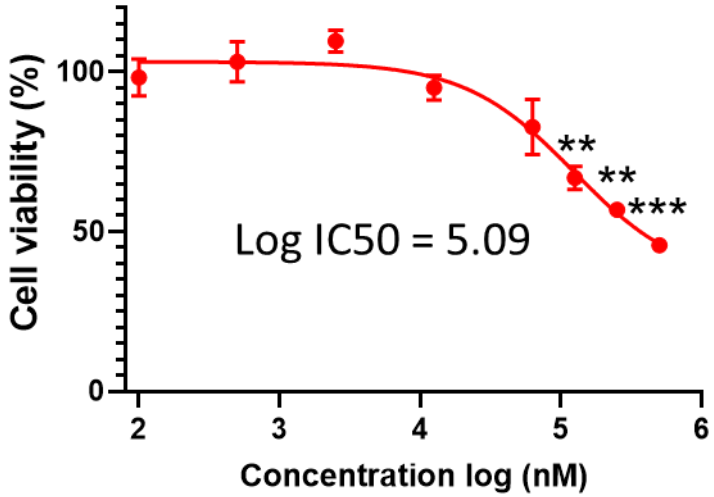

Figure S1. The cell viabilities of the HepG2 cells by treated with different concentrations of TPhP. The data were present with mean±SE, with four biological replicates at each test concentration point. Asterisks (** and ***) indicate statistical significant differences (p<0.01 and p<0.001) between high TPhP-treated groups and the 100 nM-treated group, respectively.

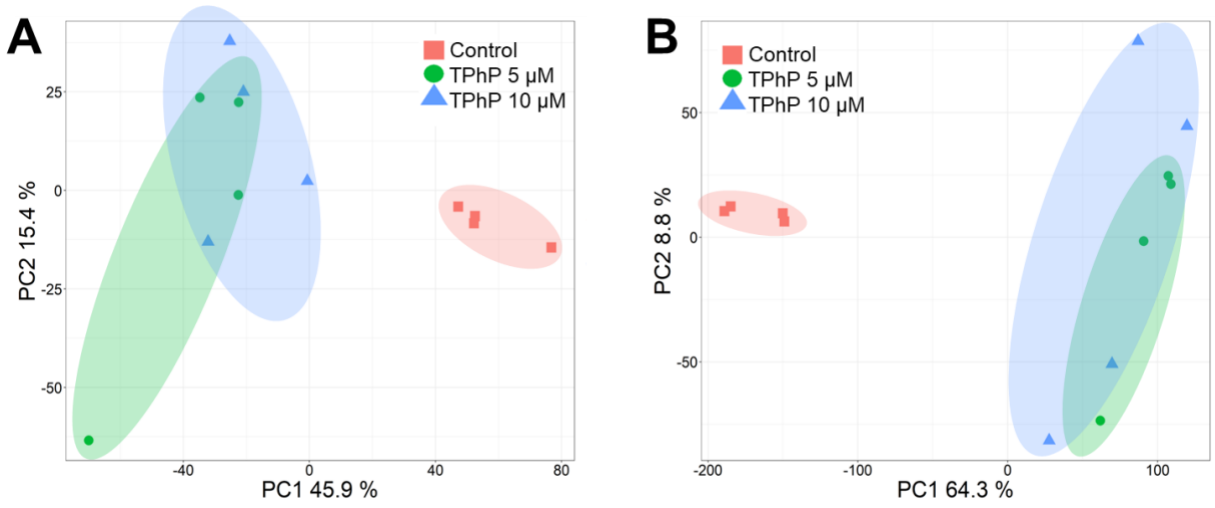

Figure S2. The metabolomics principal component analysis (PCA) of DMSO controls (Control), 5 and 10 μM TPhP treatment groups in (A) negative ion mode and (B) positive ion mode, respectively.

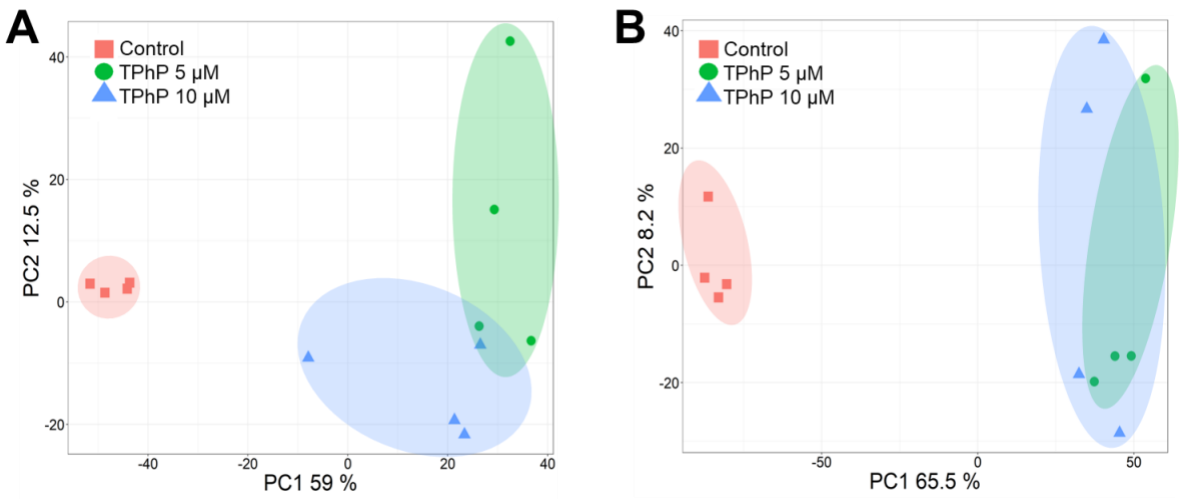

Figure S3. The lipidomics principal component analysis (PCA) of DMSO controls (Control), 5 and 10 μM TPhP treatment groups in (A) negative ion mode and (B) positive ion mode, respectively.

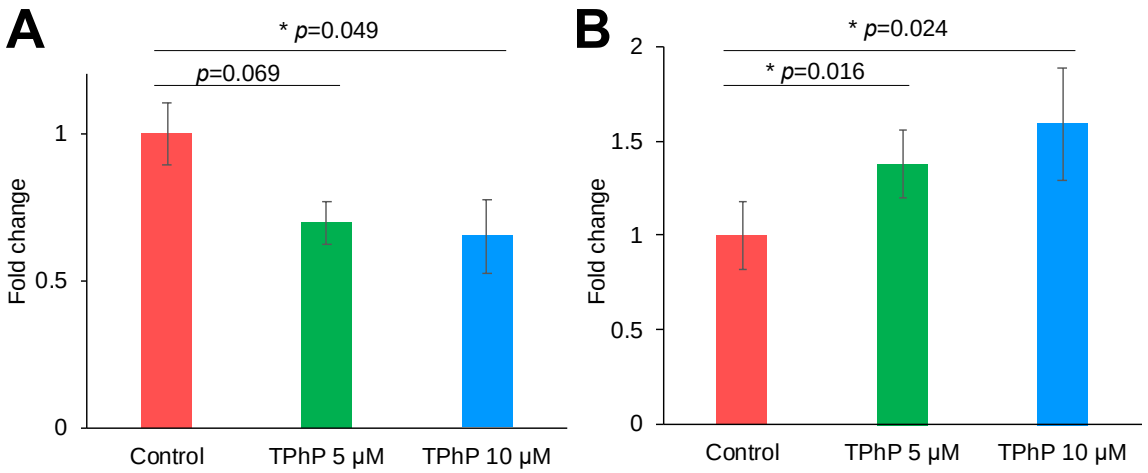

Figure S4. (A): Fold changes of acetyl-CoA in the control, 5 and 10 µM TPhP treatment groups. (B): Fold changes of 3-hydroxybutyry-CoA in the control, 5 and 10 µM TPhP treatment groups. The fold change was calculated from the ratio of the peak areas of the treatment groups and the control group. Asterisk (*) indicate statistically significant differences (p<0.05) between the TPhP-treated groups and the control group, respectively. Data are shown as mean±SE with four biological samples in each group.

