

Figure S1. Superposition diagram of total ion flow diagram (TIC diagram, a) detected by mass spectrometry, MRM metabolite detection multi peak (b) and Tlc overlap diagram (c) of the mixed QC sample. The abscissa is the retention time (RT) of the metabolite detection, and the ordinate is the ion current intensity of ion detection (the intensity unit is CPS, count per second). The multi-reaction monitoring-mode MRM metabolite detection multi-peak diagram in the figure shows the substances that can be detected in the sample, and the chromatographic peaks of different colors represent different metabolites detected. The characteristic ions of each substance are screened through the triple quadrupole, and the signal strength (CPS) of the characteristic ions is obtained in the detector. The sample machine mass spectrometry file is opened with multiquant software to integrate and

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correct the chromatographic peaks. The peak area of each chromatographic peak represents the relative content of the corresponding substance. Finally, all chromatographic peak area integration data are exported and saved. See the following table for the metabolite number, integral value and corresponding metabolite name of some metabolites detected in this experiment. The results showed that the curve overlap of total ion current detected by metabolites was high, that is, the retention time and peak intensity were consistent, indicating that the signal stability was good when mass spectrometry detected the same sample at different times. The high stability of the instrument provides an important guarantee for the repeatability and reliability of the data.

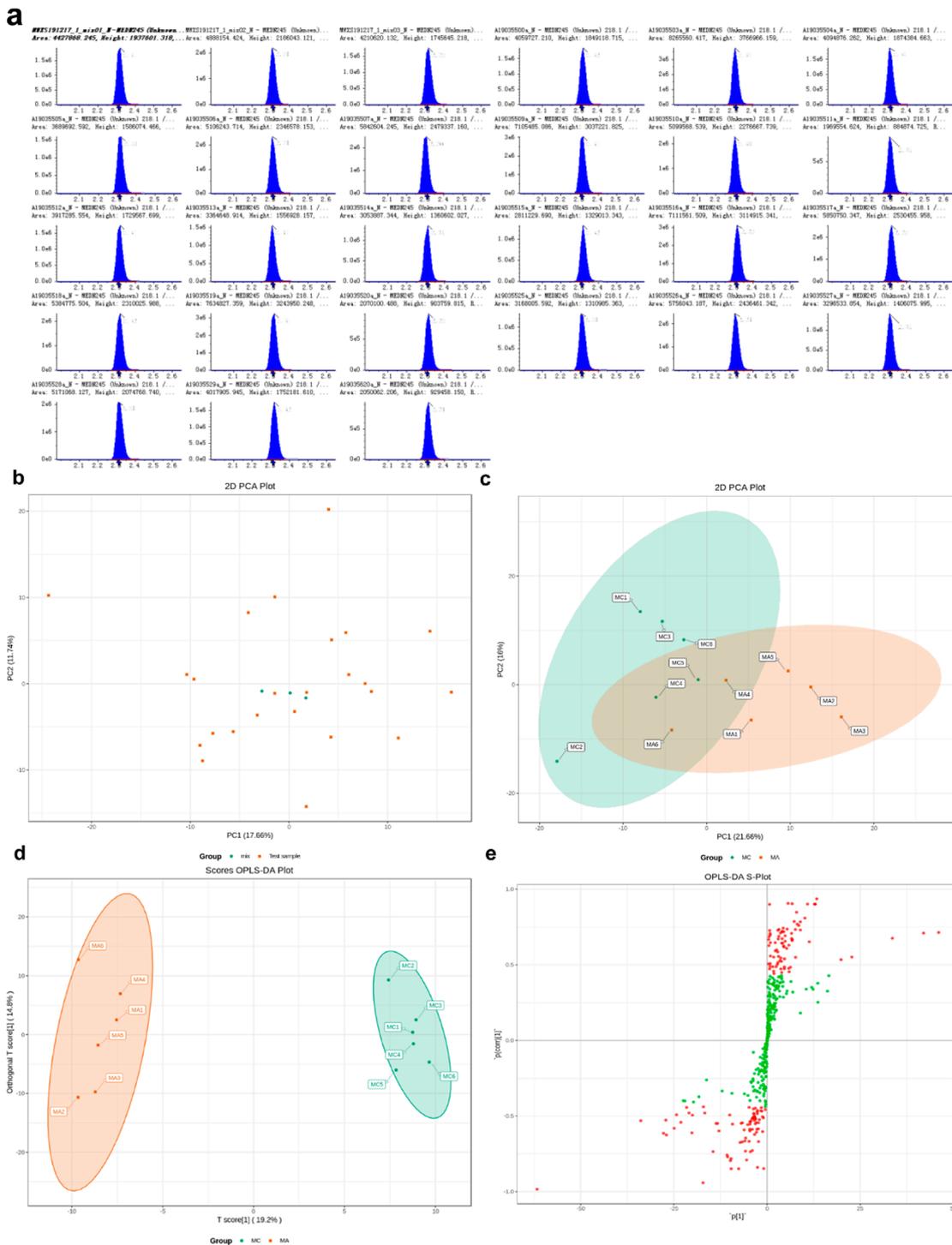


Figure S2. The integral correction chart and variability for metabolite semi-quantitative analysis. (a), integral correction chart of metabolite semi-quantitative analysis. The figure shows the semi-quantitative analysis integration correction results of randomly selected metabolites in different samples. The abscissa is the retention time (min) of metabolite detection, the ordinate is the ion current intensity (CPS) of a metabolite ion detection, and the peak area represents the relative content of the substance in the sample. (b), the overall metabolic difference between samples in each group and the variability between samples in the group. (c), the variability between different groups and among samples in the group. (d), Analytical metabolome data according to oppls-da model; we may draw a score map of each group and further show the differences between each group. The prediction parameters of the evaluation model include R2X, R2Y and Q2, wherein R2X and R2Y, respectively represent the interpretation rate of the built model to the X and Y matrices, Q2 represents the prediction ability of the model, the closer these three indicators are to 1, the more stable and reliable the model is. $Q2 > 0.5$ can be considered as an effective model, and $Q2 > 0.9$ is an excellent model. (e), the S-plot diagram of OPLS-DA; the abscissa represents the correlation coefficient between the principal component and the metabolite, and the ordinate represents the correlation coefficient between the principal component and the metabolite. The closer the metabolite is to the upper right corner and lower left corner, the more significant the difference is. The red point indicates that the VIP value of these metabolites is greater than or equal to 1, and the green point indicates that the VIP value of these metabolites is less than 1.

