

**Table S1** Summary of up-regulated and down-regulated metabolites in *ANGPTL8/betatrophin* gene knockout and wild type HepG2/IR cells

No.	RT (min)	<i>m/z</i>	Exact mass	ppm	Formula	Metabolites	VIP	<i>p</i> value
<b>Up-regulated</b>								
1	727.26	181.01	180.0634	0.49	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	D-Mannose	1.42	0.003
2	89.33	265.11	265.1123	3.44	C <sub>12</sub> H <sub>17</sub> N <sub>4</sub> O <sub>5</sub>	Thiamine	1.63	0.005
3	691.50	180.97	164.0685	4.03	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	L-Fucose	1.29	0.005
4	90.80	147.08	146.1055	9.30	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	L-Lysine	1.66	0.005
5	222.77	115.04	116.0473	13.41	C <sub>5</sub> H <sub>8</sub> O <sub>3</sub>	alpha-Ketoisovaleric acid	1.02	0.045
6	86.26	195.05	196.0583	2.14	C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>	Gluconic acid	1.47	0.005
7	128.14	162.05	162.0528	0.55	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>	2-Deoxy-scylo-inosose	1.65	0.005
8	120.62	131.97	132.0423	1.97	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>	Glutaric acid	1.36	0.005
9	125.95	187.11	188.1161	3.13	C <sub>8</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	N <sub>6</sub> -Acetyl-L-lysine	1.58	0.005
10	94.76	211.00	230.0192	0.22	C <sub>5</sub> H <sub>11</sub> O <sub>8</sub> P	alpha-D-Ribose 1-phosphate	1.49	0.005
11	125.04	256.08	256.0821	3.22	C <sub>11</sub> H <sub>14</sub> N <sub>6</sub> O <sub>6</sub>	Nicotinate D-ribonucleoside	1.29	0.005
12	828.61	111.02	111.032	0.88	C <sub>5</sub> H <sub>5</sub> N <sub>2</sub> O <sub>2</sub>	Pyrrole-2-carboxylic acid	1.15	0.020
13	740.72	301.28	301.2981	5.46	C <sub>18</sub> H <sub>39</sub> N <sub>2</sub> O <sub>2</sub>	Sphinganine	1.40	0.005
14	351.16	131.05	148.0524	0.30	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	trans-Cinnamate	1.38	0.005
<b>Down-regulated</b>								
1	390.85	137.02	138.0317	0.20	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	Salicylic acid	1.30	0.013
2	409.21	118.06	117.0578	1.44	C <sub>8</sub> H <sub>7</sub> N	Indole	1.53	0.005
3	292.70	136.06	136.0524	0.54	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	4-Hydroxyphenylacetaldehyde	1.11	0.031
4	93.10	118.05	119.0582	1.76	C <sub>4</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	L-Allothreonine	1.42	0.005
5	368.95	220.12	219.1107	1.57	C <sub>9</sub> H <sub>17</sub> N <sub>5</sub> O <sub>5</sub>	Pantothenic acid	1.08	0.045
6	151.24	104.05	104.0473	1.32	C <sub>4</sub> H <sub>8</sub> O <sub>3</sub>	2-Hydroxybutyric acid	1.57	0.005
7	91.73	102.06	101.0477	0.13	C <sub>4</sub> H <sub>7</sub> N <sub>2</sub> O <sub>2</sub>	1-Aminocyclopropanecarboxylic acid	1.45	0.005

