

Metabolic Profiling Regarding Pathogenesis of Idiopathic Pulmonary Fibrosis

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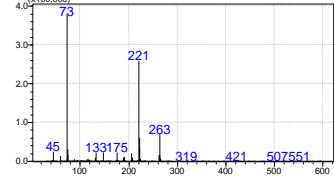
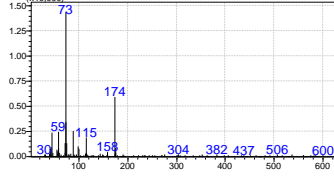
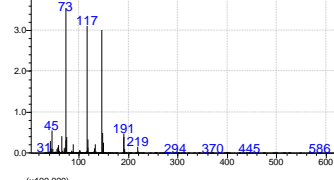
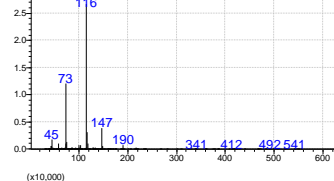
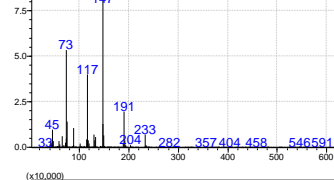
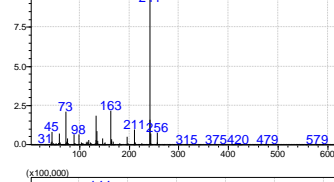
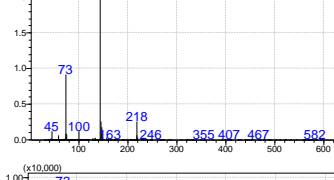
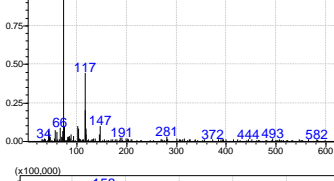
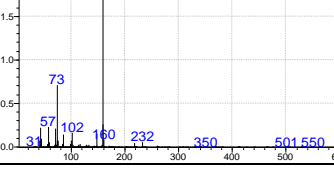
Table S-1. EI-MS spectrum of 61 authentic standards of metabolites

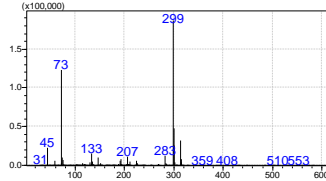
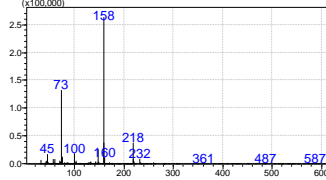
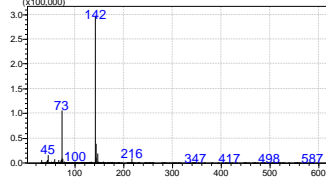
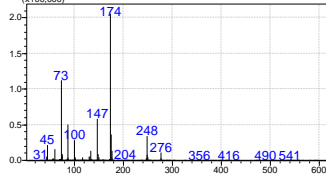
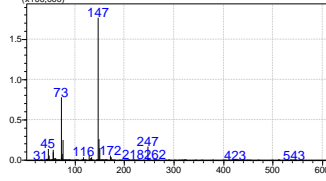
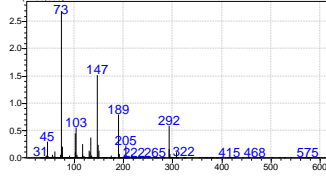
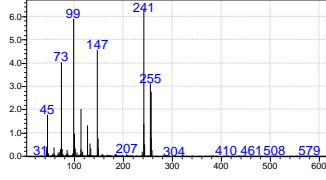
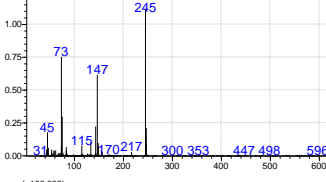
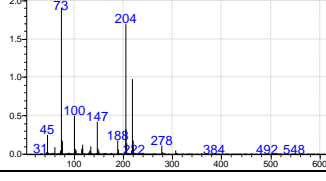
Table S-2. Statistical analyses of metabolites in control and IPF