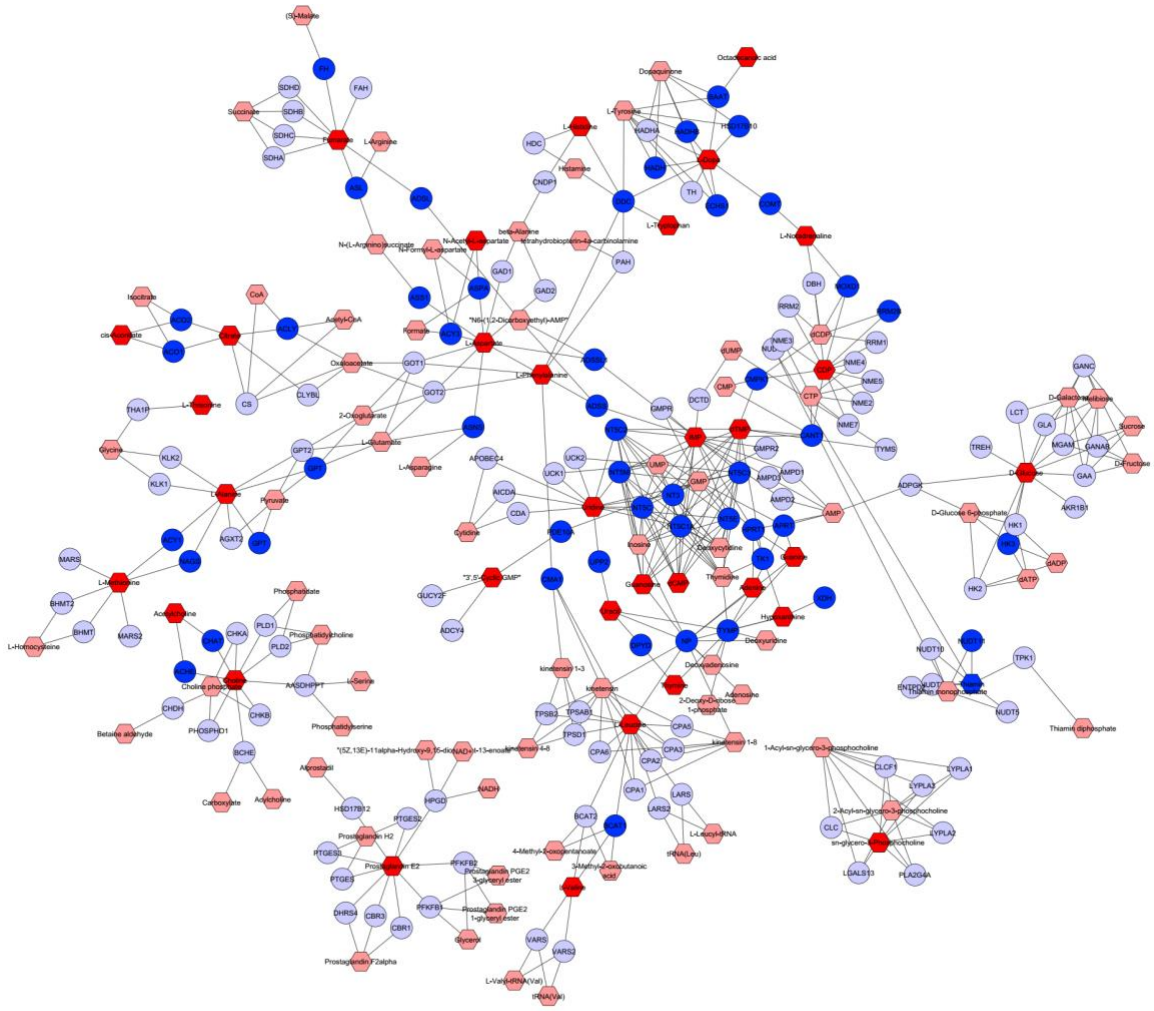

Figure S5. The affected metabolic pathways after TPhP exposure, which was generated by Metscape (embedded in Cytoscape). The nodes marked in red, pink, light blue, and dark blue represent the input metabolites, other associated metabolites, enzymes in the pathway, and the selected enzymes for docking, respectively.

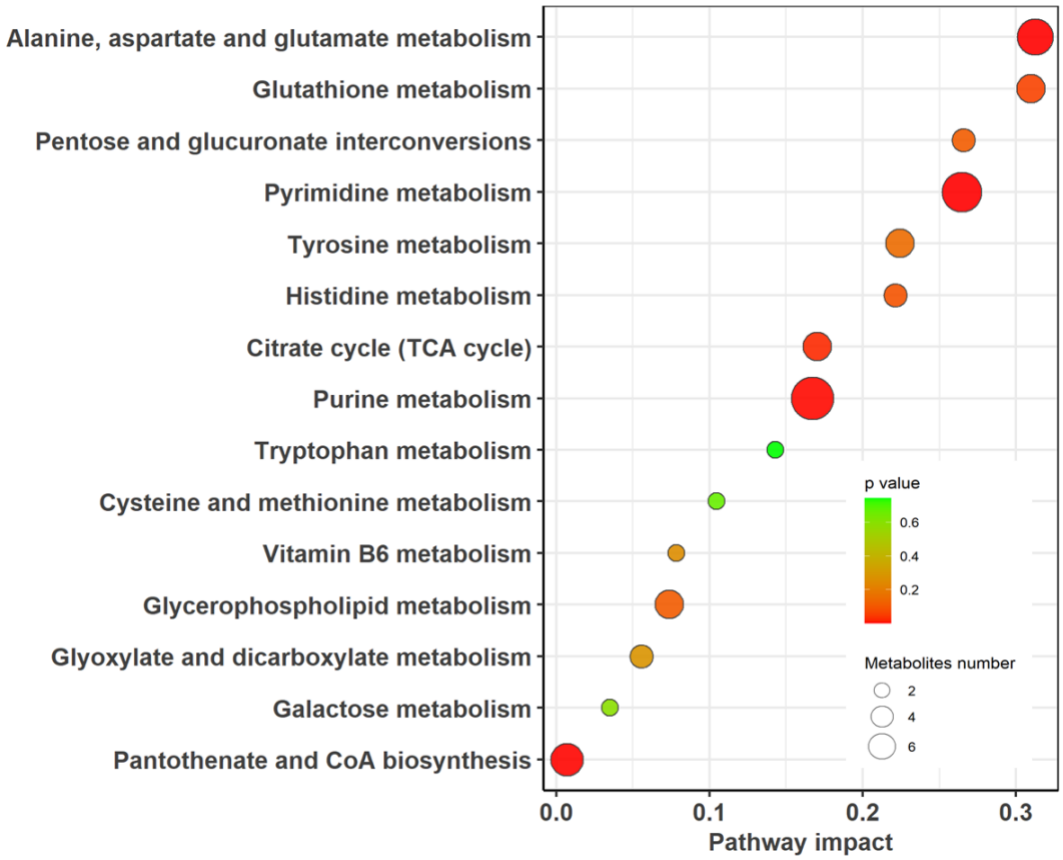

Figure S6. The metabolic pathway analysis based on the identified metabolites. The color and size of the cycle means the *p*-value and the hits metabolites in the pathway, respectively.

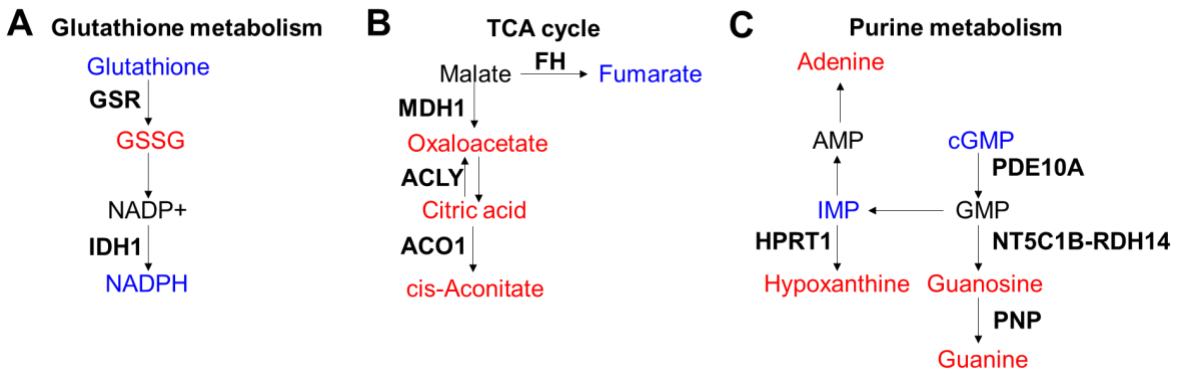

Figure S7. The metabolic pathways disturbed by TPhP: (A) glutathione metabolism, (B) tricarboxylic acid cycle (TCA cycle), and (C) purine metabolism. The metabolites marked as red, blue or black represent the ones that have been upregulated, downregulated or unchanged after TPhP exposure, respectively.