8	140.16	149.05	149.051	6.20	C5H11NO2S	L-Methionine	1.53	0.005
9	150.95	123.06	122.048	0.48	C6H6N2O	Niacinamide	1.34	0.005
10	452.50	300.20	300.2089	1.19	C20H28O2	all-trans-Retinoic acid	1.10	0.031
11	110.23	118.09	117.079	0.58	C5H11NO2	5-Aminopentanoic acid	1.63	0.005
12	84.66	132.03	133.0375	0.70	C4H7NO4	L-Aspartic acid	1.60	0.005
13	247.99	86.10	85.0891	4.61	C5H11N	Piperidine	1.65	0.005
14	96.14	89.02	90.0317	0.02	C3H6O3	L-Lactic acid	1.38	0.005
15	201.41	134.99	136.0385	2.12	C5H4N4O	Hypoxanthine	1.57	0.005
16	269.76	178.05	179.0582	0.03	C9H9NO3	Hippuric acid	1.55	0.005
17	342.88	137.05	137.0477	14.85	C7H7NO2	2-Aminobenzoic acid	1.61	0.005
18	124.58	112.05	111.0433	0.00	C4H5N3O	Cytosine	1.65	0.005
19	124.58	244.09	243.0855	0.23	C9H13N3O5	Cytidine	1.64	0.005
20	150.76	664.12	664.1169	4.82	C21H28N7O14P2	NAD	1.52	0.005
21	247.46	132.10	131.0946	1.98	C6H13NO2	L-Leucine	1.65	0.005
22	80.08	117.02	118.0266	0.23	C4H6O4	Succinic acid	1.60	0.005
23	488.20	295.13	294.1216	2.19	C14H18N2O5	Aspartame	1.63	0.005
24	145.08	112.02	112.0273	1.84	C4H4N2O2	Uracil	1.56	0.005
25	305.40	168.07	169.0739	0.07	C8H11NO3	Pyridoxine	1.57	0.005
26	139.25	243.06	244.0695	2.30	C9H12N2O6	Pseudouridine	1.56	0.005
27	325.12	251.08	252.0859	3.76	C10H12N4O4	Deoxyinosine	1.46	0.005
28	458.82	377.15	376.1383	0.52	C17H20N4O6	Riboflavin	1.63	0.005
29	792.02	105.04	104.011	0.96	C3H4O4	Hydroxypyruvic acid	1.03	0.020
30	150.31	166.05	165.046	0.00	C5H11NO3S	L-Methionine S-oxide	1.66	0.005
31	382.78	203.08	204.0899	0.02	C11H12N2O2	L-Tryptophan	1.58	0.005
32	74.29	146.03	146.0215	1.04	C5H6O5	Oxoglutaric acid	1.64	0.005
33	225.63	132.14	131.0946	1.53	C6H13NO2	L-Isoleucine	1.64	0.005

34	44.14	85.07	102.0681	2.59	C5H10O2	Ethylmethylacetic acid	1.35	0.005
35	74.32	94.05	94.0419	0.46	C6H6O	Phenol	1.66	0.005
36	298.82	125.03	126.0429	13.50	C5H6N2O2	Thymine	1.59	0.005
37	494.61	136.08	135.0684	2.74	C8H9NO	2-Phenylacetamide	1.16	0.013
38	303.41	227.07	228.0746	1.25	C9H12N2O5	Deoxyuridine	1.58	0.005
39	320.46	440.13	441.1397	1.72	C19H19N7O6	Folic acid	1.61	0.005
40	483.44	204.12	204.111	0.24	C8H16N2O4	N6-Acetyl-N6-hydroxy-L-lysine	1.62	0.005
41	97.18	122.03	121.0197	0.83	C3H7NO2S	D-Cysteine	1.60	0.005
42	102.95	220.08	221.0899	0.08	C8H15NO6	N-Acetyl-D-glucosamine	1.59	0.005
43	131.74	146.09	145.0851	0.75	C5H11N3O2	4-Guanidinobutanoic acid	1.65	0.005
44	304.53	252.11	251.102	0.04	C10H13N5O3	2-Deoxyadenosine	1.57	0.005
45	99.01	284.05	301.0563	11.97	C8H16NO9P	N-Acetyl-alpha-D-glucosamine 1-phosphate	1.61	0.005
46	392.62	193.07	193.0739	10.66	C10H11NO3	Phenylacetylglycine	1.60	0.005
47	327.90	266.09	267.0968	0.08	C10H13N5O4	Deoxyguanosine	1.57	0.005
48	129.58	192.03	192.027	1.65	C6H8O7	Isocitric acid	1.65	0.005
49	358.06	182.04	183.0532	6.66	C8H9NO4	4-Pyridoxic acid	1.52	0.005
50	106.18	324.06	323.0519	0.04	C9H14N3O8P	CMP	1.66	0.005
51	95.62	323.03	324.0359	1.93	C9H13N2O9P	UMP	1.57	0.005
52	226.73	166.07	166.063	1.02	C9H10O3	D-Phenyllactic acid	1.64	0.005
53	74.70	89.11	88.1	0.67	C4H12N2	Putrescine	1.66	0.005
54	308.26	152.06	151.0494	0.62	C5H5N5O	Guanine	1.66	0.005
55	94.94	162.11	161.1052	1.40	C7H15NO3	L-Carnitine	1.67	0.005
56	393.31	136.06	135.0545	0.68	C5H5N5	Adenine	1.66	0.005
57	317.59	282.08	283.0917	1.23	C10H13N5O5	Guanosine	1.61	0.005
58	223.09	193.07	193.0739	2.09	C10H11NO3	3-Carbamoyl-2-phenylpropionaldehyde	1.65	0.005
59	99.71	88.04	89.0477	0.36	C3H7NO2	Sarcosine	1.57	0.005