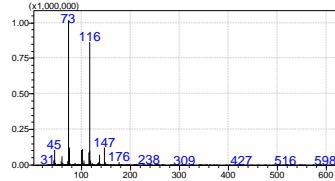
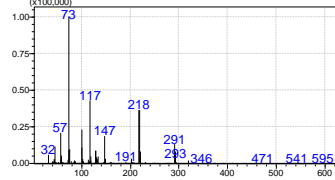
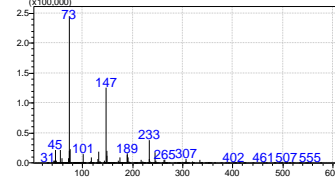
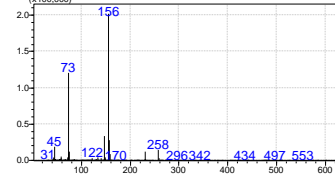
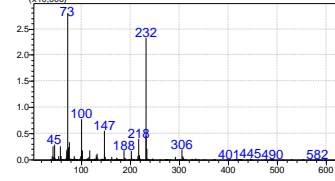
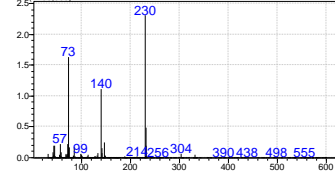
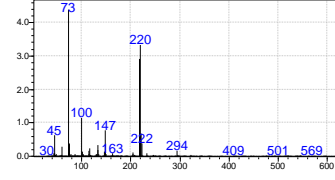
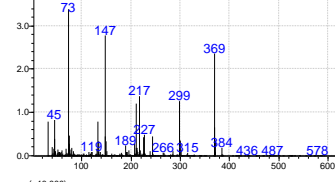
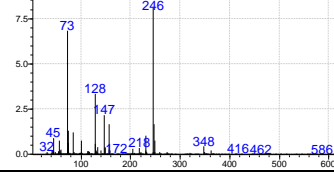
Figure S-1. Workflow of study

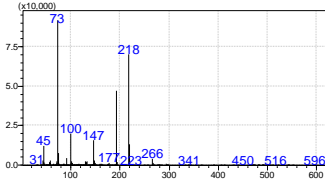
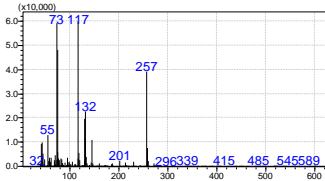
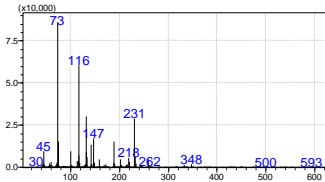
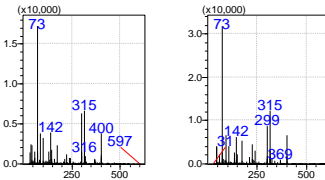
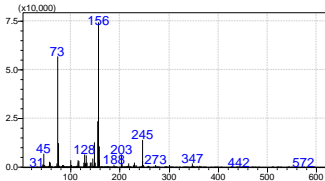
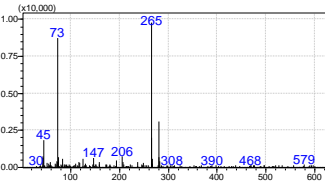
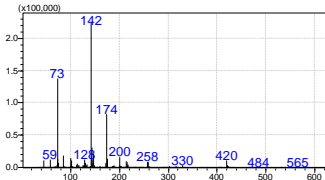
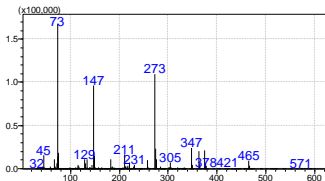
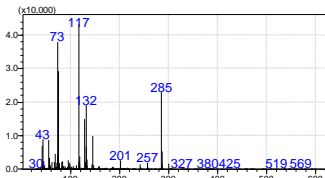
Figure S-2. ROC curve of metabolite signatures.

