

Appendix 1 to Wang H, Liang S, Wang M, et al. Potential serum biomarkers from a metabolomics study of autism.

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Table S1. Precision and repeatability of experimental method in discovery and validation study

Cohort; mode, m/z	Precision				Repeatability			
	RT, min		Intensity		RT, min		Intensity	
	Mean	RSD(%)	Mean	RSD(%)	Mean	RSD (%)	Mean	RSD (%)
Discovery								
Positive	<i>n</i> = 6				<i>n</i> = 14			
80.95	0.64	0.23	15.52	5.31	0.66	0.12	18.1	6.06
205.10	2.32	0.18	28.3	3.77	2.3	0.22	28.6	4.47
318.30	5.32	0.08	27.66	4.03	5.3	0.05	28.75	4.59
522.34	7.47	0.07	309.13	3.17	7.46	0.11	304.49	2.83
524.35	8.38	0.13	526.52	7.04	8.38	0.14	550.35	4.83
395.22	10.04	0.09	15.76	7.37	10.04	0.10	14.26	5.24
Negative	<i>n</i> = 6				<i>n</i> = 14			
72.08	0.84	0.02	30.68	2.50	0.84	0.05	31.7	1.46
246.25	4.43	0.12	4.61	6.82	4.42	0.16	5.04	5.46
200.20	6.66	0.05	21.26	7.11	6.65	0.04	22.69	3.1
502.38	7.97	0.06	10.54	2.26	7.95	0.15	9.92	2.47
782.56	9.37	0.04	122.24	5.89	9.37	0.07	119.62	3.56
281.25	10.15	0.03	11.42	4.08	10.15	0.06	11.75	4.89
Validation								
Positive	<i>n</i> = 6				<i>n</i> = 20			
167.02	0.83	0	99.61	5.36	0.83	0.01	95.66	5.36
187.01	2.84	0.06	80.14	6.87	2.85	0.1	85.43	5.87
540.32	4.94	0.11	290.03	6.32	4.89	0.11	287.03	6.32
506.31	5.19	0.02	376.69	3.74	5.23	0.02	365.75	3.74
277.22	7.16	0.04	63.85	3.74	7.14	0.04	60.59	3.74
279.23	8.81	0.15	653.34	4.65	8.83	0.16	647.37	7.65
Negative	<i>n</i> = 6				<i>n</i> = 20			
172.99	2.46	0.05	9.64	4.26	2.44	0.06	10.01	4.26
179.07	3.67	0.12	38.25	4.23	3.66	0	40.83	3.23
478.29	5.15	0.15	19.97	7.46	5.17	0.13	20.84	3.46
508.34	6.06	0.11	58.34	4.51	6.1	0.01	55.79	7.51
227.20	7.65	0.02	12.26	4.97	7.62	0.03	15.84	4.97
255.23	10.02	0.17	287.93	2.82	10.04	0.15	292.93	2.82

RSD = relative standard deviation.

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Table S2. Descriptive statistics of relative contents for 17 differential metabolites between children with autism and healthy controls in both the discovery and validation cohorts (male and female)*