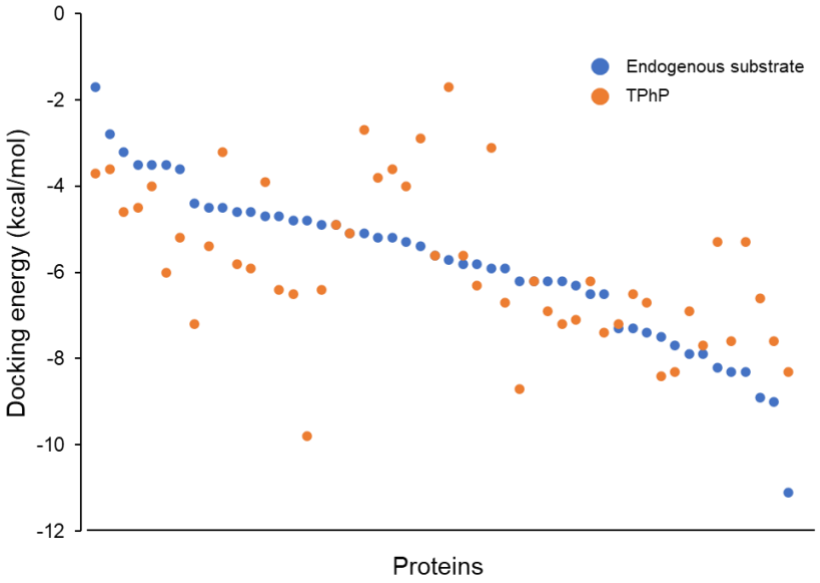

Figure S8. The affinity energies calculated between the enzymes with their corresponding endogenous substrates or TPhP.

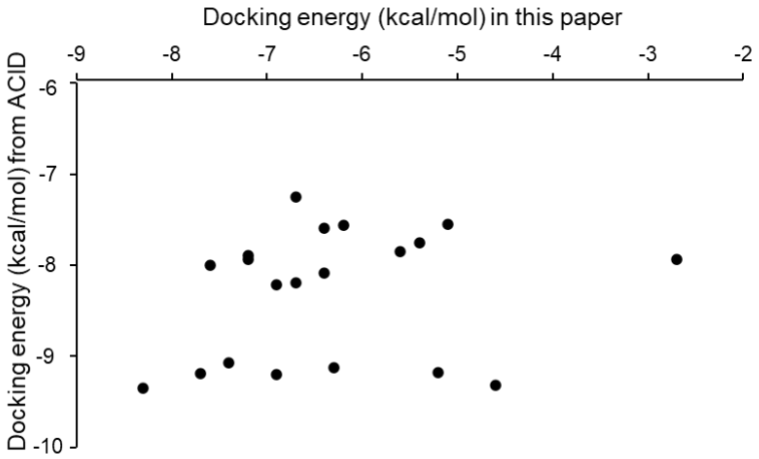

Figure S9. The scatter plot of the docking energies between the inverse docking results (ACID) and this study.

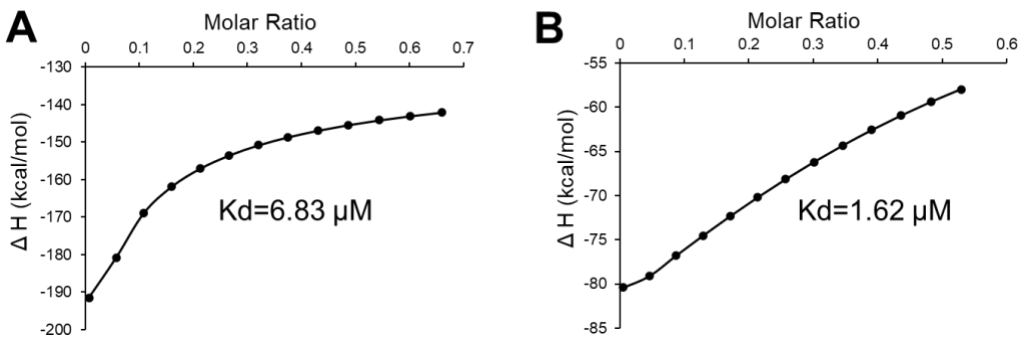

Figure S10. Interactions of TPhP with (A) HADH and (B) HSD17B10 tested by isothermal titration calorimetry (ITC). Molar ratio is the concentration ratio between TPhP and tested proteins.

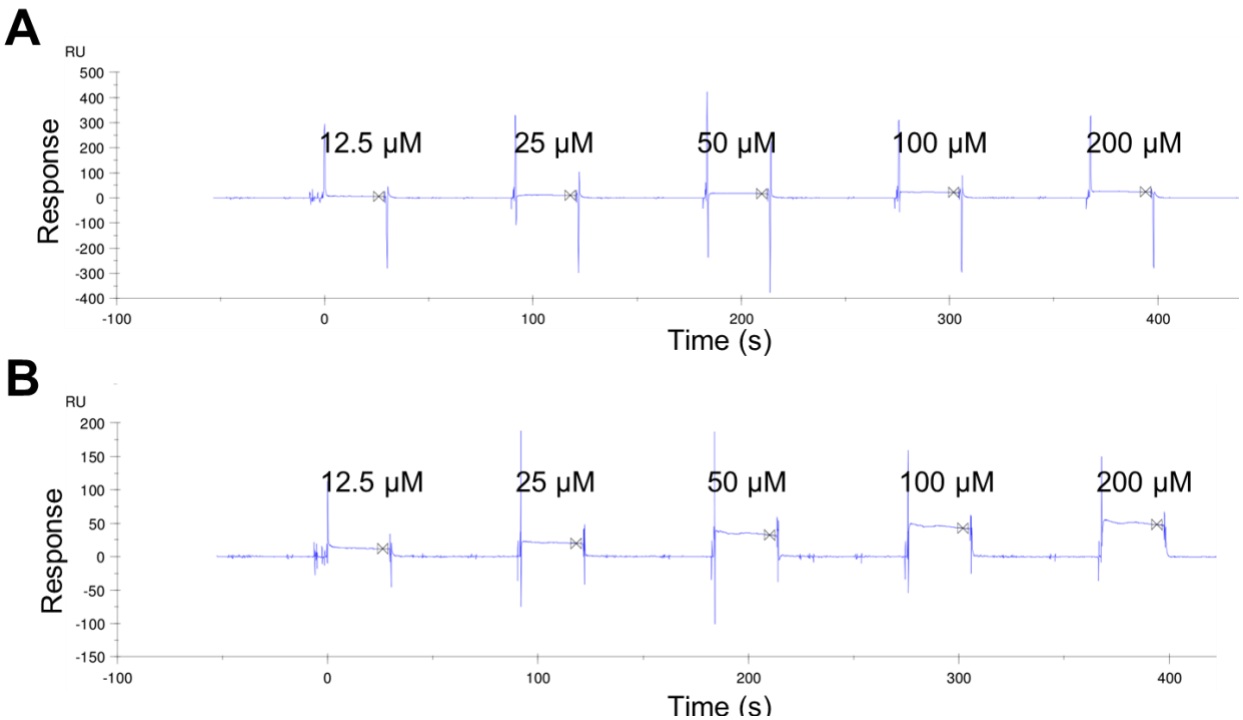

Figure S11. The binding affinities test by surface plasma resonance (SPR) of TPhP with (A) HADH and (B) HSD17B10.

