

Supporting information

Valine Supplementation Does Not Reduce Lipid Accumulation and Improve Insulin sensitivity in Mice Fed High-Fat Diet

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Supporting information

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Table S1. Summary of significantly different metabolites between HV and HW based on a OPLS-DA VIP (Variable Importance for the Projection) more than 1

Table S2. Summary of significantly different genes between HV and HW by transcriptomics

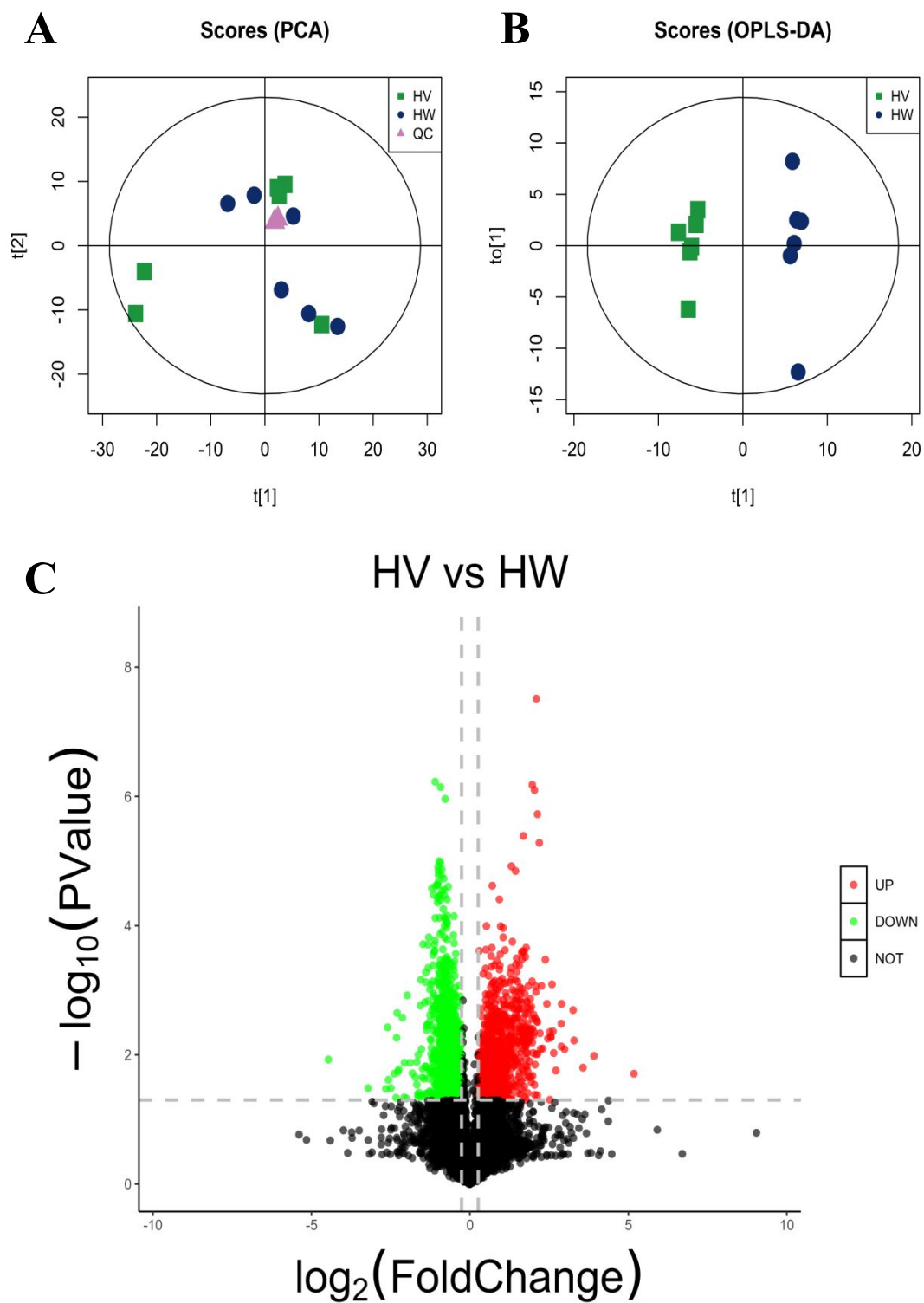


Figure S1. The PCA, OPLS-DA score plot and Volcano Plot for the metabolic profiling results of liver tissue for HV versus HW. A: PCA score plot. t[1] represents principal component 1, and t[2] represents principal component

2. B: OPLS-DA score plot. $t[1]$ represents the predicted principal component and can distinguish group variations.

C: Volcano Plot. The dots represents metabolites with $FC > 1.5$ and $P \text{ value} < 0.05$. Red dots indicate up-regulated metabolites in HV, and green dots indicate down-regulated metabolites in HV. Abbreviations: High-fat diet + valine (HV); high-fat diet + water (HW); quality control (QC); principal component analysis (PCA); orthogonal partial least squares discrimination analysis (OPLS-DA); fold change analysis (FC).