Table S-1. EI-MS spectrum of 61 authentic standards of metabolites

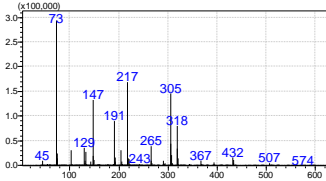
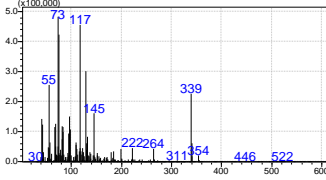
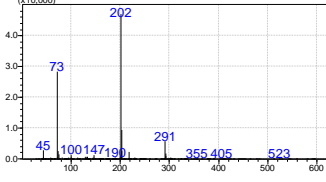
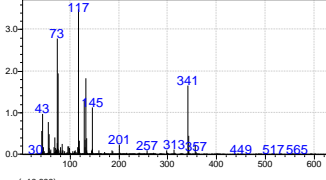
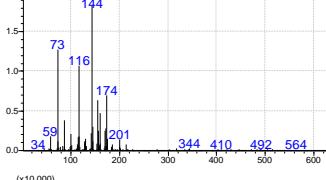
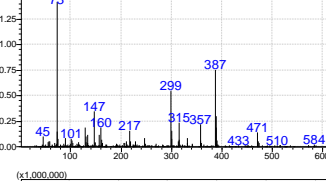
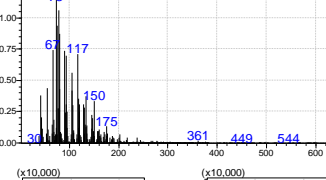
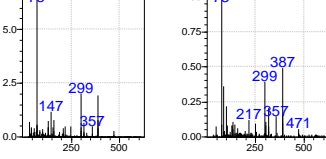
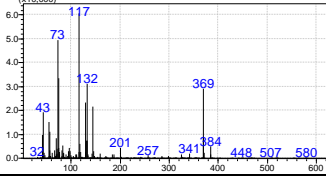
RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
5.1	Boric acid	3TMS	
6.3	Pyruvic acid	OXIME-1TMS	
6.6	Lactic acid	2TMS	
7.6	Alanine	2TMS	
9.0	3-Hydroxybutyric acid	2TMS	
9.5	mono-methyl phosphate	2TMS	
10.5	Valine	2TMS	
11.2	Diethylene glycol	2TMS	
12.0	Leucine	2TMS	

RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
12.1	Phosphoric acid	3TMS	
12.7	Isoleucine	2TMS	
12.6	Proline	2TMS	
12.9	Glycine	3TMS	
13.0	Succinic acid	2TMS	
13.6	Glyceric acid	3TMS	
13.7	Uracil	2TMS	
13.8	Fumaric acid	2TMS	
14.4	Serine	3TMS	

RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
14.7	Hypotaurine	2TMS	
15.1	Threonine	3TMS	
17.6	Malic acid	3TMS	
18.2	Pyroglutamic acid	2TMS	
18.4	Aspartic acid	3TMS	
18.5	4-Hydroxyproline	3TMS	
19.2	Cysteine	3TMS	
20.3	Phosphoenol-pyruvic acid	3TMS	
20.7	Glutamic acid	3TMS	

RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
20.7	Phenylalanine	2TMS	
21.1	Dodecanoic acid	1TMS	
21.8	Asparagine	3TMS	
23.6, 23.9	Dihydroxyacetone-phosphate	OXIME-3TMS	
24.0	Glutamine	3TMS	
24.6	Hypoxanthine	2TMS	
25.1	Ornithine	4TMS	
25.2	Citric acid	4TMS	
25.3	Myristic acid	1TMS	

RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
25.9	Anhydrosorbitol	3TMS	
27.1, 27.4	Glucose	OXIME-6TMS	
27.1	Lysine	4TMS	
27.3	Pentadecanoic acid	1TMS	
27.6	1-Hexadecanol	1TMS	
28.8	Palmitoleic acid	1TMS	
29.1	Gluconic acid	5TMS	
29.2	Hexadecanoic acid	1TMS	
29.6	scyllo-Inositol	6TMS	

RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
30.7	myo-Inositol	6TMS	
32.3	Oleic acid	1TMS	
32.6	Tryptophan	3TMS	
32.7	Stearic acid	1TMS	
33.3	Spermidine	3TMS	
34.7	Mannose-6-phosphate	OXIME-6TMS	
34.8	Arachidonic acid	1TMS	
34.9, 35.2	Glucose-6-phosphate	OXIME-6TMS	
36.0	Arachidic acid	1TMS	