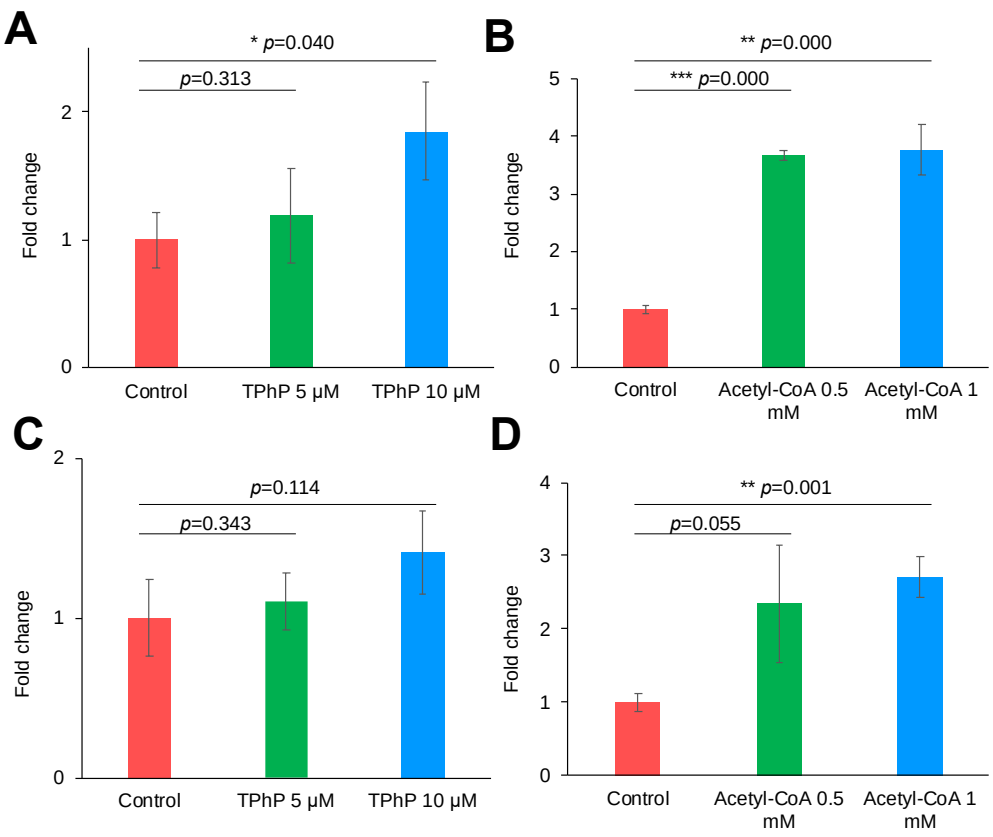

Figure S12. Fold changes of 3-hydroxybutyryl-CoA in the 0.5% DMSO Control, 5 and 10 µM TPhP, and 0.5 and 1 mM acetyl-CoA treatment groups after reaction with HADH (A and B) and HSD17B10 (C and D), respectively. The fold change was calculated from the ratio of the peak areas of the treatment groups and the control group. Asterisk indicate statistically significant differences (* for $p<0.05$, ** for $p<0.01$ and *** for $p<0.001$) between the TPhP-/acetyl-CoA-treated groups and the control group, respectively. Data are shown as mean±SE with three biological samples in each group.

Table S1. The docking results and the cellular catalytic information.

Protein name	PDB ID/Uniprot ID	Natural Substrate (NS)	Binding energy of NS (kcal/mol)	Binding energy of TPhP (kcal/mol)	Natural substrate Kd (μM) *	TPhP Kd (μM) *	Natural Substrate concentration (μM)	Km (μM)	Reference for Km value	Reference for NS concentration	Note for NS concentration
TK1	1w4r	Thymidine	-9.0	-7.6	0.2	2.6	0.5	NA		[3]	human cell
ADSL	2j91	Adenylosuccinate	-8.9	-6.6	0.3	14.3	NA	NA			
PDE10A	2our	3',5'-cyclic GMP	-8.3	-7.6	0.8	2.6	9.1	K_M=4.4 μM for cGMP	[4]	[3]	human cell mammalian cells
RRM2B	4djn	CDP	-7.9	-6.9	1.6	8.6	36	NA		[3]	human cell
PNP	1m73	Guanosine	-7.7	-8.3	2.2	0.8	4.5	NA		[3]	human Cellular Cytoplasm adult (>18 years old, 4.5 ± 2.9 μM)
UPP2	3p0f	Uridine	-7.3	-7.2	4.4	5.2	5.2	K_M=76 μM for uridine	[5]	[3]	human cell
ACO1	2b3y	Citrate	-6.5	-6.2	17.0	28.2	88	NA		[6]	human blood adult (>18 years old, 88.0 ± 33.0 μM)
ASPA	2o4h	*N*-acyl-L-aspartate	-6.5	-7.4	17.0	3.7	10	NA		[7]	Non-small cell lung cancer (5-15 μM)
IDH1	1t01	Isocitrate	-6.3	-7.1	23.8	6.2	38	K_M=65 μM for isocitrate	[8]	[9]	Cellular Cytoplasm
ACLY	5tde	Oxaloacetate	-6.2	-8.7	28.2	0.4	61	K_M=98.0 μM for citrate	[10]	[9]	human Cellular Cytoplasm adult (>18 years old)
CMPK2	1tev	CMP	-5.8	-5.6	55.4	77.7	67	K_M=3.09 mM for CMP	[11]	[3]	human cell
ASL	1k62	N-(L-Arginino)succinate	-5.6	-5.6	77.7	77.7	2.5	NA		[12]	human blood Children (6 - 18 years old, 0-5 μM)
MDH1	P40925	NAD+	-8.3	-5.3	0.8	129.1	88.7	NA		[9]	human Cellular Cytoplasm adult (>18 years old)
HPRT1	1z7g	IMP	-6.2	-6.2	28.2	28.2	23	NA		[3]	human cell
ADSS1	Q8N142	L-Aspartate	-4.8	-6.5	300.4	17.0	1270	NA		[13]	Hela cell line
GPT	P24298	L-Alanine	-4.7	-3.9	355.7	1374.4	1430	NA		[13]	Hela cell line
ASS1	2nz2	L-Aspartate	-4.7	-6.4	355.7	20.1	1270	K_M=68 μM for aspartate (at pH 7.0 and 37 degrees)	[14]	[13]	Hela cell line

FH	5upp	Malate	-4.6	-5.8	421.1	55.4	3200	K_M=1.4 mM for L-malate (at pH 8.5)	15	9	human Cellular Cytoplasm adult (>18 years old)
HSD17B10	1u7t	(2S,3S)-3-hydroxy-2-methylbutanoyl-CoA	-4.6	-5.9	428.3	46.0	45.7	K_M=25.7 µM for acetoacetyl-CoA (0.2 mM NADH, at pH 7.0 and 25 degrees)	16	17	Total acyl-CoA in MCF-7 cells (45.7 ±3.5 µM)
GSR	1dnc	Glutathione	-4.5	-5.4	498.7	109.0	800.5			18	800.50 ± 201.73 µM in human adult blood (>18 years old)
HADH	1f0y	(3S)-hydroxyacyl-CoA	-3.5	-4.5	2525.1	515.8	45.7	K_M=18.7 µM for acetoacetyl-CoA at pH 7.0	19	17	Total acyl-CoA in MCF-7 cells (45.7 ±3.5 µM)
MGLL	3hju	1-Octanoylglycerol	-3.5	-4.0	498.7	109.0	NA	K_M=110 µM for 2-arachidonoyglycerol	20		
ECHS1	2hw5	Crotonyl-CoA	-2.8	-3.6	2525.1	515.8	45.7	K_M=12.75 µM for crotonyl-CoA	21	17	Total acyl-CoA in MCF-7 cells (45.7 ±3.5 µM)
HADHB	5zqz	Acetyl-CoA	-1.7	-3.7	2656.4	1160.7	10.9	NA		22	Mitochondria (10.9 ± 0.4 µM)
OCTC	Q9UKG9	Octanoyl-CoA	-5.2	-3.8	8240.5	2243.4	45.7	NA		17	Total acyl-CoA in MCF-7 cells (45.7 ±3.5 µM)
LIPE	Q05469	glycerol 1,2-di-(9Z-octadecenoate)	-5.3	-4.0	60514.7	1862.9	NA	NA			
ACADL	P28330	Octadecanoyl-CoA	-5.9	-3.1	152.8	1573.3	45.7	K_M=8 µM for octanoyl-CoA	23	17	Total acyl-CoA in MCF-7 cells (45.7 ±3.5 µM)
CHAT	2FY2	choline	-3.6	-5.2	2281.7	152.8	NA	NA			
CANT1	1S18	UDP	-7.4	-6.7	3.7	12.1	3.5				
COMT	3BWY	S-adenosyl-L-methionine	-7.9	-7.7	1.6	2.2	NA	K_M=74 µM for 2-hydroxyestrone (at 37 degrees Celsius)	24		
ADSS	2V40	Guanosine triphosphate	-7.3	-6.5	4.4	17.0	56	NA		25	56.0 ±7.0 µM in human adult blood
NAGS	4K30	L-glutamate	-5.2	-3.6	152.8	2281.7	493	NA		26	492.6 ± 93.6 µM in human adult blood
DDC	3RBF	Pyridoxal phosphate	-4.9	-6.4	253.7	20.1	NA	NA			
DPYD	Q12882	Flavin adenine dinucleotide	-11.1	-8.3	0.0	0.8	NA	NA			
TYMP	1UOU	thymidine	-8.2	-5.3	1.0	129.1	NA	NA			
NT5C2	2JC9	Adenosine	-6.2	-6.9	28.2	8.6	23	NA		3	human cell
NT5C	4L57	Deoxyuridine monophosphate	-7.5	-8.4	3.1	0.7	2.7	NA		3	human cell
APRT	1ZN8	Adenosine monophosphate	-6.2	-7.2	28.2	5.2	82	NA		3	human cell