Metabolites	Discovery cohort									Validation cohort								
	Autism (n = 73)				Control(n = 63)				p value	Autism (n = 100)				Control(n = 100)				p value
	Mean	Min	Max	Med.	Mean	Min	Max	Med		Mean	Min	Max	Med.	Mean	Min	Max	Med	
Positive																		
L-acetylcarnitine	18.69	7.68	35.19	17.71	33.31	12.66	66.43	30.90	< 0.001	15.25	2.90	31.24	15.24	16.95	5.99	33.32	15.58	0.11
Decanoylcarnitine	13.51	2.92	39.53	12.30	22.77	5.22	43.77	21.78	< 0.001	15.31	2.98	49.42	12.96	16.81	2.89	46.40	15.98	0.018
Phytosphingosine	106.87	50.53	157.81	106.39	12.66	5.05	39.24	9.28	< 0.001	13.80	7.32	29.34	13.03	14.83	7.18	31.59	14.24	0.023
Pregnanetriol	21.90	6.93	39.53	22.64	15.97	7.28	28.85	15.27	< 0.001	21.24	6.26	58.35	19.46	17.50	3.13	44.51	15.84	0.025
LysoPC(18:3(6Z,9Z,12Z))	28.62	10.85	68.98	24.78	21.55	8.73	42.24	19.64	< 0.001	14.78	3.59	37.20	12.84	13.26	4.51	37.72	12.48	0.15
LysoPC(20:3(5Z,8Z,11Z))	108.55	49.08	202.58	101.13	95.75	35.54	189.71	88.66	0.042	59.68	27.08	129.61	55.36	56.60	15.41	105.77	54.79	0.66
Negative																		
Uric acid	56.35	13.30	97.78	55.30	76.33	47.36	113.40	73.22	< 0.001	110.32	34.75	178.99	112.27	125.90	59.70	199.18	127.57	< 0.001
9,10-Epoxyoctadecenoic acid	7.50	1.99	64.89	4.95	5.50	2.13	7.71	4.65	0.004	21.90	1.45	105.78	12.00	8.51	1.32	30.99	6.99	< 0.001
Arachidonic acid	57.17	16.78	168.0	53.94	86.23	38.68	139.70	82.62	< 0.001	64.51	4.54	173.51	62.77	67.09	13.02	177.96	63.52	0.68
Docosahexaenoic acid	17.61	5.40	41.90	16.79	47.77	19.35	81.20	47.08	< 0.001	17.77	3.92	48.19	15.45	26.48	4.86	72.51	25.61	< 0.001
Docosapentaenoic acid	6.18	0.60	29.06	5.89	14.90	5.91	26.46	14.59	< 0.001	9.66	1.17	33.19	8.29	14.14	0.65	43.89	14.25	< 0.001
Adrenic acid	3.91	0.61	9.83	4.06	10.16	3.43	19.27	9.56	< 0.001	16.13	1.33	42.72	14.85	21.77	6.07	50.24	21.77	< 0.001
Sphingosine 1-phosphate	62.23	18.37	98.15	62.36	30.56	13.02	66.53	28.95	< 0.001	49.75	20.84	119.11	49.51	28.25	9.42	64.73	26.20	< 0.001
LPA(18:2(9Z,12Z)/0:0)	28.47	4.29	67.62	26.36	12.08	4.03	26.03	11.55	< 0.001	4.78	1.38	6.41	5.30	2.49	0.43	6.15	2.02	< 0.001
LysoPE(0:0/16:0)	53.09	28.65	93.09	52.30	36.14	14.00	57.17	35.33	< 0.001	44.35	20.62	86.36	44.30	35.20	4.86	61.39	34.94	< 0.001
LysoPE(18:0/0:0)	44.88	11.99	93.84	43.64	27.29	11.63	50.14	26.65	< 0.001	34.43	17.55	64.62	32.05	27.36	12.96	56.43	26.23	< 0.001
LysoPE(0:0/20:2(11Z,14Z))	267.17	163.92	352.57	266.44	234.12	122.02	325.56	236.96	< 0.001	583.09	329.62	992.92	536.78	595.00	401.98	1020.66	571.87	0.18

*L-acetylcarnitine and decanoylcarnitine were not normally distributed in either the discovery or the validation study; we performed a nonparametric Mann-Whitney *U* test. The others 15 metabolites were assessed using a *t* test and a nonparametric Mann-Whitney *U* test; those results were similar, so we provide only the nonparametric test results.

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Table S3: Descriptive statistics of relative contents for 17 typical differential metabolites between children with autism and healthy controls in both the discovery and validation cohorts (male)