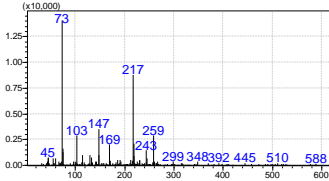
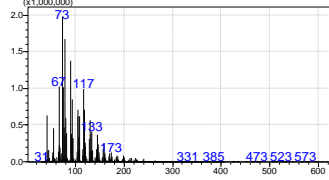
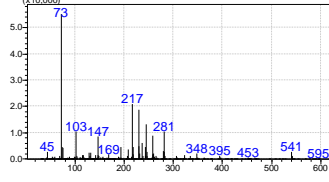
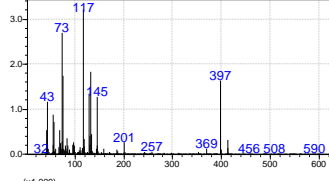
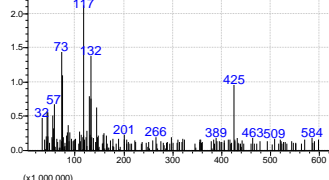
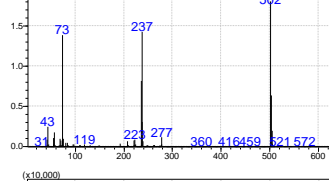
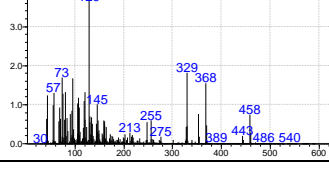
RT (min)	Metabolites	Derivatization	EI-MS spectrum of authentic standard
36.4	Uridine	3TMS	
37.8	Docosahexaenoic acid	1TMS	
38.4	Inosine	4TMS	
39.0	Behenic acid	1TMS	
41.8	Lignoceric acid	1TMS	
46.0	D-alpha-Tocopherol	1TMS	
46.2	Cholesterol	1TMS	