ACHE	1B41	acetylcholine	-3.2	-4.6		421.1	0.034	NA		[27]	Human cerebrospinal fluid Adult (>18 years old) 0.034 ± 0.009 μM
ASNS	6GQ3	L-glutamine	-5.4	-2.9	109.0	7446.0	1270	NA		[13]	Hela cell line
NT5E	4H1Y	Adenosine	-5.9	-6.7	46.8	12.1	97	NA		[3]	human cell
ACO2	Q99798	citrate	-4.5	-3.2	498.7	4485.2	190	NA		[6]	human Cellular Cytoplasm adult (>18 years old, 88.0 ± 33.0 μM)
NT5C3	2VKQ	cytidine 5′-monophosphate	-4.9	-4.9	253.7	253.7	67	K_M=66 μM for CMP	[28]	[3]	human cell
CMPK1	1TEV	cytidine 5′-monophosphate	-5.1	-5.1	180.9	180.9	67	NA		[3]	human cell
NT5M	1Q91	uracil	-4.8	-9.8	300.4	0.1	2.1	NA		[29]	2.10 ± 1.02 μM in human adult blood
BAAT	Q14032	glycine	-3.5	-6		39.5	790	K_M=5.6 mM for glycine toward choloyl-CoA	[30]	[13]	Hela cell line
BCAT1	2COI	L-leucine	-4.4	-7.2	590.5	5.2	730	NA		[13]	Hela cell line
XDH	2E1Q	xanthine	-5.8	-6.3	55.4	23.8	172	NA		[3]	human cell
ACY3	Q96HD9	N-Acetyl-L-aspartate	-5.7	-1.7	65.6	56559.8	0.81	NA		[31]	Cerebrospinal Fluid 0.81 ± 0.38 μM
ACY1	1Q7L	N-Acetyl-L-aspartate	-5.1	-2.7	180.9	10439.7	0.81	NA		[31]	Cerebrospinal Fluid 0.81 ± 0.38 μM

*Calculated based on the formula Kd=e^(ΔG/RT), in which, R=1.986 cal/K, T is regard as 298 K (25 ℃).

Table S2. The molecular docking parameters for the proteins.

Protein name	PDB ID/Uniprot ID	center_x	center_y	center_z	size_x	size_y	size_z	energy_range	exhaustiveness	num_modes
GSR	1dnc	61.708	-20.858	41.16	15	15	15	3	100	9
IDH1	1t01	-17.014	-87.291	27.626	15	15	15	3	100	9
ACO1	2b3y	35.6	29.1	6.97	15	15	15	3	100	9
FH	5upp	-28.827	-82.47	-20.24	15	15	15	3	100	9
MDH1	P40925	31.426	23.47	4.961	18	18	18	3	100	9
ACLY	5tde	24.439	-3.418	39.24	15	15	15	3	100	9
ASPA	2o4h	-43.606	8.379	-42.911	25	25	25	3	100	9
GPT	P24298	-31.346	-14.32	16.51	15	15	15	3	100	9
ADSS1	Q8N142	38.942	17.39	4.59	18	18	18	3	100	9
ASS1	2nz2	-0.478	32.337	21.849	8	8	8	3	100	9
ADSL	2j91	27.898	32.418	8.389	15	15	15	3	100	9
ASL	1k62	51.352	16.109	53.17	15	15	15	3	100	9
CMPK2	1tev	41.37	20.28	16.65	10	10	10	3	100	9
RRM2B	4djn	0.265	9.99	94.337	40	40	40	3	100	9
UPP2	3p0f	14.279	-2.401	56.243	15	15	15	3	100	9
TK1	1w4r	54.454	75.241	2.822	15	15	15	3	100	9
PDE10A	2our	5.509	10.699	42.552	15	15	15	3	100	9
PNP	1m73	55.652	50.954	23.991	15	15	15	3	100	9
HPRT1	1z7g	-90.729	23.945	-19.4	24	24	24	3	100	9
HADH	1f0y	12.425	1.755	-21.653	22.5	15	15	3	100	9
HSD17B10	1u7t	60.373	41.845	23.038	15	15	22.5	3	100	9
OCTC	Q9UKG9	-30.653	140.45	72.365	22.5	22.5	22.5	3	10	9
ACADL	P28330	-21.681	79.23	42.629	15	15	15	3	100	9
ECHS1	2hw5	-21.039	41.253	58.725	15	15	15	3	100	9
HADHB	5zqz	130.972	123.998	139.988	15	15	15	3	100	9

LIPE	Q05469	16.591	35.136	6.84	25	25	25	3	10	9
MGLL	3hju	-17.037	-19.119	-21.372	15	15	15	3	100	9
ACHE	1B41	105.602	110.022	-124.881	15	15	15	3	10	9
ACO2	Q99798	35.888	34.114	70.798	15	15	15	3	10	9
ACY1	1Q7L	11.16	11.688	27.129	15	15	15	3	10	9
ACY3	Q96HD9	13.802	-36.216	8.582	15	15	15	3	10	9
ADSS	2V40	55.986	18.66	19.188	15	15	15	3	10	9
APRT	1ZN8	34.143	10.325	0.81	15	15	15	3	10	9
ASNS	6GQ3	24.845	17.001	12.404	15	15	15	3	10	9
BAAT	Q14032	-15.203	4.909	9.992	15	15	15	3	10	9
BCAT1	2COI	-37.178	-2.704	-15.214	15	15	15	3	10	9
CANT1	1S1D	12.166	42.672	88.791	15	15	15	3	10	9
CHAT	2FY3	6.848	3.373	67.263	8.1	8.1	8.1	3	10	9
CMPK1	1TEV	48.592	30.024	25.04	15	15	15	3	10	9
COMT	3BWY	-8.769	-4.218	-16.177	15	15	15	3	10	9
DDC	3RCH	46.186	13.57	-11.518	15	15	15	3	10	9
DPYD	Q12882	57.091	80.499	92.414	24	24	24	3	10	9
NAGS	4K30	37.878	119.052	117.209	15	15	15	3	10	9
NT5C	6g2n	7.847	2.545	5.014	15	15	15	3	10	9
NT5C2	2JC9	-23.353	32.906	50.673	15	15	15	3	10	9
NT5C3A	2VKQ	29.23	0.387	13.982	15	15	15	3	10	9
NT5E	4H2G	12.34	17.159	34.691	15	15	15	3	10	9
NT5M	1Q91	12.244	41.051	24.696	15	15	15	3	10	9
PNP	1M73	-16.465	89.392	-0.684	15	15	15	3	10	9
TYMP	1UOU	-2.703	2.672	28.611	15	15	15	3	10	9
XDH	2E1Q	30.226	14.144	61.171	15	15	15	3	10	9

Table S3. The dysregulated metabolites identified from the 5 and 10 µM TPhP treatment groups.