Metabolites	Discovery cohort									Validation cohort								
	Autism (n = 59)				Control (n = 51)				p value	Autism (n = 86)				Control (n = 81)				p value
	Mean	Min	Max	Med.	Mean	Min	Max	Med		Mean	Min	Max	Med.	Mean	Min	Max	Med	
Positive																		
L-acetylcarnitine	18.75	7.68	35.19	17.39	30.02	12.66	61.81	29.00	< 0.001	15.15	2.90	31.24	15.29	17.66	5.99	33.32	16.47	0.026
Decanoylcarnitine	14.02	2.92	39.53	12.39	22.90	8.02	43.77	21.50	< 0.001	15.55	2.98	49.42	13.00	17.26	2.89	46.40	17.09	0.021
Phytosphingosine	105.18	50.53	157.80	105.73	12.36	5.05	31.31	9.79	< 0.001	13.83	7.32	29.34	13.21	14.28	7.18	31.59	13.97	0.32
Pregnanetriol	21.58	6.93	39.53	22.59	15.58	7.28	26.45	14.52	< 0.001	21.66	6.26	58.35	19.47	17.64	5.26	44.51	15.50	0.027
LysoPC(18:3(6Z,9Z,12Z))	27.52	10.85	68.51	23.72	22.21	11.55	42.24	19.96	0.040	14.71	3.59	37.20	12.67	13.36	4.51	37.72	12.64	0.29
LysoPC(20:3(5Z,8Z,11Z))	108.80	49.08	202.58	105.19	100.00	55.22	165.13	93.24	0.07	59.60	27.08	129.61	55.92	56.21	15.41	105.77	55.00	0.62
Negative																		
Uric acid	55.66	13.30	97.78	54.17	74.33	47.36	111.51	72.69	< 0.001	110.13	34.75	178.99	112.44	126.53	69.38	199.18	125.94	0.003
9,10-Epoxyoctadecenoic acid	7.74	1.99	64.89	4.95	5.36	2.13	7.00	4.58	0.035	24.36	1.44	105.78	15.72	8.09	1.33	30.99	6.97	< 0.001
Arachidonic acid	56.64	16.78	168.02	53.93	79.02	38.68	123.69	80.24	< 0.001	65.60	14.56	173.51	62.50	64.25	13.02	147.00	58.82	0.82
Docosahexaenoic acid	17.46	6.36	41.90	16.24	44.48	19.35	81.20	41.12	< 0.001	17.69	3.92	48.19	15.38	25.82	4.86	57.97	24.86	< 0.001
Docosapentaenoic acid	6.14	0.60	29.05	5.84	15.52	5.91	26.46	15.01	< 0.001	9.79	1.17	33.19	8.52	14.07	1.89	43.89	13.79	< 0.001
Adrenic acid	3.99	0.61	9.83	4.08	10.25	3.43	19.27	9.59	< 0.001	16.28	1.33	42.72	14.83	21.30	6.07	50.24	19.78	0.0026
Sphingosine 1-phosphate	60.92	18.37	98.15	60.74	30.79	16.57	66.53	28.38	< 0.001	49.76	21.95	119.11	49.47	26.55	9.42	60.07	24.57	< 0.001
LPA(18:2(9Z,12Z)/0:0)	28.17	4.29	67.62	25.98	12.56	4.03	14.87	12.19	< 0.001	4.71	1.38	6.25	5.21	2.35	0.44	5.66	1.95	< 0.001
LysoPE(0:0/16:0)	52.47	28.65	81.69	52.09	35.61	14.00	54.47	35.40	< 0.001	44.56	20.62	86.36	44.15	34.42	4.86	61.39	34.51	< 0.001
LysoPE(18:0/0:0)	45.50	11.99	93.84	45.09	26.76	11.63	50.14	27.17	< 0.001	34.09	17.55	64.27	31.67	27.27	12.96	56.43	26.08	< 0.001
LysoPE(0:0/20:2(11Z,14Z))	268.64	163.92	352.57	267.07	237.41	122.02	325.56	239.50	< 0.001	579.52	329.62	992.92	537.73	590.62	401.99	1020.66	573.92	0.26

*L-acetylcarnitine and decanoylcarnitine were not normally distributed in either the discovery or the validation study; we performed a nonparametric Mann-Whitney *U* test. The others 15 metabolites were assessed using a *t* test and a nonparametric Mann-Whitney *U* test; those results were similar, so we provide only the nonparametric test results.

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Table S4: Receiver operator characteristic analysis of validation stage using a discovery model

Sample	AUC (95%CI)	Best cutoff (P of logistic)	Sensitivity, %	Specificity, %	Maximum of Youden index*
Overall	0.898 (0.856–0.940)	0.3515	90.0	74.0	0.640
Male-only	0.915 (0.874–0.957)	0.327	82.6	86.4	0.690

AUC = area under the curve.
*Sensitivity + specificity - 1.