60	223.28	385.13	384.1216	1.66	C14H20N6O5S	S-Adenosylhomocysteine	1.66	0.005
61	96.03	489.11	488.1073	6.11	C14H26N4O11P2	Citicoline	1.55	0.005
62	104.42	362.05	363.058	2.09	C10H14N5O8P	GMP	1.59	0.005
63	446.18	359.08	360.0845	2.32	C18H16O8	Rosmarinic acid	1.60	0.005
64	153.19	176.06	175.0481	0.15	C6H9NO5	N-Acetyl-L-aspartic acid	1.65	0.005
65	446.18	296.08	297.0896	0.24	C11H15N5O3S	5-Methylthioadenosine	1.61	0.005
66	143.47	608.08	607.0816	7.82	C17H27N3O17P2	UDP-N-acetyl-D-mannosamine	1.58	0.005
67	324.45	346.06	347.0631	1.18	C10H14N5O7P	3-AMP	1.61	0.005
68	99.78	130.06	131.0695	10.78	C4H9N3O2	Creatine	1.59	0.005
69	198.08	321.05	322.0566	0.69	C10H15N2O8P	dTMP	1.53	0.005

Notes: The metabolites were confirmed based on their exact molecular weights and characterized by comparisons with reference standards or MS/MS fragment information. RT, retention time; *m/z*, mass-to-charge ratio; VIP, variable importance in the project.