Ion mode	*m/z*	Identified metabolite	Rt (min)	Fold change 5 µM	Fold change 10 µM	*p*-value 5 µM	*p*-value 10 µM	Adjust *p*-value 5 µM	Adjust *p*-value 10 µM
ESI negative	282.0851	Guanosine	9.0	14.0	15.9	0.002	0.002	0.012	0.037
	111.0200	Uracil	6.4	11.4	12.8	0.002	0.005	0.012	0.038
	306.0586	dCMP	6.3	12.0	12.5	0.001	0.004	0.010	0.038
	173.0095	Aconitic acid	22.5	1.2	3.5	0.909	0.046	0.909	0.072
	193.0373	D-Glucuronic acid	11.0	2.5	3.3	0.265	0.032	0.294	0.064
	251.2016	7,10-hexadecadienoic acid	9.6	3.2	2.6	0.040	0.232	0.072	0.301
	218.1037	Pantothenic Acid	12.4	2.5	2.1	0.038	0.075	0.072	0.106
	191.0202	Citric acid	20.0	1.5	2.0	0.016	0.312	0.052	0.373
	196.0595	L-Dopa	8.6	2.2	2.0	0.079	0.012	0.112	0.047
	243.0579	Uridine	6.9	1.9	1.9	0.055	0.013	0.082	0.047
	134.0471	Adenine	6.3	1.6	1.9	0.047	0.037	0.072	0.064
	611.1453	Glutathione, oxidized	19.9	2.7	1.8	0.036	0.299	0.072	0.365
	283.2647	Stearic acid	8.2	1.7	1.6	0.143	0.014	0.178	0.047
	351.2213	PGE2	8.6	1.9	1.5	0.045	0.032	0.072	0.064
	402.0113	Cytidine diphosphate	23.6	1.3	1.4	0.025	0.348	0.066	0.401
	174.0021	N-acetylaspartate	12.4	1.1	1.3	0.534	0.042	0.552	0.069
	297.2442	FA(18:1(OH))	9.5	1.8	1.2	0.355	0.022	0.380	0.056
	125.0332	Thymine	5.4	2.2	1.1	0.036	0.941	0.072	0.946
	271.1909	Pentadecanedioic acid	9.5	0.7	1.0	0.016	0.829	0.052	0.857
	118.0510	Threonine/Homoserine	10.6	0.8	1.0	0.039	0.946	0.072	0.946
	306.0774	Glutathione	19.3	0.8	0.9	0.017	0.402	0.052	0.446
	115.0037	Fumaric acid	13.4	0.7	0.8	0.000	0.074	0.000	0.106
	203.0830	Tryptophan	9.3	0.8	0.8	0.032	0.147	0.072	0.195
	116.0725	Valine	8.9	0.8	0.8	0.038	0.128	0.072	0.177
	179.0562	Glucose	8.2	0.8	0.8	0.047	0.524	0.072	0.571

	130.0510	N-Acetyl-L-alanine	17.7	0.8	0.8	0.296	0.035	0.322	0.064
	344.0326	cGMP	16.0	0.6	0.8	0.001	0.255	0.010	0.324
	132.0302	Aspartic Acid	13.4	0.8	0.8	0.046	0.146	0.072	0.195
	744.1048	NADPH	13.2	0.8	0.8	0.004	0.020	0.022	0.055
	88.0406	Alanine	13.9	0.8	0.7	0.115	0.030	0.149	0.064
	156.0298	Aminomuconic acid	17.7	0.9	0.6	0.653	0.023	0.664	0.056
	154.0624	L-Histidine	10.3	0.7	0.6	0.109	0.048	0.145	0.073
	563.0689	dTDP-D-glucose	19.2	0.6	0.6	0.126	0.017	0.160	0.052
	256.0959	Glycerophosphocholine	8.5	0.7	0.5	0.248	0.034	0.281	0.064
	171.0065	Glycerol 2-phosphate	8.5	0.7	0.5	0.097	0.016	0.131	0.051
	312.0506	Cyclic dAMP	9.6	0.5	0.5	0.036	0.046	0.072	0.072
	242.0804	Cytidine	8.5	0.6	0.5	0.001	0.003	0.010	0.037
	303.0381	Thymidine 3',5'-cyclic monophosphate	19.1	0.6	0.4	0.008	0.019	0.038	0.055
	166.0497	Vitamin B6	7.8	0.6	0.3	0.047	0.007	0.072	0.043
	137.0461	Hypoxanthine	2.6	2.7	3.0	0.047	0.028	0.072	0.064
	119.0718	hydroxy isovaleric acid	2.1	2.3	2.3	0.002	0.014	0.012	0.047
	152.0569	Guanine	2.6	2.5	2.0	0.069	0.001	0.100	0.031
	104.1070	Choline	0.8	1.5	1.9	0.039	0.371	0.072	0.419
	173.0786	2-Octenedioic acid	2.6	1.7	1.7	0.010	0.003	0.044	0.037
	130.1590	Octylamine	3.4	1.1	1.3	0.399	0.037	0.420	0.064
	89.0595	Butyric acid	2.9	1.6	1.2	0.030	0.673	0.072	0.720
	335.2957	Docosatrienoic Acid	6.8	1.3	0.9	0.024	0.716	0.066	0.753
	115.0752	Pyroterebic acid	1.7	0.7	0.9	0.021	0.286	0.061	0.356
	146.1176	Acetylcholine	0.8	0.8	0.8	0.001	0.009	0.010	0.047
	109.0659	*p*-cresol	1.9	0.6	0.7	0.001	0.000	0.010	0.000
	349.0550	IMP	1.3	0.5	0.7	0.015	0.346	0.052	0.401
	242.2842	1-Hexadecylamine	6.6	0.7	0.7	0.033	0.014	0.072	0.047
ESI	150.0583	L-Methionine	3.4	0.6	0.7	0.164	0.005	0.200	0.038
positive	170.0813	Norepinephrine	1.4	0.8	0.7	0.249	0.037	0.281	0.064

132.1017	L-Leucine/ L-Isoleucine	1.0	0.6	0.7	0.229	0.037	0.269	0.064
265.1118	Thiamine	0.8	0.7	0.6	0.093	0.010	0.129	0.047
275.1866	3-Hydroxytetradecanedioic acid	6.3	0.5	0.6	0.017	0.051	0.052	0.076
310.0904	Inodxyl glucuronide	1.2	0.4	0.6	0.002	0.021	0.012	0.056
280.2636	Linoleamide	5.8	0.7	0.6	0.216	0.040	0.258	0.068
105.0555	hydroxy butyric acid	1.2	0.7	0.5	0.006	0.006	0.031	0.041
218.1387	Propionyl-L-carnitine	2.4	0.5	0.5	0.017	0.010	0.052	0.047

Table S4. The dysregulated lipids identified from the 5 and 10 µM TPhP treatment groups.

Ion mode	*m/z*	Identified lipids	Rt (min)	Fold change 5 µM	Fold change 10 µM	*p*-value 5 µM	*p*-value 10 µM	Adducts	Adjust *p*-value 5 µM	Adjust *p*-value 10 µM
ESI negative	654.5588	CAR (30:0)	6.9	0.9	0.6	0.635	0.003	[M-H]-	0.811	0.007
	731.6033	CE (20:4)	8.2	1.7	0.7	0.082	0.013	[M-H]-	0.196	0.020
	708.6076	Cer (t42:2)	7.3	2.9	0.0	0.030	0.049	[M-H]-	0.093	0.049
	683.5756	DG (36:0)	6.9	1.0	0.6	0.818	0.001	[M+Formate]-	0.922	0.004
	221.1521	FA (14:3)	0.5	0.4	0.4	0.000	0.001	[M+OAc]-	0.000	0.004
	526.2932	LPE (22:5)	1.1	4.4	3.4	0.045	0.045	[M+OAc]-	0.129	0.047
	627.5589	MG (34:0)	7.1	1.3	1.2	0.020	0.025	[M+OAc]-	0.070	0.032
	845.5339	PG (42:8)	5.0	1.4	1.4	0.001	0.003	[M-H]-	0.009	0.007
	953.5362	PI (40:7)	4.6	0.9	0.8	0.107	0.030	[M+FA-H]-	0.241	0.035
	858.5233	PS (42:8)	4.7	1.0	0.8	0.907	0.027	[M-H]-	0.940	0.033
ESI positive	478.3897	ACar (22:3)	3.3	2.5	3.5	0.000	0.000	[M+H-H2O]+	0.000	0.000
	812.5506	BMP (38:6)	6.7	1.1	1.3	0.071	0.015	[M]+	0.186	0.022
	400.3497	CAr (16:0)	2.1	0.6	1.6	0.092	0.040	[M+H]+	0.216	0.043
	428.3819	CAr (18:0)	4.6	0.9	1.2	0.392	0.049	[M+H-H2O]+	0.565	0.049
	506.4205	CAr (24:3)	4.0	1.0	1.2	0.793	0.026	[M+H-H2O]+	0.903	0.033
	520.5083	Cer (d34:1)	5.8	1.2	1.5	0.013	0.000	[M+H]+	0.050	0.000
	594.6003	Cer (d38:1)	7.1	1.0	1.3	0.831	0.000	[M+H]+	0.928	0.000
	608.5725	Cer (t38:2)	6.3	1.0	1.2	0.502	0.029	[M+H]+	0.696	0.035
	566.5697	Cer-NS (d36:1)	6.6	1.0	1.2	0.563	0.004	[M+H]+	0.753	0.008
	780.6306	CerP (d44:1)	5.5	0.8	0.6	0.023	0.003	[M+NH4]+	0.076	0.007
	577.5192	DG (34:1)	4.7	2.0	2.9	0.001	0.002	[M+H]+	0.009	0.006
	575.5041	DG (34:2)	7.9	1.2	3.0	0.731	0.004	[M+H]+	0.858	0.008
	640.5876	DG (36:1)	7.3	1.4	1.8	0.341	0.024	[M+H]+	0.523	0.031