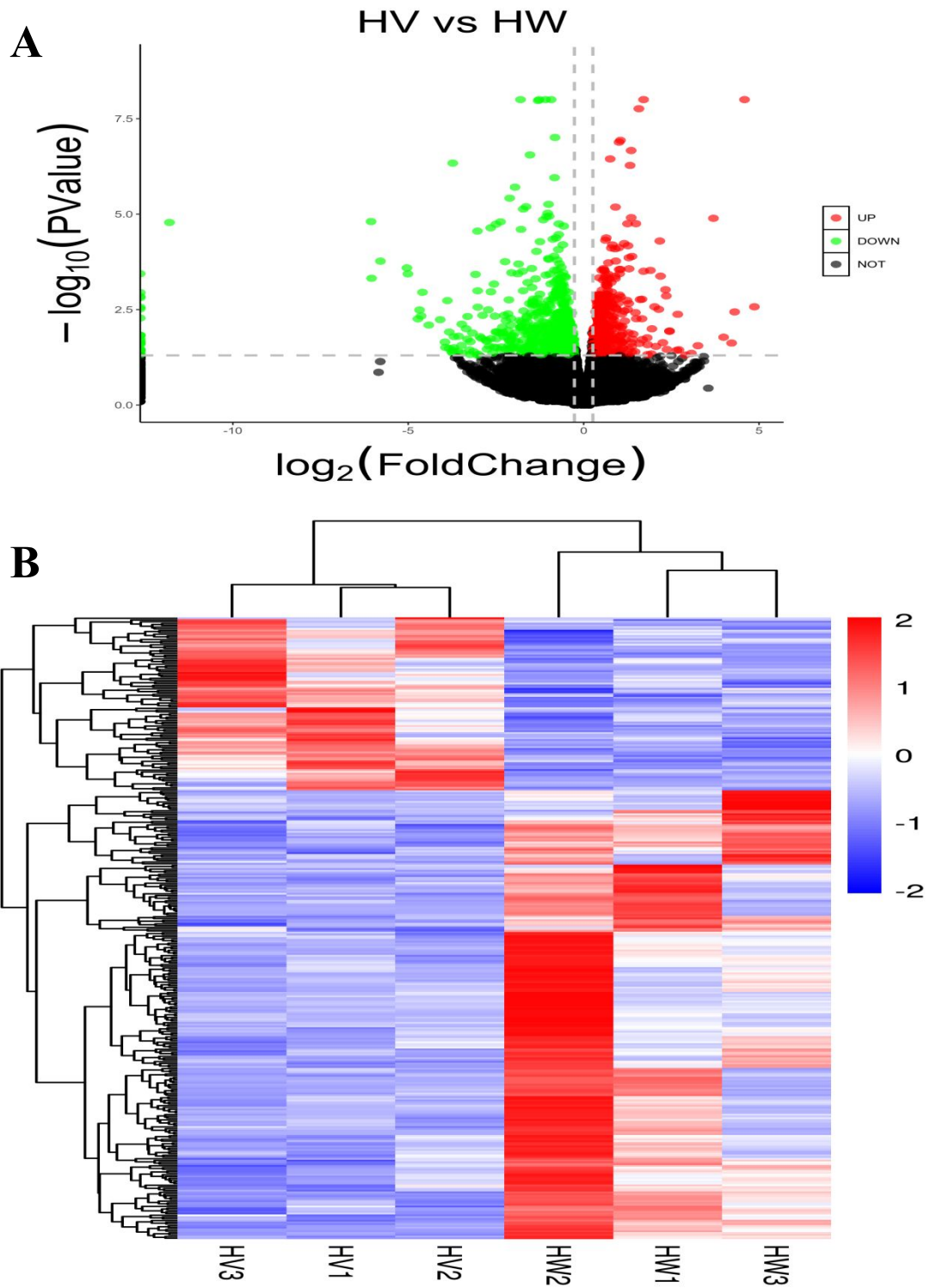


Figure S2. Volcano Plot and Hierarchical Clustering of differential genes for HV versus HW. A: Volcano Plot.

The black dots represent the genes with no significant difference, the red dots represent the up-regulated genes with significant difference, and the green dots represent the down-regulated genes with significant difference. B:

The Hierarchical Clustering of differential genes. Red indicates the up-regulated genes in HV, and blue indicates the down-regulated genes in HV. Scaled expression values are color-coded according to the legend on the right.

Abbreviations: High-fat diet + valine (HV); high-fat diet + water (HW).