**Table S2** Metabolic pathways analyzed in the study

Pathway	Total	Hits	Raw p	-LOG(p)	Holm adjust	FDR	Impact	compounds	pathway
Pyrimidine metabolism	60	9	0.000	8.76	0.01	0.01	0.36	C00105;C00055;C00475;C00380;C00106;C00526;C00364;C00178;C02067	hsa00240
Lysine degradation	47	5	0.021	3.85	1.00	0.19	0.31	C00047;C02727;C00489;C03955;C00431	hsa00310
Alanine, aspartate and glutamate metabolism	24	4	0.008	4.80	0.62	0.11	0.26	C01042;C00049;C00026;C00042	hsa00250
Pantothenate and CoA biosynthesis	27	4	0.013	4.37	0.93	0.14	0.25	C00864;C00141;C00049;C00106	hsa00770
Phenylalanine metabolism	45	7	0.001	7.26	0.06	0.03	0.23	C05607;C02505;C00423;C01586;C05598;C00042;C00805	hsa00360
Cysteine and methionine metabolism	56	7	0.003	5.93	0.20	0.05	0.20	C01234;C00170;C02989;C00073;C00021;C00793;C00049	hsa00270
Tryptophan metabolism	79	3	0.518	0.66	1.00	1.00	0.19	C00078;C00463;C00108	hsa00380
Vitamin B6 metabolism	32	3	0.096	2.35	1.00	0.43	0.18	C00314;C00026;C00847	hsa00750
Retinol metabolism	22	1	0.540	0.62	1.00	1.00	0.18	C00777	hsa00830
Citrate cycle (TCA cycle)	20	3	0.030	3.52	1.00	0.24	0.16	C00042;C00026;C00311	hsa00020
Riboflavin metabolism	21	1	0.523	0.65	1.00	1.00	0.15	C00255	hsa00740
Sphingolipid metabolism	25	1	0.586	0.53	1.00	1.00	0.14	C00836	hsa00600
Thiamine metabolism	24	1	0.571	0.56	1.00	1.00	0.12	C00378	hsa00730
Valine, leucine and isoleucine biosynthesis	27	3	0.064	2.75	1.00	0.36	0.12	C00123;C00141;C00407	hsa00290
Aminoacyl-tRNA biosynthesis	75	6	0.042	3.16	1.00	0.31	0.11	C00049;C00073;C00047;C00407;C00123;C00078	hsa00970
Arginine and proline metabolism	77	7	0.015	4.18	1.00	0.15	0.10	C00049;C00300;C01035;C00134;C00431;C00213;C05942	hsa00330
Lysine biosynthesis	32	3	0.096	2.35	1.00	0.43	0.10	C00049;C00047;C00026	hsa00300
Pentose phosphate pathway	32	2	0.303	1.19	1.00	0.99	0.09	C00620;C00257	hsa00030
Purine metabolism	92	10	0.001	6.92	0.08	0.03	0.08	C00559;C05512;C00147;C00144;C00262;C00242;C00330;C00387;C01367;C00620	hsa00230
Phenylalanine, tyrosine and tryptophan biosynthesis	27	3	0.064	2.75	1.00	0.36	0.07	C00463;C00078;C00108	hsa00400
Glyoxylate and dicarboxylate metabolism	50	4	0.091	2.39	1.00	0.43	0.06	C00168;C00026;C00311;C00042	hsa00630
Fructose and mannose metabolism	48	2	0.498	0.70	1.00	1.00	0.05	C00159;C00507	hsa00051

Glycine, serine and threonine metabolism	48	6	0.005	5.22	0.41	0.09	0.05	C00049;C00213;C00300;C00168;C05519;C00078	hsa00260
Pyruvate metabolism	32	1	0.677	0.39	1.00	1.00	0.05	C00256	hsa00620
Glycerophospholipid metabolism	39	1	0.748	0.29	1.00	1.00	0.04	C00307	hsa00564
Nicotinate and nicotinamide metabolism	44	4	0.063	2.77	1.00	0.36	0.04	C00049;C05841;C00153;C00003	hsa00760
Valine, leucine and isoleucine degradation	40	3	0.158	1.85	1.00	0.60	0.04	C00123;C00141;C00407	hsa00280
Amino sugar and nucleotide sugar metabolism	88	4	0.361	1.02	1.00	1.00	0.02	C00140;C04501;C01170;C00159	hsa00520
Drug metabolism - cytochrome P450	86	1	0.954	0.05	1.00	1.00	0.02	C16587	hsa00982
Butanoate metabolism	40	2	0.404	0.91	1.00	1.00	0.02	C00042;C00026	hsa00650
Glutathione metabolism	38	1	0.739	0.30	1.00	1.00	0.02	C00134	hsa00480
Tyrosine metabolism	76	4	0.266	1.33	1.00	0.94	0.02	C03765;C00146;C00042;C01850	hsa00350
Propanoate metabolism	35	3	0.118	2.14	1.00	0.50	0.00	C00042;C01234;C05984	hsa00640
Nitrogen metabolism	39	3	0.149	1.90	1.00	0.60	0.00	C00078;C00049;C00108	hsa00910
beta-Alanine metabolism	28	3	0.070	2.66	1.00	0.37	0.00	C00049;C00864;C00106	hsa00410
One carbon pool by folate	9	1	0.271	1.30	1.00	0.94	0.00	C00504	hsa00670
D-Glutamine and D-glutamate metabolism	11	1	0.321	1.14	1.00	0.99	0.00	C00026	hsa00471
Biotin metabolism	11	1	0.321	1.14	1.00	0.99	0.00	C00047	hsa00780
Cyanoamino acid metabolism	16	1	0.431	0.84	1.00	1.00	0.00	C00049	hsa00460
Histidine metabolism	44	2	0.452	0.79	1.00	1.00	0.00	C00049;C00026	hsa00340
Galactose metabolism	41	1	0.766	0.27	1.00	1.00	0.00	C00159	hsa00052
Folate biosynthesis	42	1	0.774	0.26	1.00	1.00	0.00	C00504	hsa00790
Ascorbate and aldarate metabolism	45	1	0.797	0.23	1.00	1.00	0.00	C00026	hsa00053
Fatty acid metabolism	50	1	0.830	0.19	1.00	1.00	0.00	C00489	hsa00071