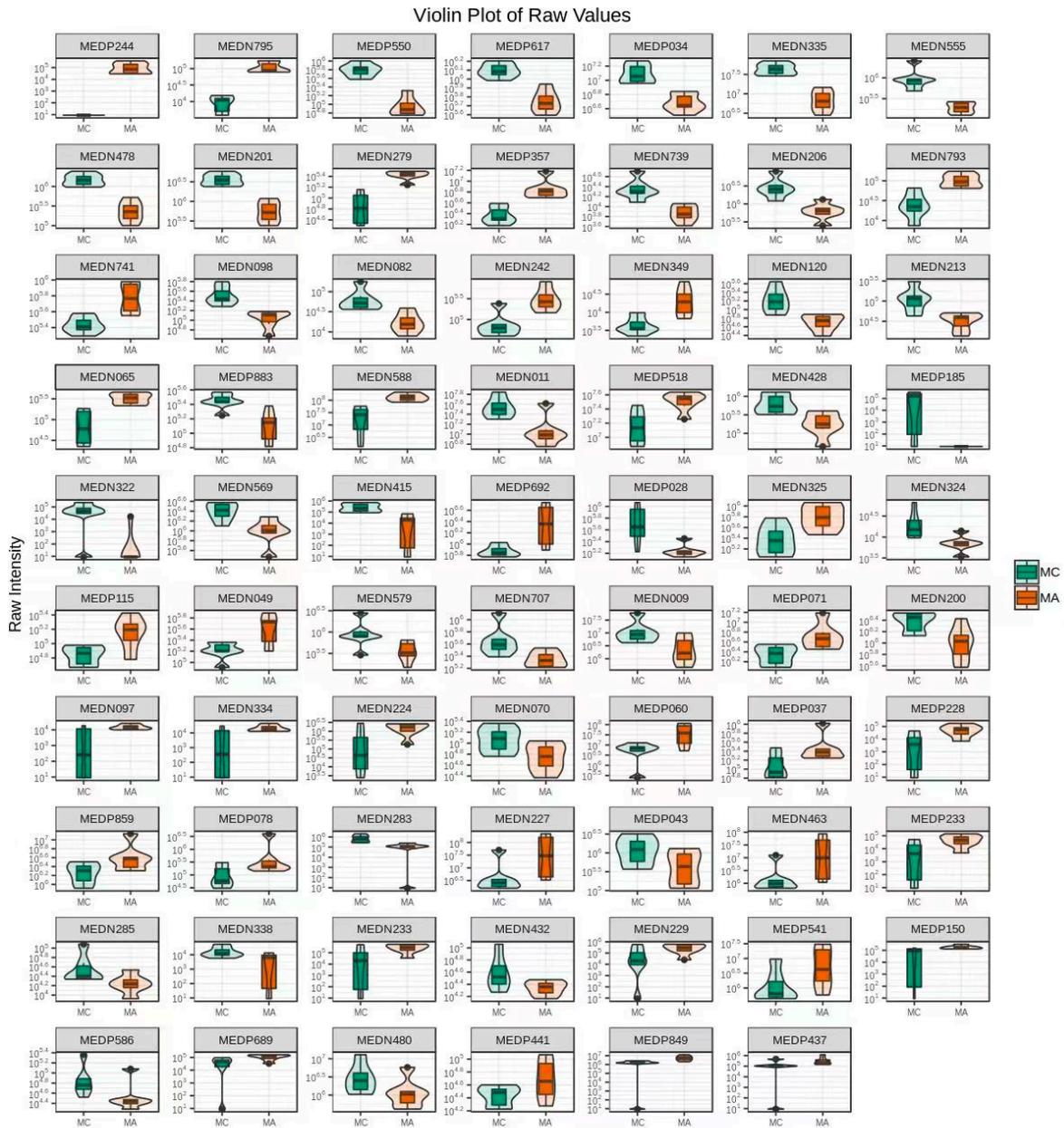