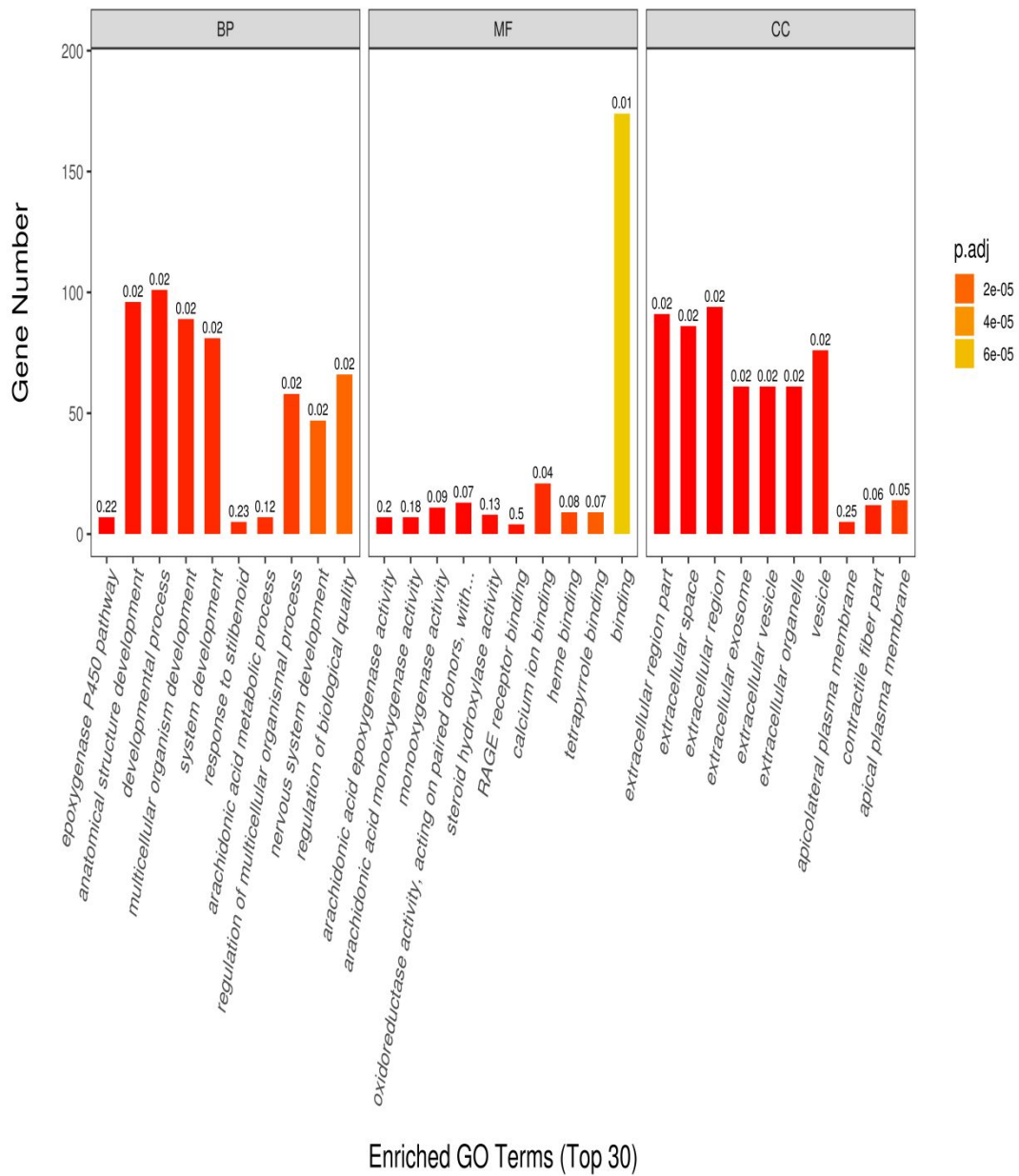


Figure S3. Gene ontology (GO) enrichment histogram of differential genes. Abbreviations of classifications: BP: biological processes; MF: molecular functions; CC: cellular components.

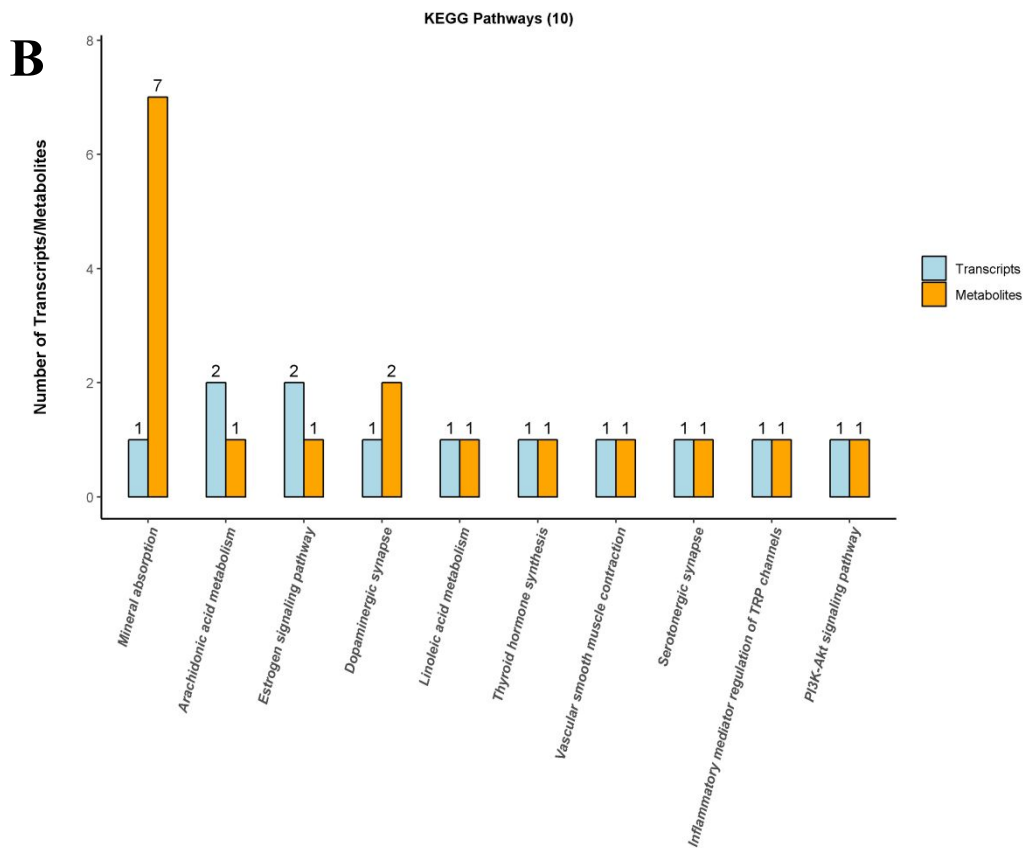
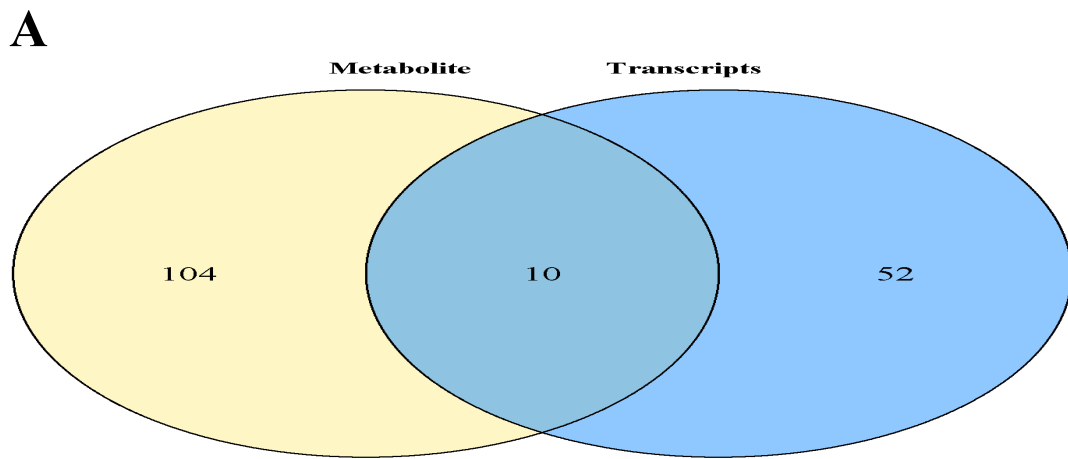