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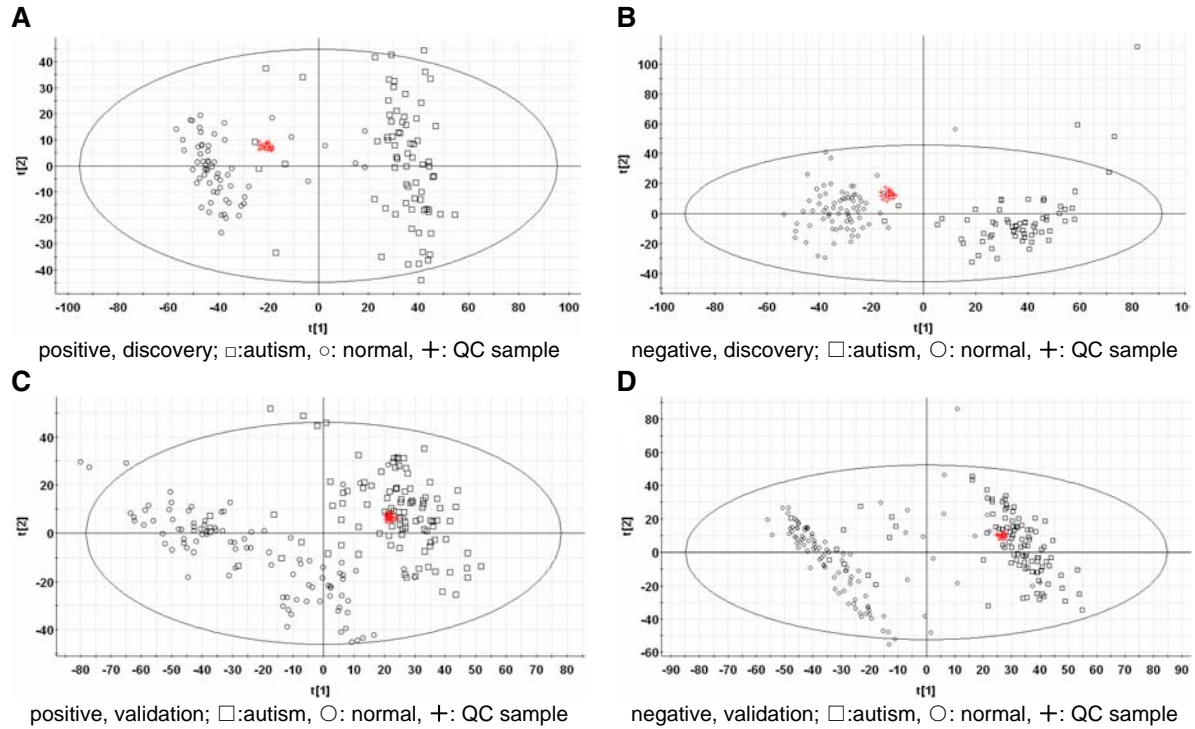


Fig. S1. Two-dimensional PCA scores plots of autism, control and quality control (QC) samples in the discovery and validation stages. $t[1]$ = first principal component; $t[2]$ = second principal component. The QC samples were tightly clustered in the PCA score plot and showing minimal analytical variation.