### Supplementary Figure legends

**Figure S1** ANGPTL8/betatrophin increases blood TG levels in KM mice. **(A)** TG concentration 30 min after recombinant ANGPTL8/betatrophin injection (i.p.) into 8-week-old mice (n = 6). **(B)** TG concentration, 0, 10, 30 and 60 min after recombinant ANGPTL8/betatrophin injection (i.p.) into 36-week-old mice (n = 6). The experiment was repeated three times. Data represent the mean  $\pm$  standard deviation (SD). r-betatrophin, recombinant ANGPTL8/betatrophin.

**Figure S2** Multivariate statistical analysis of metabolite profiles in HepG2/IR samples. **(A)** PCA score plot (n = 6). **(B-C)** PLS-DA score plots of metabolite profiles in positive ion mode and negative ion mode (n = 6). The ellipse area represents the 95% confidence interval by Hotelling T2 test. The red circle represents each sample in the wild type HepG2/IR group; the green triangle represents each sample in the *ANGPTL8/betatrophin* gene knockout HepG2/IR group. WT, wild type HepG2/IR; A, *ANGPTL8/betatrophin* gene knockout HepG2/IR.

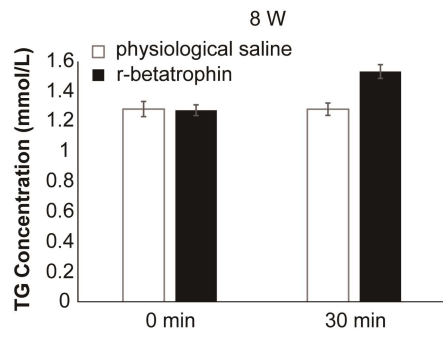
**Figure S3** Metabolite correlation analysis. Color-marked part and the blank part were  $p < 0.05$  and  $p > 0.05$ , respectively. Positive correlations (red) with a correlation of up to 1 and negative correlations (blue) with a correlation of up to -1.

**Figure S4** Box-and-whisker plots showing the relative levels of up-regulated metabolites. Results of metabolite changed trends were expressed as normalized ratios (“\*”,  $p < 0.05$ ; “\*\*\*”,  $p < 0.01$ ). WT, wild type HepG2/IR; A, *ANGPTL8/betatrophin* knockout HepG2/IR.

**Figure S5** Box-and-whisker plots showing the relative levels of down-regulated metabolites. Results of metabolite changed trends were expressed as normalized ratios (“\*”,  $p < 0.05$ ; “\*\*\*”,  $p < 0.01$ ). WT, wild type HepG2/IR; A, *ANGPTL8/betatrophin* knockout HepG2/IR.