Figure S4. A: The Venn of differential genes and differential metabolites involved in pathways. Blue for transcriptomics, yellow for metabolomics; the cross region of the circle represents the number of metabolic pathways involved in both omics. The sum of all figures inside the circle represents the sum of the number of pathways involved in differentially expressed genes and differentially expressed metabolites, respectively. The cross region of the circle represents the number of pathways involved in both omics. B: The top 10 pathways with the highest number of genes and metabolites involved. Each column represents a KEGG pathway, blue for

transcriptomics, orange for metabolomics. From left to right, by number of genes or metabolites, from high to low.
The higher the column, the more molecules the biological pathway is annotated in the sample.

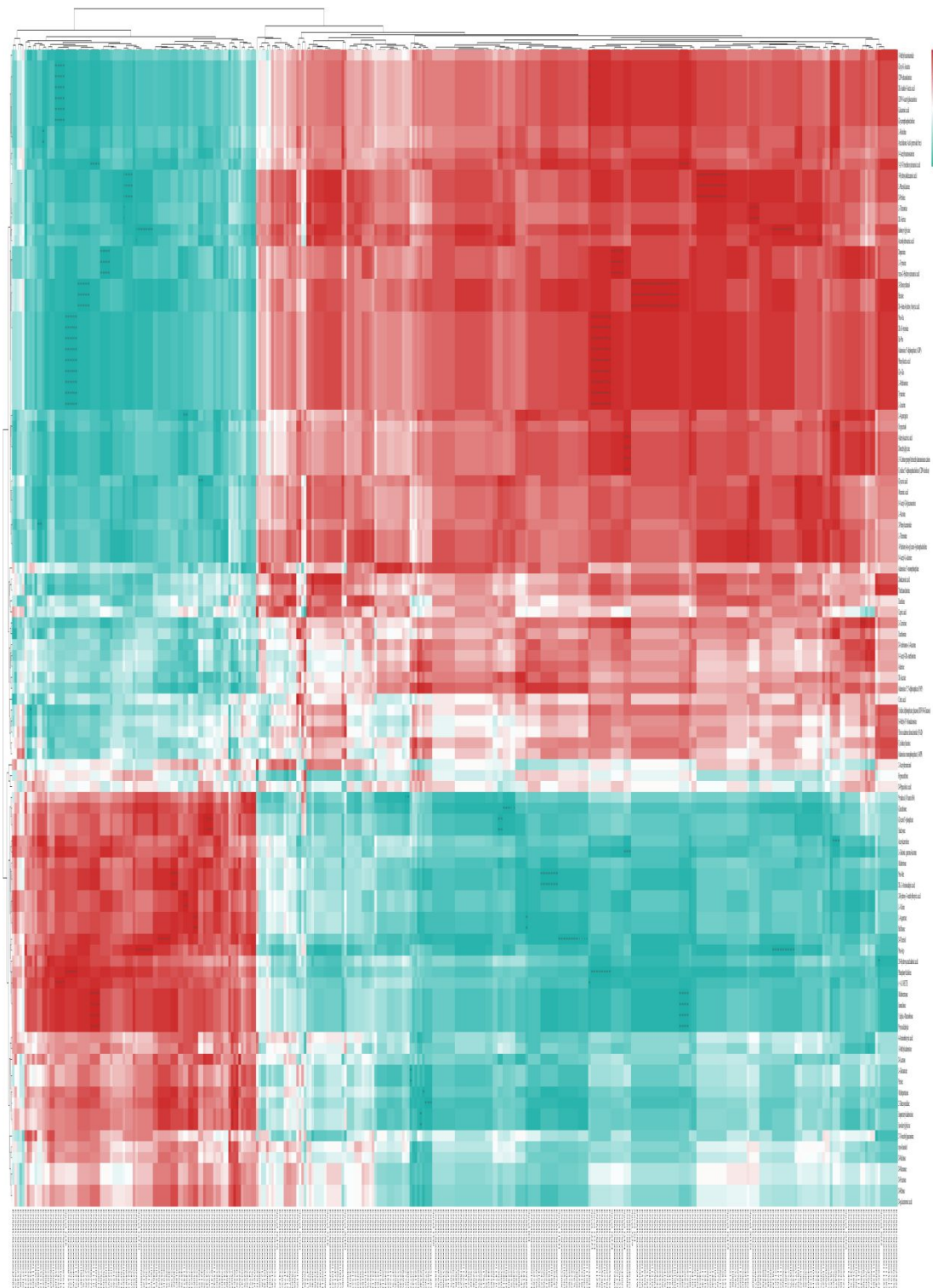


Figure S5. Correlation analysis Spearman differential genes and differential metabolites. Each row represents a significant difference in metabolites and each column represents a significant difference in genes. The left branch indicates the result of clustering the differential metabolites, and the upper branch indicates the result of clustering the differential genes. Each lattice in the figure contains two kinds of information (correlation coefficient r and p