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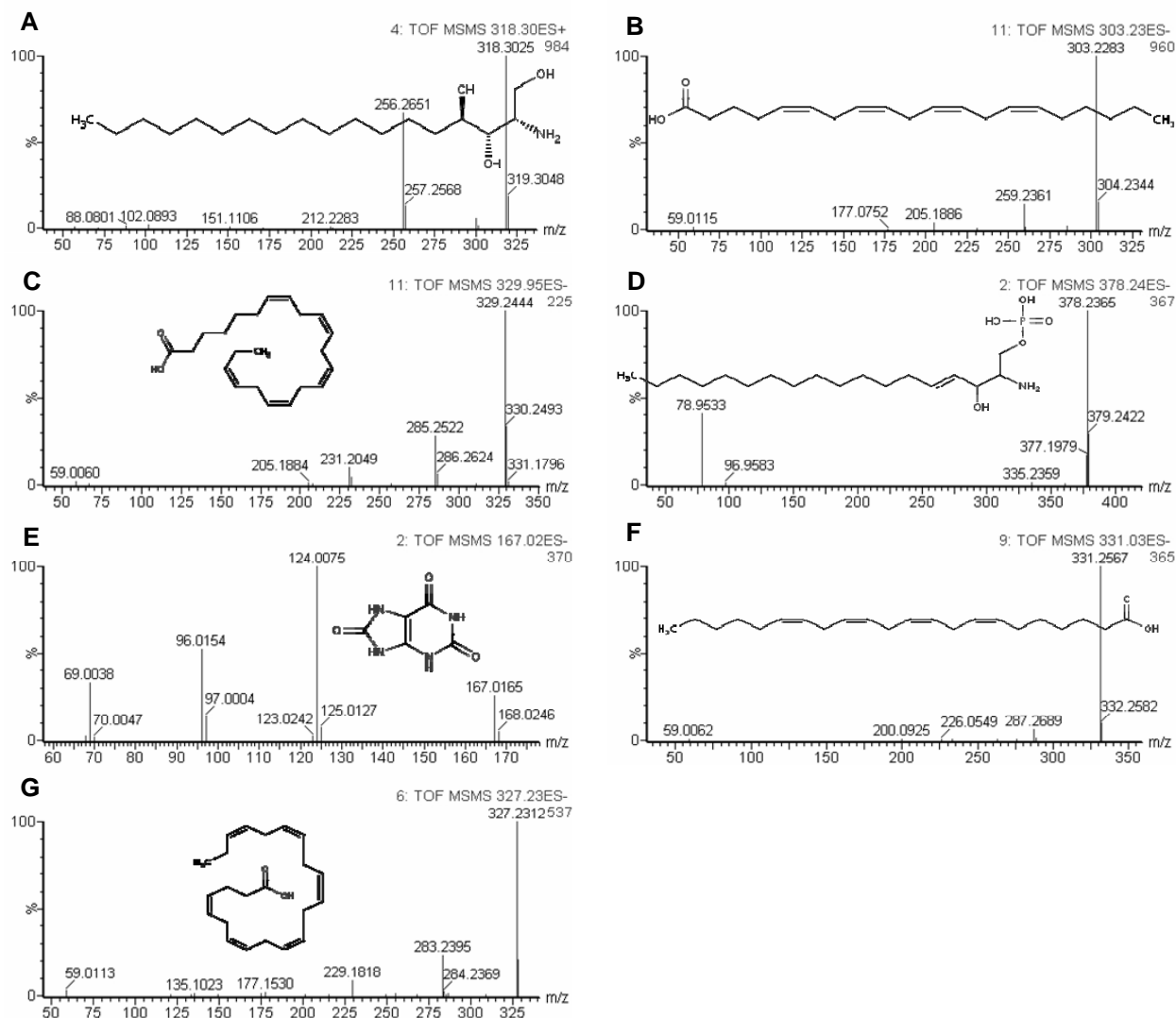


Fig. S2. Chemical structure and mass fragment information of 7 principal metabolites identified by available library databases and reference compounds: (A) phytosphingosine, (B) arachidonic acid, (C) docosapentaenoic acid, (D) sphingosine 1-phosphate, (E) uric acid, (F) adrenic acid and (G) docosahexaenoic acid.

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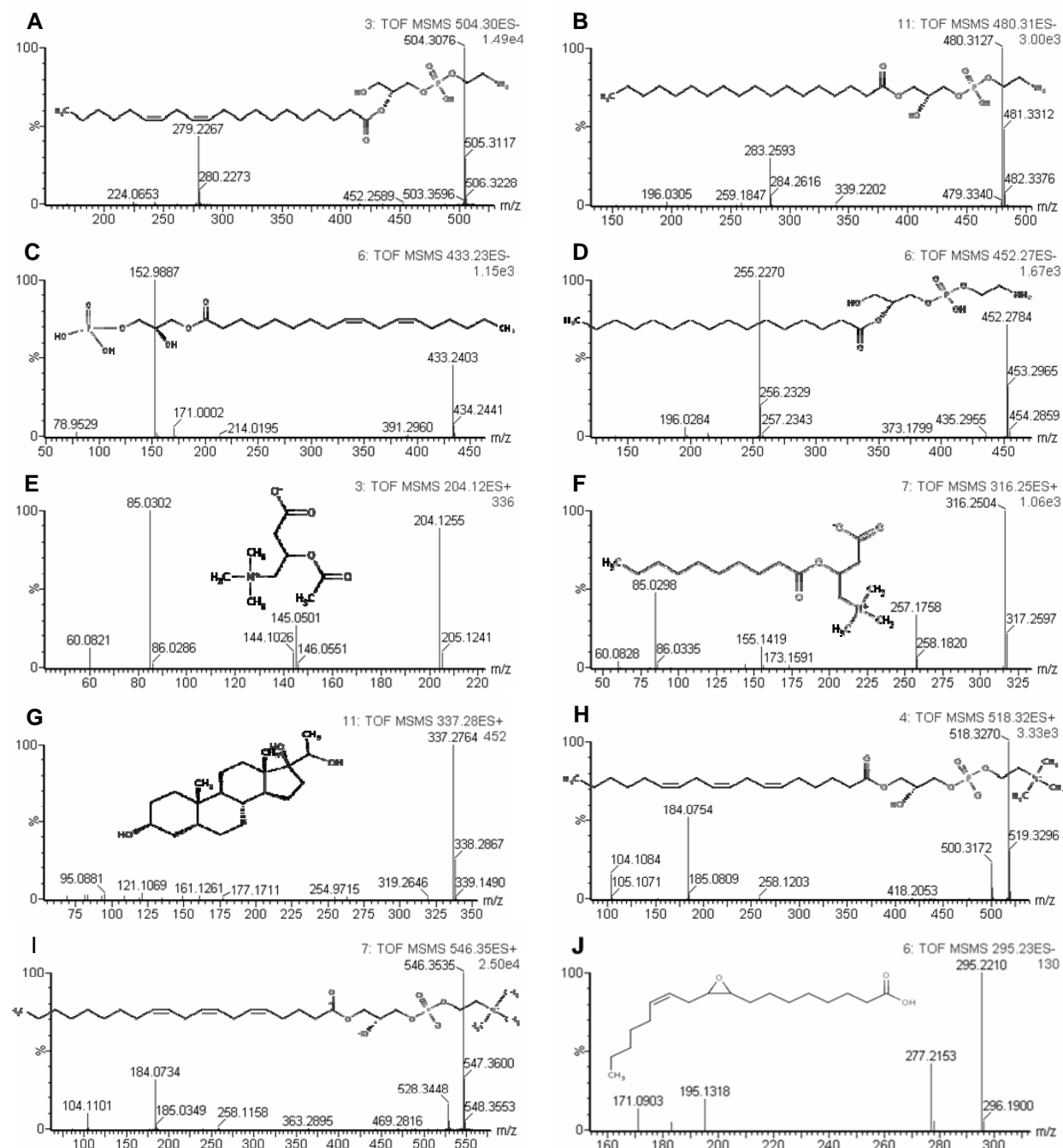