603.5344	DG (36:2)	9.2	3.2	7.5	0.059	0.001	[M+NH4]+	0.162	0.004
599.5029	DG (36:4)	4.1	1.3	2.2	0.007	0.001	[M+H]+	0.035	0.004
662.5715	DG (38:4)	6.9	1.2	2.3	0.570	0.027	[M+NH4]+	0.753	0.033
651.5346	DG (40:6)	5.8	1.0	1.5	0.693	0.022	[M+NH4]+	0.838	0.030
707.5959	DG (44:6)	7.2	0.9	1.3	0.739	0.033	[M+H]+	0.858	0.037
468.3106	LPC (14:0)	0.8	0.2	4.6	0.279	0.013	[M+H-H2O]+	0.498	0.020
494.3299	LPC (16:1)	1.0	0.6	1.8	0.078	0.039	[M]+	0.191	0.042
524.3836	LPC (18:0)	2.4	0.7	0.6	0.007	0.009	[M+H]+	0.035	0.016
482.3257	LPE (18:0)	1.9	1.3	2.0	0.097	0.001	[M+Na]+	0.223	0.004
502.3021	LPE (20:4)	6.1	2.8	2.9	0.009	0.031	[M+H]+	0.038	0.036
526.2926	LPE (22:6)	5.4	1.3	2.5	0.376	0.049	[M+H]+	0.562	0.049
372.3192	MG (18:2)	3.4	0.9	1.2	0.592	0.038	[M+NH4]+	0.765	0.042
692.5242	PA (34:1)	4.6	0.8	1.4	0.172	0.048	[M+H]+	0.351	0.049
718.546	PA (36:2)	5.7	1.1	1.3	0.532	0.032	[M+Na]+	0.720	0.036
744.5583	PA (38:3)	5.8	1.1	1.3	0.295	0.026	[M+NH4]+	0.498	0.033
606.4585	PC (24:1e)	2.6	0.9	1.3	0.393	0.023	[M]+	0.565	0.030
678.5085	PC (28:0)	4.3	1.0	1.7	0.907	0.002	[M+NH4]+	0.940	0.006
676.5276	PC (28:1)	5.9	2.0	3.8	0.009	0.000	[M+H]+	0.038	0.000
706.5647	PC (30:0)	5.6	0.3	0.4	0.000	0.002	[M+H]+	0.000	0.006
704.5229	PC (30:1)	4.6	2.0	5.3	0.258	0.004	[M+H]+	0.484	0.008
700.4895	PC (30:3)	4.3	1.0	2.9	0.985	0.001	[M+H-H2O]+	0.985	0.004
734.5719	PC (32:0)	5.5	1.2	1.4	0.004	0.000	[M+NH4]+	0.029	0.000
718.5754	PC (32:1e)	5.5	0.6	0.7	0.005	0.012	[M+H]+	0.030	0.019
730.5459	PC (32:2)	5.4	1.5	2.1	0.000	0.000	[M+NH4]+	0.000	0.000
728.5402	PC (32:3)	5.5	0.8	0.4	0.034	0.004	[M+H]+	0.100	0.008
760.5867	PC (34:1)	6.3	1.0	1.2	0.682	0.018	[M+NH4]+	0.834	0.026
758.5746	PC (34:2)	5.8	1.5	1.8	0.001	0.000	[M+H]+	0.009	0.000
754.5374	PC (34:4)	4.6	0.9	1.5	0.392	0.007	[M+H]+	0.565	0.013
752.5255	PC (34:5)	4.2	1.5	3.8	0.299	0.002	[M+Na]+	0.498	0.006
772.6207	PC (36:0)	6.1	0.6	0.6	0.003	0.005	[M+NH4]+	0.023	0.010
782.5695	PC (36:4)	5.2	1.1	1.3	0.074	0.000	[M+H]+	0.189	0.000

780.5538	PC (36:5)	4.8	1.0	1.5	0.905	0.005	[M+H]+	0.940	0.010
766.5747	PC (36:5e)	5.2	0.7	0.7	0.001	0.001	[M+H]+	0.009	0.004
778.5441	PC (36:6)	4.8	1.2	1.7	0.583	0.046	[M+H]+	0.762	0.048
818.6649	PC (38:0)	6.0	1.0	1.3	0.707	0.042	[M+NH4]+	0.838	0.045
812.6156	PC (38:3)	5.9	1.0	1.3	0.665	0.030	[M+H]+	0.831	0.035
808.5852	PC (38:5)	5.3	0.9	1.2	0.403	0.021	[M+H]+	0.572	0.029
794.6054	PC (38:5e)	5.6	0.5	0.5	0.001	0.003	[M+H]+	0.009	0.007
806.5694	PC (38:6)	5.1	1.6	1.9	0.000	0.000	[M+H]+	0.000	0.000
804.5536	PC (38:7)	4.6	1.0	1.8	0.664	0.003	[M+H]+	0.831	0.007
826.5433	PC (40:10)	4.0	0.8	1.5	0.243	0.039	[M+NH4]+	0.474	0.042
834.6001	PC (40:6)	6.2	1.0	0.8	0.984	0.006	[M+H-H2O]+	0.985	0.011
832.5849	PC (40:7)	5.2	1.0	1.2	0.260	0.002	[M+H]+	0.484	0.006
830.5687	PC (40:8)	4.7	1.0	1.4	0.850	0.007	[M+H]+	0.931	0.013
828.5539	PC (40:9)	4.4	1.1	1.8	0.704	0.006	[M+H]+	0.838	0.011
854.5715	PC (42:10)	4.6	1.0	1.6	0.953	0.002	[M+NH4]+	0.971	0.006
864.6463	PC (42:5)	6.2	1.1	1.5	0.338	0.002	[M+H-H2O]+	0.523	0.006
862.6313	PC (42:6)	5.8	1.0	1.2	0.322	0.001	[M+H]+	0.523	0.004
848.6527	PC (42:6e)	6.6	1.0	1.3	0.954	0.008	[M+H]+	0.971	0.014
878.5719	PC (44:12)	4.6	1.1	2.1	0.341	0.004	[M+H]+	0.523	0.008
876.683	PC (44:6e)	7.0	0.3	6.5	0.174	0.004	[M+H]+	0.351	0.008
690.5618	PC (O-30:1)	4.5	0.9	1.5	0.349	0.037	[M+Na]+	0.528	0.041
746.6065	PC (P-34:0)	6.0	0.7	0.8	0.007	0.023	[M+H]+	0.035	0.030
720.5543	PE (34:0)	5.2	0.9	1.2	0.329	0.032	[M+H]+	0.523	0.036
735.5737	PE (34:1)	5.5	1.2	1.6	0.003	0.000	[M+H]+	0.023	0.000
740.5245	PE (36:4)	5.3	1.1	1.6	0.279	0.001	[M+H]+	0.498	0.004
768.577	PE (38:4)	6.1	0.8	0.8	0.009	0.022	[M+H-H2O]+	0.038	0.030
766.5591	PE (38:5)	4.7	1.1	1.7	0.523	0.003	[M+H]+	0.716	0.007
792.5584	PE (40:6)	5.8	1.1	1.3	0.019	0.000	[M+H]+	0.068	0.000
830.6798	PE (42:1)	6.3	2.0	4.2	0.023	0.001	[M+H]+	0.076	0.004
820.5865	PE (42:6)	5.4	1.0	1.2	0.876	0.022	[M+H-H2O]+	0.934	0.030
818.569	PE (42:7)	4.9	1.0	1.3	0.674	0.009	[M+NH4]+	0.833	0.016