value). Correlation coefficient r expressed in color. $r > 0$ means positive correlation, in red, $r < 0$, in blue, the deeper the color, the stronger the correlation. $P < 0.05$, expressed as *; $P < 0.001$, expressed as ***.

Table S1. Summary of significantly different metabolites between HV and HW based on a OPLS-DA VIP (Variable Importance for the Projection) more than 1

name	description	Fold		p-value	m/z	rt(s)	KEGG.I D
		VIP	chang e				
M104T400	Dimethylglycine	2.0173	0.1920	1.7284E-08	104.07	400.03	C01026
M229T244	Ile-Pro	1.7201	0.5477	1.9894E-05	229.15	244.15	
M222T256	N-Acetyl-D-glucosamine	3.6270	0.6720	3.5309E-05	222.10	255.56	C00140
M204T254	N-Acetylmannosamine	3.7405	0.7052	0.0001	204.09	254.28	C00645
M162T328	DL-2-Aminoadipic acid	1.3556	2.2228	0.0001	162.08	327.60	C00956
M146T258	DL-O-tyrosine	1.1389	0.7282	0.0002	146.06	257.58	
M464T489	Adenylsuccinic acid	1.2467	0.2527	0.0007	464.08	489.28	C03794
M120T256_2	Tyramine	10.6932	0.7527	0.0008	120.08	255.58	C00483
M447T443	CDP-ethanolamine	1.3754	0.4224	0.0008	447.07	443.34	C00570
M188T257	DL-Indole-3-lactic acid	3.3469	0.7211	0.0009	188.07	257.44	
M131T256	Phenyllactic acid	1.1339	0.7713	0.0009	131.05	255.58	C05607
M364T392	2'-O-methylguanosine	1.7213	6.4327	0.0010	364.06	391.76	

name	description	Fold			m/z	rt(s)	KEGG.I D
		VIP	chang	p-value			
M187T337	Pro-Ala	1.6434	0.4857	0.0010	187.1 1	337.3 6	
M489T439	Cytidine 5'-diphosphocholine (CDP-choline)	2.8065	0.5448	0.0011	489.1 1	439.1 8	C00307
M428T457	Adenosine 5'-diphosphate (ADP)	1.0002	0.8234	0.0012	428.0 4	457.0 8	C00008
M166T256_ 2	L-Phenylalanine	7.8093	0.7651	0.0019	166.0 9	255.5 8	C00079
M198T278	D-Mannose	2.2034	1.6844	0.0025	198.1 0	278.1 3	C00159
M121T112	Purine	1.7964	1.6777	0.0025	121.0 5	111.7 0	C15587
M285T308	Xanthosine	1.7257	0.7046	0.0028	285.0 8	308.3 1	C01762
M608T429	UDP-N-acetylglucosamine	1.9253	0.5377	0.0031	608.0 9	428.9 9	C00043
M153T308	Oxypurinol	2.1899	0.7017	0.0033	153.0 4	308.3 1	C07599
M522T446_ 2	Maltotriose	16.482 1	3.2292	0.0037	522.2 0	446.1 7	C01835
M165T299	trans-2-Hydroxycinnamic acid	2.7744	0.7546	0.0038	165.0 5	299.3 3	
M428T487	Adenosine 3',5'-diphosphate (PAP)	1.6502	0.4430	0.0039	428.0 4	486.6 4	C00054
M132T261	L-Leucine	5.5898	0.8224	0.0040	132.1 0	260.9 7	C00123

name	description	Fold			m/z	rt(s)	KEGG.I D
		VIP	chang	p-value			
M136T299	Dopamine	2.3987	0.7661	0.0046	136.0 8	299.3 3	C03758
M182T299_2	L-Tyrosine	3.2766	0.7583	0.0047	182.0 8	299.3 3	C00082
M258T426	Glycerophosphocholine	6.6027	0.3538	0.0050	258.1 1	425.6 1	C00670
M649T487	Stachyose	2.6248	5.0610	0.0055	649.2 2	486.7 6	C01613
M310T426	Pro-Met	2.4572	1.5186	0.0064	310.1 1	425.5 8	
M116T308_2	D-Proline	7.1607	0.8258	0.0065	116.0 7	307.9 3	C00763
M336T63_1	Isopentenyladenosine	1.9029	1.4729	0.0094	336.1 7	62.81	
M150T282	L-Methionine	2.6940	0.8003	0.0095	150.0 6	281.7 0	C00073
M196T46	2-Phenylacetamide	1.4285	0.6713	0.0112	196.1 0	45.78	
M786T394	Flavin adenine dinucleotide (FAD)	1.1451	0.8678	0.0116	786.1 7	394.2 5	C00016
M360T424	Isomaltose	1.4754	1.4447	0.0137	360.1 5	424.3 7	C00252
M522T365	Raffinose	1.2418	2.0479	0.0147	522.1 9	365.4 9	C00492
M213T415_2	Triethanolamine	4.1421	0.5891	0.0159	213.1 2	414.8 0	C06771