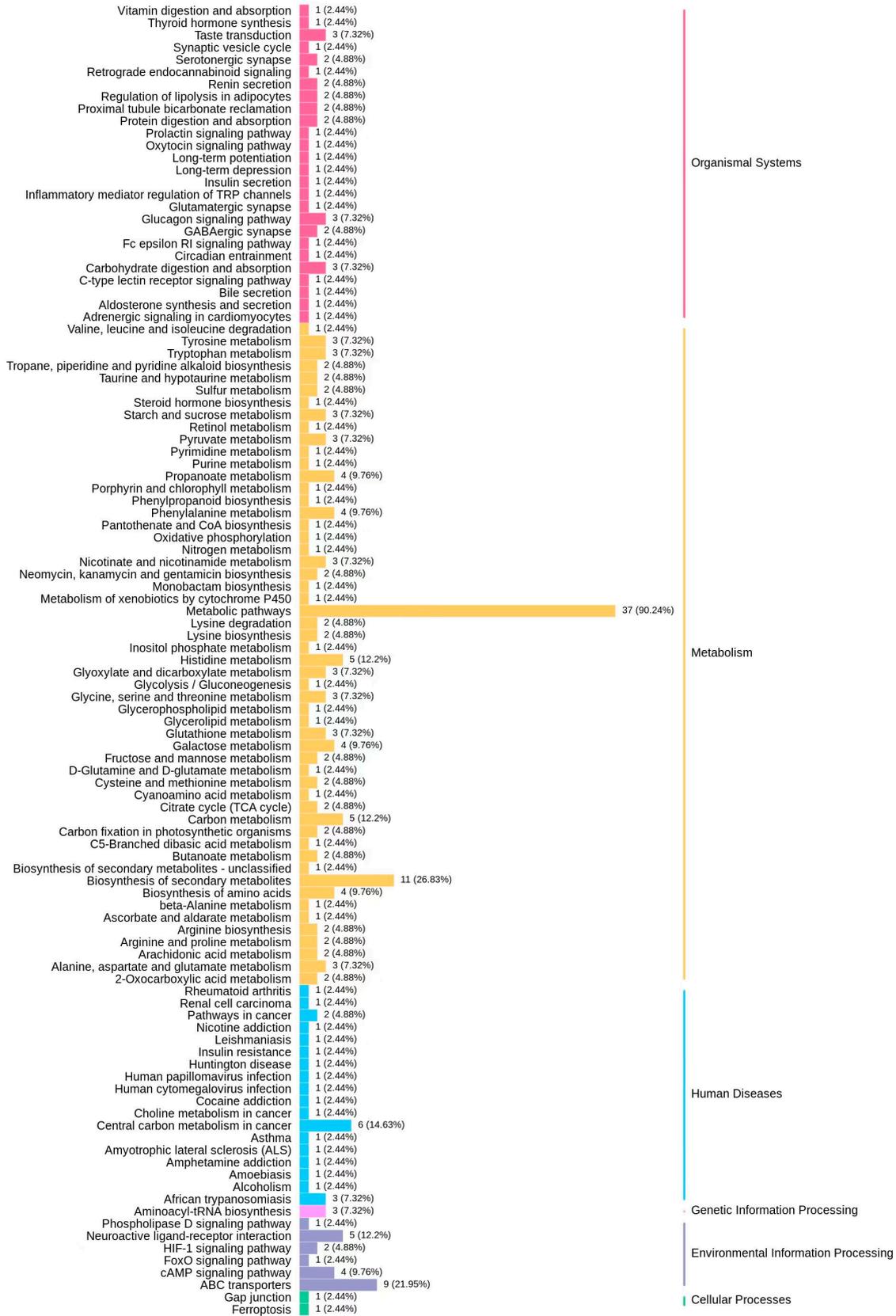