Fig. S3. Chemical structure and mass fragment information of 10 principal metabolites, those were supported by available library databases: (A) LysoPE(0:0/20:2(11Z,14Z)), (B) LysoPE(18:0/0:0), (C) LPA(18:2(9Z,12Z)/0:0), (D) LysoPE(0:0/16:0), (E) L-acetylcarnitine, (F) decanoylcarnitine, (G) pregnanetriol, (H) LysoPC(18:3(6Z,9Z,12Z)), (I) LysoPC(20:3(5Z,8Z,11Z)) and (J) 9,10-epoxyoctadecenoic acid.

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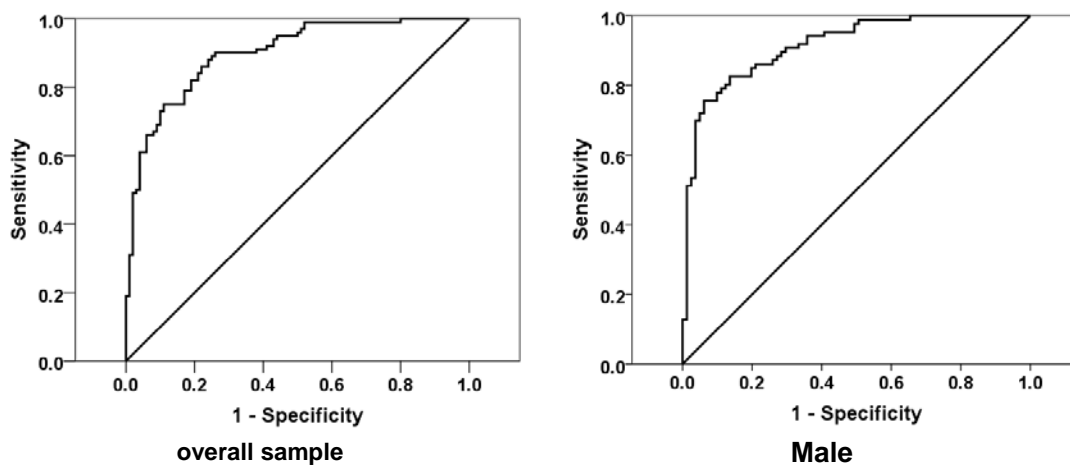


Fig. S4: Receiver operating characteristic curves of the validation stage using the discovery model. The variables of the discovery model in the overall sample were sex, age, docosahexaenoic acid and sphingosine 1-phosphate. The variables in the male-only sample were age, docosahexaenoic acid and sphingosine 1-phosphate.