name	description	Fold			m/z	rt(s)	KEGG.I D
		VIP	chang	p-value			
M118T272_ 2	Betaine	21.995 2	0.5443	0.0163	118.0 9	271.5 6	C00719
M325T387	3.alpha.-Mannobiose	17.755 4	1.7243	0.0168	325.1 1	386.7 8	
M829T505	Maltopentaose	1.4116	2.4245	0.0175	829.2 9	504.9 2	
M204T303	Acetylcarnitine	2.3464	1.2581	0.0176	204.1 2	303.3 4	C02571
M667T388	Maltotetraose	1.0107	1.8244	0.0180	667.2 3	387.7 3	C02052
M162T353	L-Carnitine	22.666 2	0.8250	0.0210	162.1 1	353.2 0	C00318
M303T48	20-Hydroxyarachidonic acid	6.8430	1.8239	0.0282	303.2 3	47.53	
M145T388	Pyruvaldehyde	4.4061	1.6134	0.0306	145.0 5	387.8 7	C00546
M104T369	4-Aminobutyric acid	2.0924	1.2430	0.0327	104.0 7	369.2 2	C00334
M496T195	1-Palmitoyl-sn-glycero-3-phosphocholine	18.126 1	0.8774	0.0338	496.3 4	194.7 0	
M156T398	L-Histidine	5.3730	0.7730	0.0362	156.0 8	397.8 5	C00135
M146T374_ 2	(3-Carboxypropyl)trimethylammonium cation	5.2519	0.7777	0.0363	146.1 2	374.3 6	
M231T424	Pro-Asp	1.0615	1.1575	0.0393	231.1 0	424.2 5	

name	description	Fold			m/z	rt(s)	KEGG.I D
		VIP	chang	p-value			
M137T311	1-Methylnicotinamide	1.7283	0.4833	0.0421	137.0 7	310.9 5	C02918
M160T382	Cyclohexylamine	12.243 9	0.6489	0.0456	160.1 3	381.7 3	C00571
M132T336	2-Ethoxyethanol	1.9021	0.5651	0.0459	132.1 0	336.1 1	C14687
M133T376	L-Asparagine	1.5707	0.6487	0.0459	133.0 6	376.4 4	C00152
M134T428	L-Aspartate	1.1315	1.7521	0.0488	134.0 4	427.9 8	C00049
M137T131	Hypoxanthine	2.3476	0.7410	0.0539	137.0 5	131.1 1	C00262
M269T34	Arachidonic Acid (peroxide free)	1.0672	0.6967	0.0568	269.2 3	33.65	C00219
M348T467	Adenosine monophosphate (AMP)	2.0092	0.5660	0.0575	348.0 7	466.5 8	C00020
M184T489_ 2	Phosphorylcholine	12.797 3	1.1169	0.0580	184.0 7	489.2 8	C00588
M348T404	Adenosine 3'-monophosphate	1.0756	0.8739	0.0622	348.0 7	403.9 1	C01367
M130T305	D-Pipecolinic acid	1.0304	1.1567	0.0633	130.0 9	305.4 2	
M173T432	Glycerol 3-phosphate	1.4229	1.6552	0.0693	173.0 2	432.2 6	C00093
M298T100	S-Methyl-5'-thioadenosine	5.5914	0.6766	0.0765	298.1 0	100.2 3	C00170

name	description	Fold			m/z	rt(s)	KEGG.I D
		VIP	chang	p-value			
M282T289	1-Methyladenosine	1.7594	1.3104	0.0790	282.1 2	288.8 7	C02494
M148T425	L-Glutamate	1.4642	1.1514	0.0833	148.0 6	425.0 4	C00025
M136T249	Adenine	1.5493	0.5748	0.0894	136.0 6	249.0 4	C00147
M205T420	Gly-Glu	1.5339	0.7652	0.0973	205.0 8	420.2 2	
M194T275	2-Acetylresorcinol	1.0800	1.6211	0.0990	194.0 8	275.3 9	

VIP>1 and P value<0.05 metabolites, as metabolites with significant differences; while VIP>1 and 0.05<P value<0.1 as differential metabolites.

Table S2. Summary of significantly different genes between HV and HW by transcriptomics

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG00000004 0660	Cyp2b9	123.54	5.14	4.6145	1.99E-19
ENSMUSG00000007 2849	Serpina1e	47463.43	100919.29	-1.0883	6.84E-16
ENSMUSG00000008 7412	Gm15501	171.94	52.59	1.7074	2.40E-12
ENSMUSG00000000 6445	Epha2	603.59	1147.66	-0.9267	3.62E-12
ENSMUSG00000009 0877	Hspa1b	277.7	966.3	-1.7991	6.56E-12
ENSMUSG00000003 0827	Fgf21	199.48	479.65	-1.2649	9.93E-12
ENSMUSG00000005 2302	Tbc1d30	152.91	376.09	-1.2998	6.24E-10
ENSMUSG00000002 4986	Hhex	961.11	322.5	1.5744	7.31E-09
ENSMUSG00000003 0852	Tacc2	520.59	917.06	-0.8162	8.81E-08
ENSMUSG00000002 5396	Hsd17b6	2199.25	1062.34	1.0502	1.06E-07
ENSMUSG00000003 5105	Egln3	368.19	182.77	1.0129	1.20E-07
ENSMUSG00000006 0807	Serpina6	2100.05	820.92	1.3548	2.06E-07