Figure S3. The data distribution and its probability density via the violin diagram. The middle box represents the quartile range, the thin line extending from it represents the 95% confidence interval, the black horizontal line in the middle is the median, and the outer shape represents the distribution density of the data.

KEGG Classification



0 6 12 20 28 36 44 52 60 68 76 84 92
Percent (%)

Figure S4. A metabolite with significant difference; the classification diagram is made according to the types of pathways in KEGG, according to the annotation results of KEGG. The ordinate is the name of the KEGG metabolic pathway, and the abscissa is the number of metabolites annotated to the pathway and its proportion to the total number of metabolites annotated.

Table S1. The numbers of the significant expression metabolites and partial calculation results of orthogonal partial least squares discriminant analysis (OPLS-DA) metabolite database mapping table.

Index	Compounds	Class	VIP/ty pe	KEG G CID	HMDB	Pubch em CID
003	MEDN Glycine	Amino acid metabolom ics	1.165	C000 37	HMDB00 123	750
005	MEDN L- Threonine	Amino acid metabolom ics	0.556	C001 88	HMDB00 167	6288
009	MEDN L- Aspartic Acid	Amino acid metabolom ics	1.489 ↓	C000 49	HMDB00 191	5960
011	MEDN L- Glutamic Acid	Amino acid metabolom ics	1.675 ↓	C000 25	HMDB00 148	33032
012	MEDN L- Histidine	Amino acid metabolom ics	0.059	C001 35	HMDB00 177	6274
013	MEDN L- Isoleucine	Amino acid metabolom ics	1.196	C004 07	HMDB00 172	6306
018	MEDN L-Serine	Amino acid metabolom ics	0.310	C000 65	HMDB00 187	5951

Index	Compounds	Class	VIP/type	KEG G CID	HMDB	Pubchem CID
		Amino				
019	MEDN L-Tryptophan	acid metabolomics	0.519	C000 78	HMDB00 929	6305
		Amino				
028	MEDN 4-Hydroxy-L-Glutamic Acid	acid metabolomics	0.329	C030 79	HMDB02 273	5460078
		Organic acid and its derivatives				
032	MEDN Allantoin	Organic acid and its derivatives	0.193	C015 51	HMDB00 462	204

Note: PLS-DA based on the results of opsls-da, the obtained multivariable analysis of the variable importance in projection (VIP) of the opsls-da model.