857.5877	PE (44:10)	4.9	1.1	1.5	0.289	0.001	[M+NH4]+	0.498	0.004
650.522	PE-Cer (d32:1)	5.3	1.0	1.7	0.858	0.025	[M+H]+	0.931	0.032
722.5804	PE-Cer (t36:1)	7.8	0.8	0.8	0.010	0.015	[M+H]+	0.041	0.022
740.5557	PG (32:0)	6.2	0.8	0.8	0.005	0.016	[M+H]+	0.030	0.023
764.5646	PG (34:2)	0.5	0.6	0.4	0.194	0.044	[M+H]+	0.385	0.046
762.5286	PG (34:3)	4.6	0.9	1.3	0.291	0.027	[M+H]+	0.498	0.033
814.5692	PG (38:5)	5.5	0.9	0.7	0.001	0.015	[M+H]+	0.009	0.022
738.5436	PG (P-32:1(OH))	5.8	0.8	0.8	0.012	0.010	[M+H]+	0.048	0.017
833.5139	PI (34:3)	4.4	1.0	1.7	0.877	0.006	[M+H]+	0.934	0.011
832.5067	PS (38:5)	4.8	0.7	0.3	0.061	0.005	[M+NH4]+	0.163	0.010
808.5142	PS (38:6)	4.6	0.8	3.4	0.752	0.012	[M+H]+	0.865	0.019
844.6186	PS (40:2)	6.1	0.3	0.4	0.001	0.003	[M+H-H2O]+	0.009	0.007
822.5658	PS (40:4)	5.0	0.3	0.1	0.000	0.000	[M+H]+	0.000	0.000
834.5273	PS (40:7)	4.2	0.9	1.6	0.457	0.003	[M+Na]+	0.641	0.007
725.5569	SM (d36:4)	4.8	1.0	1.2	0.288	0.001	[M+NH4]+	0.498	0.004
787.6723	SM (d40:1)	7.2	1.1	1.4	0.332	0.002	[M+H]+	0.523	0.006
815.6996	SM (d42:1)	7.0	1.3	1.7	0.033	0.001	[M+H-H2O]+	0.100	0.004
708.6318	TG (40:2)	5.5	0.7	0.6	0.030	0.011	[M+H]+	0.093	0.018
738.6698	TG (42:1)	6.1	0.9	0.8	0.077	0.021	[M+H]+	0.191	0.029
792.7072	TG (46:2)	8.5	3.9	8.8	0.261	0.014	[M+H]+	0.484	0.021
820.738	TG (48:2)	8.8	1.4	2.2	0.119	0.002	[M+H]+	0.263	0.006
850.7848	TG (50:1)	9.2	1.5	2.0	0.017	0.001	[M+H]+	0.063	0.004
846.7533	TG (50:3)	8.8	1.3	2.1	0.125	0.000	[M+H]+	0.271	0.000
799.6309	TG (50:9)	6.2	0.6	0.6	0.005	0.010	[M+H]+	0.030	0.017
862.7834	TG (51:2)	9.1	1.5	2.1	0.059	0.000	[M+H-H2O]+	0.162	0.000
876.7996	TG (52:2)	9.2	1.3	1.6	0.008	0.001	[M+H]+	0.038	0.004
870.7568	TG (52:5)	8.8	4.2	12.7	0.132	0.001	[M+H]+	0.281	0.004
849.6546	TG (54:12)	6.5	1.1	2.2	0.852	0.003	[M+H]+	0.931	0.007
881.756	TG (54:5)	9.2	1.7	2.1	0.006	0.001	[M+H]+	0.035	0.004

Reference:

(1) Liu, X.; Sadhukhan, S.; Sun, S.; Wagner, G. R.; Hirschey, M. D.; Qi, L.; Lin, H.; Locasale, J. W. *Mol. Cell. Prot: MCP* **2015**, *14*, 1489-1500.

(2) Yang, X.; Ma, Y.; Li, N.; Cai, H.; Bartlett, M. G. *Anal. Chem.* **2017**, *89*, 813-821.

(3) Traut, T. W. *Mol. Cell. Biochem.* **1994**, *140*, 23.

(4) Wang, H.; Liu, Y.; Hou, J.; Zheng, M.; Robinson, H.; Ke, H. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 5782-5787.

(5) Johansson, M. *Biochem. Biophys. Res. Commun.* **2003**, *307*, 41-46.

(6) https://hmdb.ca/metabolites/HMDB0000094#concentrations.

(7) Bogner-Strauss, J. G. *Front. Endocrinol.* **2017**, *8*, 240.

(8) Dang, L.; White, D. W.; Gross, S.; Bennett, B. D.; Bittinger, M. A.; Driggers, E. M.; Fantin, V. R.; Jang, H. G.; Jin, S.; Keenan, M. C.; Marks, K. M.; Prins, R. M.; Ward, P. S.; Yen, K. E.; Liau, L. M.; Rabinowitz, J. D.; Cantley, L. C.; Thompson, C. B.; Vander Heiden, M. G.; Su, S. M. *Nature* **2009**, *462*, 739-744.

(9) Zupke, C.; Sinskey, A. J.; Stephanopoulos, G. *Appl. Microbiol. Biotechnol.* **1995**, *44*, 27-36.

(10) Lord, K. A.; Wang, X. M.; Simmons, S. J.; Bruckner, R. C.; Loscig, J.; O'Connor, B.; Bentley, R.; Smallwood, A.; Chadwick, C. C.; Stevis, P. E.; Ciccarelli, R. B. *Protein Expr. Purif.* **1997**, *9*, 133-141.

(11) https://www.uniprot.org/uniprot/Q5EBM0.

(12) https://hmdb.ca/metabolites/HMDB0000052#concentrations.

(13) Piez, K. A.; Eagle, H. *J. Biol. Chem.* **1958**, *231*, 533-545.

(14) Berning, C.; Bieger, I.; Pauli, S.; Vermeulen, T.; Vogl, T.; Rummel, T.; Höhne, W.; Koch, H. G.; Rolinski, B.; Gempel, K.; Häberle, J. *Hum. Mutat.* **2008**, *29*, 1222-1227.

(15) Ajalla Aleixo, M. A.; Rangel, V. L.; Rustiguel, J. K.; de Pádua, R. A. P.; Nonato, M. C. *The FEBS J.* **2019**, *286*, 1925-1940.

(16) Shafqat, N.; Marschall, H. U.; Filling, C.; Nordling, E.; Wu, X. Q.; Björk, L.; Thyberg, J.; Mårtensson, E.; Salim, S.; Jörnvall, H.; Oppermann, U. *Biochem. J.* **2003**, *376*, 49-60.

(17) Haynes, C. A.; Allegood, J. C.; Sims, K.; Wang, E. W.; Sullards, M. C.; Merrill, A. H., Jr. *J. lipid Res.* **2008**, *49*, 1113-1125.

(18) Nakayama, Y.; Kinoshita, A.; Tomita, M. *Theor. Biol. Med. Model.* **2005**, *2*, 18.

(19) Barycki, J. J.; O'Brien, L. K.; Bratt, J. M.; Zhang, R.; Sanishvili, R.; Strauss, A. W.; Banaszak, L. J. *Biochemistry* **1999**, *38*, 5786-5798.

(20) Navia-Paldanius, D.; Savinainen, J. R.; Laitinen, J. T. *J. lipid Res.* **2012**, *53*, 2413-2424.

(21) Yamada, K.; Aiba, K.; Kitaura, Y.; Kondo, Y.; Nomura, N.; Nakamura, Y.; Fukushi, D.; Murayama, K.; Shimomura, Y.; Pitt, J.; Yamaguchi, S.; Yokochi, K.; Wakamatsu, N. *J. Med. Genet.* **2015**, *52*, 691-698.

(22) Ronowska, A.; Szutowicz, A.; Bielarczyk, H.; Gul-Hinc, S.; Klimaszewska-Łata, J.; Dyś, A.; Zyśk, M.; Jankowska-Kulawy, A. *Front. Cell. Neurosci.* **2018**, *12*.

(23) Nandy, A.; Kieweg, V.; Kräutle, F. G.; Vock, P.; Küchler, B.; Bross, P.; Kim, J. J.; Rasched, I.; Ghisla, S. *Biochemistry* **1996**, *35*, 12402-12411.

(24) Dawling, S.; Roodi, N.; Mernaugh, R. L.; Wang, X.; Parl, F. F. *Cancer Res.* **2001**, *61*, 6716-6722.

(25) https://hmdb.ca/metabolites/HMDB0001273#concentrations.

(26) https://hmdb.ca/metabolites/HMDB0060475#concentrations.

(27) Jia, J. P.; Jia, J. M.; Zhou, W. D.; Xu, M.; Chu, C. B.; Yan, X.; Sun, Y. X. *Chin. Med. J. (Engl.)* **2004**, *117*, 1161-1164.

(28) Amici, A.; Ciccioli, K.; Naponelli, V.; Raffaelli, N.; Magni, G. *Cell. Mol. Life Sci.* **2005**, *62*, 1613-1620.

(29) https://hmdb.ca/metabolites/HMDB0000300#concentrations.

(30) Johnson, M. R.; Barnes, S.; Kwakye, J. B.; Diasio, R. B. *J. Biol. Chem.* **1991**, *266*, 10227-10233.

(31) https://hmdb.ca/metabolites/HMDB0000812#concentrations.