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG0000010 5703	Gm43305	246.86	712.4	-1.5277	2.71E-07
ENSMUSG0000002 8194	Ddah1	2720.09	1610.73	0.7561	3.48E-07
ENSMUSG0000005 3469	Tg	2.26	30	-3.7158	4.52E-07
ENSMUSG0000002 3087	Noct	264.06	105.51	1.3229	5.19E-07
ENSMUSG0000004 8856	Slc25a47	12100.24	21497.87	-0.8292	1.10E-06
ENSMUSG0000002 5004	Cyp2c40	18.55	71.97	-1.9578	1.95E-06
Novel.16475	-	16.79	72.37	-2.1081	3.81E-06
ENSMUSG0000003 8880	Mrps34	255.38	511.07	-1.0002	5.52E-06
ENSMUSG0000000 2944	Cd36	2658.58	1416.48	0.9084	6.53E-06
ENSMUSG0000001 8830	Myh11	76.82	239.58	-1.6389	6.38E-06
ENSMUSG0000005 7278	Snrpg	33.49	110.52	-1.7243	7.36E-06
ENSMUSG0000002 0423	Btg2	115.05	234.26	-1.0284	9.43E-06
ENSMUSG0000003 8599	Capn8	148.48	293.6	-0.9806	1.14E-05
ENSMUSG0000007	Thbd	81.35	169.54	-1.057	1.17E-05

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
4743					
ENSMUSG0000009 1867	Cyp2a22	143.61	56.06	1.3601	1.23E-05
ENSMUSG0000007 5044	Slc22a29	22.49	1.73	3.7431	1.28E-05
ENSMUSG0000005 5116	Arntl	172.94	383.97	-1.15	1.40E-05
ENSMUSG0000002 9188	Slc34a2	8.57	48.49	-2.502	1.84E-05
ENSMUSG0000004 7517	Dmbt1	0.68	2434.8	-11.8243	1.65E-05
ENSMUSG0000002 0000	Moxd1	14.53	969.53	-6.0562	1.56E-05
ENSMUSG0000011 2774	Gm36041	113.85	40.53	1.494	1.77E-05
ENSMUSG0000011 5919	Gm31583	1113.79	1818.23	-0.7071	1.79E-05
ENSMUSG0000007 9507	H2-Q1	139.83	58.71	1.2508	1.78E-05
Novel.11841	-	11.72	60.5	-2.3639	1.59E-05
ENSMUSG0000005 7342	Sphk2	1200.76	1778.18	-0.5661	2.05E-05
ENSMUSG0000002 0911	Krt19	12.45	78.1	-2.6467	2.29E-05
Novel.19562	-	16.16	55.65	-1.7867	2.50E-05

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG00000004 2784	Muc1	7.39	60.25	-3.0158	2.78E-05
ENSMUSG00000005 0445	Cyp8b1	12243.39	20015.67	-0.7091	3.43E-05
ENSMUSG00000002 6249	Serpine2	384.34	662.11	-0.7828	4.18E-05
ENSMUSG00000001 0601	Apol7a	2184.02	1396.87	0.6448	4.19E-05
ENSMUSG00000002 9009	Mthfr	372.33	683.83	-0.8761	4.51E-05
ENSMUSG00000002 9369	Afm	2519.37	1637.84	0.6213	4.82E-05
ENSMUSG00000004 9124	Gm8186	61.41	13.64	2.1732	5.05E-05
ENSMUSG00000005 6966	Gjc3	42.19	100.03	-1.2431	5.26E-05
ENSMUSG00000005 2117	D630039A 03Rik	344.95	154.81	1.1538	5.89E-05
ENSMUSG00000002 5405	Inhbc	1417.87	801.59	0.8232	6.40E-05
ENSMUSG00000003 7465	Klf10	410.02	618.25	-0.5931	6.33E-05
ENSMUSG00000008 7579	Hectd2os	263.4	109.68	1.2661	6.72E-05
ENSMUSG00000005 5301	Adh7	608.92	352.07	0.7897	7.69E-05

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG0000005 2133	Sema5b	177.01	88.45	1.0024	7.57E-05
ENSMUSG0000007 3155	1810058I2 4Rik	751.02	402.82	0.8988	8.09E-05
ENSMUSG0000002 5316	Banp	143.57	236.39	-0.7211	8.44E-05
ENSMUSG0000003 4634	Ly6d	54.03	137.09	-1.3443	9.43E-05
ENSMUSG0000002 9695	Aass	7698.27	13793.35	-0.8414	0.0001264
ENSMUSG0000004 5776	Lrtm1	201.55	77.64	1.3759	0.00012765
ENSMUSG0000004 0270	Bach2	182.15	74.49	1.2889	0.00013853
ENSMUSG0000002 6475	Rgs16	471.16	945.78	-1.0066	0.00014493
ENSMUSG0000005 0234	Gja4	66.93	133.49	-0.9909	0.00015234
ENSMUSG0000001 9768	Esr1	242.66	410.44	-0.7599	0.00015697
ENSMUSG0000005 1359	Ncald	292.44	154.99	0.9153	0.00016933
ENSMUSG0000002 4028	Tff2	0.3	16.62	-5.5506	0.00017033
ENSMUSG0000009 2008	Cyp2c69	8.33	39.32	-2.2422	0.00017538