**Figure S1**

**A**



**B**

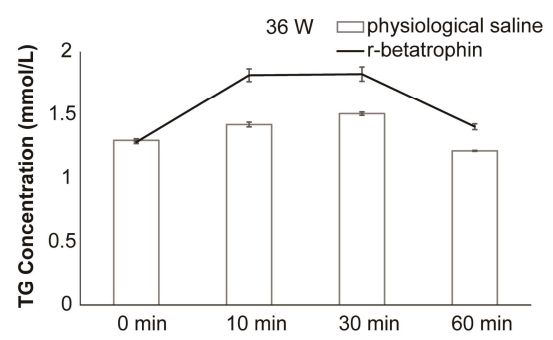




Figure S2

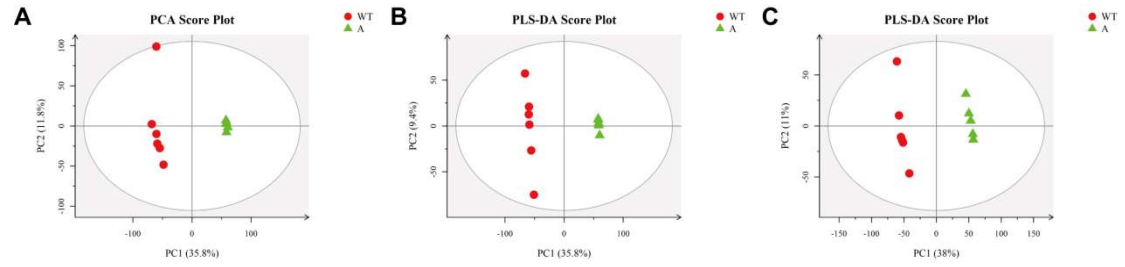