Table S2. The shared and specific proteins in the detected tissues of the ‘iron prawn’ *M. rosenbergii*.

Comparison group	Index	Compounds	Class	L log2FC	T type
MA vs. MB	ME	Epinephrine	Hormones	19	u
	DP185			.36	p
	ME	4-Pyridoxic Acid	Pyridine and pyridine derivatives	16	u
	DP118			.31	p
	ME	N1-Acetylspermine	Organic acid and its derivatives	6.	u
	DP576			85	p
	ME	5-Aminovaleric Acid	Organic acid and its derivatives	3.	u
	DP297			18	p
	ME	(5-L-Glutamyl)-L-Amino Acid	Amino acid metabolomics	3.	u
	DP028			16	p
	ME	Guanidineacetic Acid	Organic acid and its derivatives	2.	u
	DP313			97	p
	ME	8-Hydroxyguanosine	Nucleotide metabolomics	2.	u
	DP158			92	p
	ME	Indoleacrylic acid	Organic acid and its derivatives	2.	u
	DN551			57	p
	ME	Kinurenine	Organic acid and its derivatives	2.	u
	DN322			50	p
	ME	D-Fructose-1,6-Biphosphate-Trisodium Salt	Carbohydrate metabolomics	2.	u
	DN485			04	p
ME	Lysopc 18:0	Lipids others	-	d	
DP342		phospholipid	2.00	own	
ME	Cysteine glutathione disulfide	Amino acid metabolomics	-	d	
DN822			2.11	own	
ME	Lysopc 17:0	Lipids others	-	d	
DP434		phospholipid	2.30	own	
ME	Lysopc 20:2	Lipids others	-	d	
DP352		phospholipid	2.33	own	

	ME	Lysopc 20:1		Lipids	others	-	d
	DP350			phospholipid		2.37	own
	ME	Guanosine	3',5'-Cyclic	Nucleotide		-	d
	DN161	Monophosphate		metabolomics		2.69	own
	ME	Sn-Glycero-3-Phosphocholine		Cholines		-	d
	DP442					2.81	own
	ME	Phe-Phe		Amino	acid	-	d
	DP078			metabolomics		2.88	own
	ME	δ-Valerolactam		Pyridine	and	-	d
	DP541			pyridine derivatives		3.00	own
	ME	Ginkgoic acid		Organic	acid and	-	d
	DN636			its derivatives		3.07	own
	ME	L- tyrosine methyl ester		Amino	acid	-	d
	DP664			metabolomics		3.46	own
	ME	N-Acetyl-5-Hydroxytryptamine		Tryptamines	and	-	d
	DP212			its derivatives		3.66	own
	ME	S-Sulfo-L-Cysteine		Amino	acid	-	d
	DN071			metabolomics		11.86	own
MC	ME	All-Trans-13,14-Dihydroretinol		Coothersenzyme		13	u
vs. MA	DP244			factor and vitamin		.89	p
	ME	PGF2α [9α,11α,15S-trihydroxy-		Oxidized lipid		3.	u
	DN795	prosta-5Z,13E-dien-1-oic acid]				54	p
	ME	D-Fructose	6-Phosphate-	Carbohydrate		3.	u
	DN463	Disodium Salt		metabolomics		45	p
	ME	D-Glucose	6-Phosphate	Carbohydrate		3.	u
	DN227			metabolomics		11	p
	ME	Methionine Sulfoxide		Amino	acid	3.	u
	DP060			metabolomics		00	p
	ME	Phe-Phe		Amino	acid	2.	u
	DP078			metabolomics		79	p
	ME	D-Melezitose		Carbohydrate		2.	u
	DP228			metabolomics		51	p
	ME	PGD2 [9α,15S-dihydroxy-11-oxo-		Oxidized lipid		2.	u
	DN349	prosta-5Z,13E-dien-1-oic acid]				50	p
	ME	Glycerol 3-phosphate		Lipids_fatty acids		2.	u
	DN588					32	p
	ME	δ-Valerolactam		Pyridine	and	2.	u
	DP541			pyridine derivatives		31	p
	ME	Raffinose		Carbohydrate		2.	u
	DP233			metabolomics		01	p
	ME	L-Aspartic Acid		Amino	acid	-	d
	DN009			metabolomics		2.23	own
	ME	Citramalic Acid		Amino	acid	-	d
	DN206			metabolomics		2.26	own
	ME	Hydroxyphenyllactic acid		Carbohydrate		-	d
	DN555			metabolomics		2.43	own
	ME	Aminomalonic Acid		Organic	acid and	-	d
	DN478			its derivatives		2.56	own
	ME	Methylmalonic Acid		Organic	acid and	-	d
	DN335			its derivatives		2.59	own
	ME	Succinic Acid		Amino	acid	-	d
	DN201			metabolomics		2.59	own
	ME	18-Hydroxycorticosterone		Lipids		-	d
	DP550					2.72	own