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG0000003 8217	Tlcd2	1364.41	836.35	0.706	0.00018776
ENSMUSG0000004 0740	Slc25a34	220.76	355.85	-0.6878	0.00019858
ENSMUSG0000003 3998	Kcnk1	8.78	31.75	-1.8464	0.00020168
ENSMUSG0000003 6966	Spryd3	392.85	610.26	-0.6358	0.00024561
ENSMUSG0000001 8623	Mmp7	2.24	73.57	-5.0295	0.00025507
ENSMUSG0000002 7070	Lrp2	20.21	56.29	-1.4661	0.00026473
ENSMUSG0000007 4207	Adh1	20826.11	14071.44	0.5656	0.00026806
ENSMUSG0000003 4774	Dsg1c	131.52	53.61	1.2916	0.00027002
ENSMUSG0000002 6655	Fam107b	1563.56	1049.05	0.5762	0.00028559
ENSMUSG0000006 3730	Hsd3b2	963.89	476.82	1.0154	0.00028213
ENSMUSG0000003 0170	Wnt5b	132.54	223.09	-0.7527	0.00029317
ENSMUSG0000003 0731	Syt3	101.59	49.17	1.0459	0.00027977
ENSMUSG0000005 6131	Pgm3	473.72	843.14	-0.8319	0.00029308

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG0000003 9114	Nrn1	1767.48	1236.69	0.5149	0.00029367
ENSMUSG0000004 2686	Jph1	32.41	8.67	1.9044	0.00029745
ENSMUSG0000007 2940	Gm10443	22.64	75.57	-1.7327	0.00031249
ENSMUSG0000003 6611	Eepd1	420.19	278.1	0.5944	0.00030778
ENSMUSG0000002 4558	Mapk4	36.05	11.02	1.7124	0.00031346
ENSMUSG0000002 2878	Adipoq	0.35	7.46	-4.3974	0.0003418
ENSMUSG0000005 4641	Mmrn1	15.32	63.73	-2.0448	0.00034202
ENSMUSG0000003 0494	Rhpn2	330.05	495.07	-0.5849	0.00034303
ENSMUSG0000002 5656	Arhgef9	86.58	42.54	1.0223	0.00036244
ENSMUSG0000004 7730	Fcgbp	2.24	72.17	-5.0021	0.00036664
ENSMUSG0000004 7767	Atg16l2	184.87	360.34	-0.9624	0.00037013
ENSMUSG0000003 1995	St14	11.5	42.23	-1.8726	0.00036634
ENSMUSG0000004 5136	Tubb2b	2.94	24.91	-3.0817	0.00037834

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
ENSMUSG0000005 2957	Gas1	189.35	116.24	0.7014	0.00037914
ENSMUSG0000002 2824	Muc13	0	9.65	-5.7279	0.00036228
ENSMUSG0000003 5372	1810055G 02Rik	992.56	1602.76	-0.691	0.00037457
Novel.12253	-	42.61	89.9	-1.082	0.00039433
ENSMUSG0000004 5092	S1pr1	927.73	597.53	0.6346	0.00039961
ENSMUSG0000002 4900	Cpt1a	14986.72	18943.49	-0.338	0.0004132
ENSMUSG0000010 6705	Gm2602	27.98	6.12	2.1702	0.00042091
ENSMUSG0000000 3477	Inmt	5477.94	2942.16	0.8968	0.00042467
ENSMUSG0000006 0803	Gstp1	11422.05	16576.07	-0.5373	0.00042524
ENSMUSG0000005 4484	Tmem62	320.47	501.49	-0.6454	0.00044922
ENSMUSG0000005 8207	Serpina3k	174954.88	248807.53	-0.508	0.00044591
ENSMUSG0000003 1924	Cyb5b	6379.97	4194.94	0.6049	0.00045396
ENSMUSG0000001 6382	Pls3	1431.09	1049.75	0.4472	0.00046349
ENSMUSG0000002	Mme	901.86	544.9	0.7275	0.00048001

gene_ID	symbol	HV_readcount	HW_readcount	log2FoldChange	pvalue
7820					
ENSMUSG00000003 4591	Slc41a2	405.48	216.32	0.9052	0.0004843
ENSMUSG00000002 0159	Gabrp	0.95	62.78	-6.0237	0.00047831
ENSMUSG00000003 7139	Myom3	37.24	96.12	-1.3635	0.00049347
ENSMUSG00000002 4843	Chka	655.27	1088.59	-0.7316	0.00049562
ENSMUSG00000004 1261	Car8	1529.11	1042.2	0.5529	0.0005328
ENSMUSG00000009 2274	Neat1	5660.49	9574.84	-0.7583	0.00052896
ENSMUSG00000003 1725	Ces1f	7997.95	5008.39	0.6753	0.0005434
ENSMUSG00000000 2250	Ppard	137.56	212.19	-0.6239	0.0005603
ENSMUSG00000004 2099	Kank3	135.47	215.82	-0.6707	0.00056509

P < 0.05 as significant difference criteria

Table S3 Composition of experimental diets (%)

Ingredient	LFD ¹	HFD ²
Casein	18.96	25.84
L-Cystine	0.28	0.39
Corn Starch	29.86	0
Maltodextrin 10	3.32	16.15
Sucrose	33.17	8.90
Cellulose	4.74	6.46
Soybean Oil	2.37	3.23
Lard	1.90	31.66
Mineral Mix	0.95	1.29
DiCalcium	1.23	1.68
Calcium	0.52	0.71
Potassium Citrate	1.56	2.13
Vitamin Mix	0.95	1.29
Choline	0.19	0.26
Yellow Dye	0.005	
Blue Dye		0.006
Total	100	100

¹Containing 67.3% carbohydrate, 4.3% fat, and 19.2% protein on an as-fed basis. 10% of the total energy was provided by fat (mainly lard).

²Containing 26.3% carbohydrate, 34.9% fat, and 26.2% protein on an as-fed basis. 60% of the total energy was provided by fat (mainly lard).