Figure S3

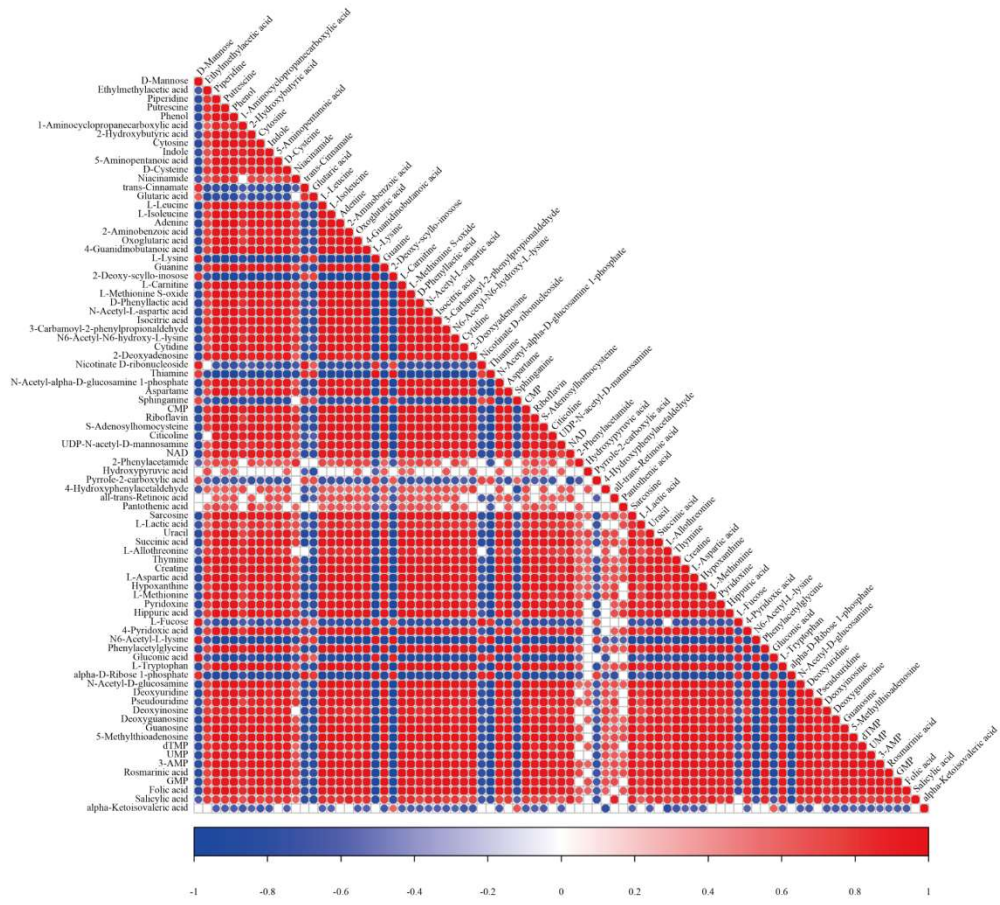


Figure S4

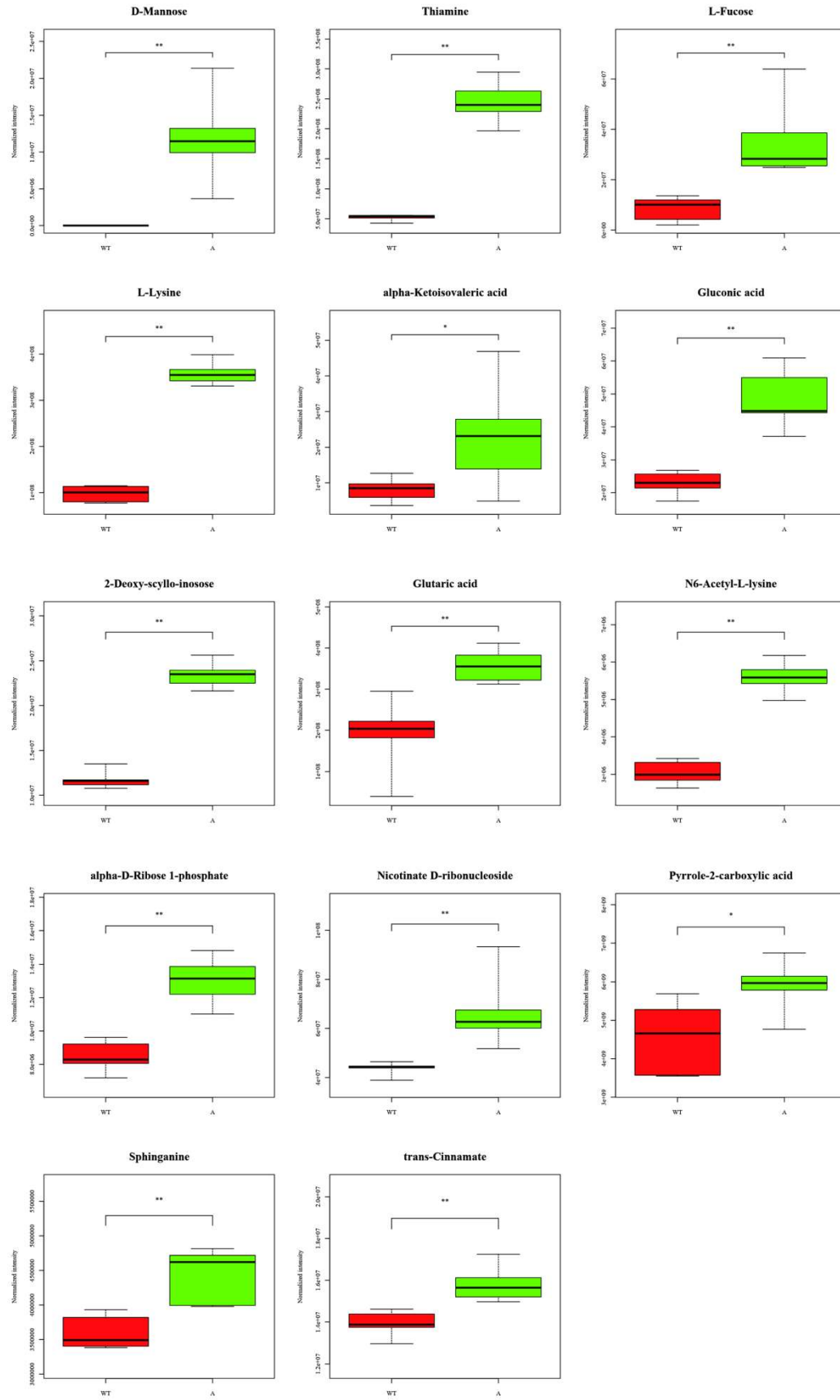


Figure S5