	ME	2-Hydroxybutanoic Acid	Organic acid and	-	d
	DN283		its derivatives	2.85	own
	ME	2-(Formylamino)Benzoic Acid	Benzene and	-	d
	DN415		substituted derivatives	3.85	own
	ME	Kinurenine	Organic acid and	-	d
	DN322		its derivatives	4.70	own
	ME	Epinephrine	Hormones	-	d
	DP185			14.08	own
MC	ME	All-Trans-13,14-Dihydroretinol	Coothersenzyme	11	u
vs. MD	DP244		factor and vitamin	.58	p
	ME	Raffinose	Carbohydrate	2.	u
	DP233		metabolomics	51	p
	ME	D-Trehalose	Carbohydrate	2.	u
	DN224		metabolomics	50	p
	ME	Lactose	Carbohydrate	2.	u
	DN229		metabolomics	41	p
	ME	(5-L-Glutamyl)-L-Amino Acid	Amino acid	2.	u
	DP028		metabolomics	34	p
	ME	2-Hydroxybutanoic Acid	Organic acid and	-	d
	DN283		its derivatives	2.04	own
	ME	Menaquinone	Coothersenzyme	-	d
	DP391		factor and vitamin	2.36	own
	ME	Allysine	Organic acid and	-	d
	DN487		its derivatives	2.61	own
	ME	Hyodeoxycholic Acid	Bile acids	-	d
	DN109			2.80	own
	ME	Methyl jasmonate	Lipids_fatty acids	-	d
	DP630			2.96	own
	ME	D-Xylose	Carbohydrate	-	d
	DN225		metabolomics	3.31	own
	ME	N1-Acetylspermine	Organic acid and	-	d
	DP576		its derivatives	10.21	own
MD	ME	N1-Acetylspermine	Organic acid and	15	u
vs. MB	DP576		its derivatives	.79	p
	ME	Indoleacrylic acid	Organic acid and	5.	u
	DN551		its derivatives	67	p
	ME	Guanidineacetic Acid	Organic acid and	4.	u
	DP313		its derivatives	01	p
	ME	5-Aminovaleric Acid	Organic acid and	3.	u
	DP297		its derivatives	89	p
	ME	Isoxanthopterin	Pteridines and	3.	u
	DP384		derivatives	39	p
	ME	D-Xylose	Carbohydrate	3.	u
	DN225		metabolomics	27	p
	ME	TranexamicAcid	Organic acid and	3.	u
	DP689		its derivatives	06	p
	ME	4-Guanidinobutyric Acid	Organic acid and	3.	u
	DP296		its derivatives	03	p
	ME	Hyodeoxycholic Acid	Bile acids	2.	u
	DN109			97	p
	ME	Methyl jasmonate	Lipids_fatty acids	2.	u
	DP630			90	p
	ME	Acetylcholine Chloride	Cholines	2.	u
	DP123			78	p

ME	8-Hydroxyguanosine	Nucleotide	2.	u
DP158		metabolomics	63	p
ME	Dl-Glyceraldehyde3-Phosphate	Organic acid and	2.	u
DN480		its derivatives	60	p
ME	Urocanic Acid	Organic acid and	2.	u
DP357		its derivatives	41	p
ME	Creatine	Organic acid and	2.	u
DN305		its derivatives	30	p
ME	2-Methylbutyroylcarnitine	Lipids_fatty acids	2.	u
DP618			19	p
ME	D-Glucose 6-Phosphate	Carbohydrate	2.	u
DN227		metabolomics	13	p
ME	D-Fructose 6-Phosphate-	Carbohydrate	2.	u
DN463	Disodium Salt	metabolomics	02	p
ME	Lysope 18:1	Lipids others	-	d
DN362		phospholipid	2.07	own
ME	Cysteine glutathione disulfide	Amino acid	-	d
DN822		metabolomics	2.09	own
ME	Lysope 14:0	Lipids others	-	d
DN368		phospholipid	2.16	own
ME	Lysopc 18:3	Lipids others	-	d
DP498		phospholipid	2.16	own
ME	Lysopa 16:0	Lipids others	-	d
DN372		phospholipid	2.23	own
ME	Methylmalonic Acid	Organic acid and	-	d
DN335		its derivatives	2.27	own
ME	Lysopc 18:2	Lipids others	-	d
DP346		phospholipid	2.28	own
ME	Lysopc 16:0	Lipids others	-	d
DP338		phospholipid	2.29	own
ME	Succinic Acid	Amino acid	-	d
DN201		metabolomics	2.30	own
ME	Riboflavin	Coothersenzym	-	d
DP250		factor and vitamin	2.31	own
ME	Aminomalonic Acid	Organic acid and	-	d
DN478		its derivatives	2.31	own
ME	Lysopc 17:0	Lipids others	-	d
DP434		phospholipid	2.33	own
ME	Lysope 16:0	Lipids others	-	d
DN366		phospholipid	2.36	own
ME	L-Aspartic Acid	Amino acid	-	d
DN009		metabolomics	2.37	own
ME	Kinurenine	Organic acid and	-	d
DN322		its derivatives	2.49	own
ME	LysoPE(16:1(9Z)-0:0)	Lipids_fatty acids	-	d
DP638			2.51	own
ME	Lysopc 15:0	Lipids others	-	d
DP494		phospholipid	2.57	own
ME	Ginkgoic acid	Organic acid and	-	d
DN636		its derivatives	2.62	own
ME	Lysopc 18:1	Lipids others	-	d
DP344		phospholipid	2.63	own
ME	PAF C-16	Lipids	-	d
DP537			2.75	own

ME	Lysopc 18:0	Lipids	others	-	d
DP342		phospholipid		2.78	own
ME	Lysopc 14:0	Lipids	others	-	d
DP336		phospholipid		3.00	own
ME	18-Hydroxycorticosterone	Lipids		-	d
DP550				3.01	own
ME	Lysopc 20:2	Lipids	others	-	d
DP352		phospholipid		3.14	own
ME	Lysopc 16:1	Lipids	others	-	d
DP340		phospholipid		3.16	own
ME	N-Acetyl-5-Hydroxytryptamine	Tryptamines	and	-	d
DP212		its derivatives		3.40	own
ME	N6-Succinyl Adenosine	Nucleotide		-	d
DN428		metabolomics		4.07	own
ME	Lysopc 20:1	Lipids	others	-	d
DP350		phospholipid		4.44	own
ME	S-Sulfo-L-Cysteine	Amino	acid	-	d
DN071		metabolomics		12.61	own