Table S-2. Statistical analyses of metabolites in control and IPF

Metabolites	RSD of QC (%)	Internal standard	Control		IPF		P value	FDR	VIP
			Mean	Stdev	Mean	Stdev			
Cysteine	5.1	Tryptophan d ₅	1.0	0.3	2.3	0.8	3.7E-07	2.0E-05	1.7E+00
Malic acid	2.9	Fructose ¹³ C ₆	1.0	0.2	1.5	0.3	6.4E-05	5.7E-04	1.6E+00
Glycine	11.0	Glycine d ₅	1.0	0.2	1.7	0.4	1.5E-05	3.9E-04	1.6E+00
Serine	6.1	Tryptophan d ₅	1.0	0.3	1.9	0.6	6.4E-05	5.7E-04	1.5E+00
Proline	3.7	Tryptophan d ₅	1.0	0.3	2.0	0.7	8.4E-05	6.4E-04	1.5E+00
Leucine	3.9	Tryptophan d ₅	1.0	0.3	1.9	0.6	4.8E-05	5.7E-04	1.5E+00
Isoleucine	4.2	Tryptophan d ₅	1.0	0.3	1.8	0.6	1.4E-04	6.9E-04	1.5E+00
Pyroglutamic acid	7.7	Fructose ¹³ C ₆	1.0	0.3	1.8	0.6	1.1E-04	6.9E-04	1.5E+00
Phenylalanine	3.6	Tryptophan d ₅	1.0	0.3	1.9	0.7	1.4E-04	6.9E-04	1.4E+00
Glutamic acid	8.6	Tryptophan d ₅	1.0	0.4	2.0	0.8	4.8E-05	5.7E-04	1.4E+00
Glucose	1.0	Fructose ¹³ C ₆	1.0	0.3	0.5	0.3	2.3E-04	1.0E-03	1.4E+00
Dihydroxyacetone phosphate	10.5	Fructose ¹³ C ₆	1.0	0.2	1.3	0.3	1.5E-03	4.1E-03	1.3E+00
Inosine	6.2	Alanine d ₇	1.0	0.4	1.6	0.4	5.6E-04	2.3E-03	1.3E+00
Threonine	4.1	Alanine d ₇	1.0	0.2	1.4	0.3	1.5E-03	4.1E-03	1.3E+00
Aspartic acid	4.3	Tryptophan d ₅	1.0	0.4	1.9	0.9	1.4E-04	6.9E-04	1.3E+00
Uracil	6.1	Alanine d ₇	1.0	0.5	1.6	0.5	2.2E-03	5.7E-03	1.3E+00
Alanine	1.6	Alanine d ₇	1.0	0.2	1.3	0.3	1.5E-03	4.1E-03	1.3E+00
Lactic acid	9.0	Tryptophan d ₅	1.0	0.3	1.9	0.9	1.5E-03	4.1E-03	1.3E+00
Hypoxanthine	5.1	Tryptophan d ₅	1.0	0.4	1.5	0.4	1.5E-03	4.1E-03	1.2E+00
Arachidonic acid	25.8	Glycine d ₅	1.0	0.4	2.0	1.1	2.6E-03	6.2E-03	1.1E+00
Valine	3.6	Alanine d ₇	1.0	0.2	1.3	0.2	1.5E-03	4.1E-03	1.1E+00
mono-methylphosphate	6.7	Stearic acid d ₃₅	1.0	0.3	1.6	0.7	2.9E-02	5.3E-02	1.1E+00
Tryptophan	2.6	Tryptophan d ₅	1.0	0.4	1.5	0.5	4.4E-03	9.9E-03	1.1E+00
Uridine	26.9	Stearic acid d ₃₅	1.0	0.4	1.7	0.8	1.9E-02	3.8E-02	1.0E+00
Mannose-6-phosphate	3.3	Fructose ¹³ C ₆	1.0	0.6	1.7	0.8	1.0E-03	4.0E-03	1.0E+00
Glucose-6-phosphate	3.0	Fructose ¹³ C ₆	1.0	0.6	1.7	0.7	2.6E-03	6.2E-03	1.0E+00
Spermidine	14.4	Stearic acid d ₃₅	1.0	0.3	0.7	0.2	1.3E-02	2.7E-02	9.9E-01
Succinic acid	5.3	Fructose ¹³ C ₆	1.0	0.3	0.8	0.2	9.6E-03	2.1E-02	9.2E-01
4-Hydroxyproline	2.2	Tryptophan d ₅	1.0	0.3	1.8	1.5	4.1E-02	7.2E-02	7.9E-01
Phosphoric acid	2.4	Fructose ¹³ C ₆	1.0	0.2	1.1	0.2	4.6E-02	7.8E-02	7.8E-01
1-Hexadecanol	5.0	Fructose ¹³ C ₆	1.0	0.1	1.2	0.3	1.5E-02	3.0E-02	7.8E-01

The metabolite intensity is expressed as mean and stdev (Standard deviation) after normalization with average value of variables in control subjects. Statistically significant values are shown in red color. RSD: Relative Standard Deviation, FDR: False Discovery Rate, VIP: Variable Importance Projection, N.A.: Not assessed.

Identity	RSD of QC (%)	Internal standard	Control		IPF		P value	FDR	VIP
			Mean	Stdev	Mean	Stdev			
Ornithine	10.2	Tryptophan d ₅	1.0	0.3	0.8	0.2	8.0E-02	1.3E-01	7.7E-01
Anhydrosorbitol	2.1	Fructose ¹³ C ₆	1.0	0.5	1.3	0.4	3.3E-02	5.9E-02	7.3E-01
Behenic acid	20.3	Stearic acid d ₃₅	1.0	0.2	0.9	0.2	1.2E-01	1.8E-01	6.6E-01
Diethylene glycol	22.6	Benzoic acid d ₅	1.0	0.5	0.7	0.3	5.2E-02	8.5E-02	6.6E-01
Stearic acid	3.5	Stearic acid d ₃₅	1.0	0.1	1.1	0.2	2.9E-01	4.1E-01	4.8E-01
Citric acid	5.4	Citric acid d ₄	1.0	0.2	1.1	0.4	3.4E-01	4.6E-01	4.7E-01
Phosphoenolpyruvic acid	7.3	Citric acid d ₄	1.0	0.2	1.1	0.4	2.4E-01	3.3E-01	4.2E-01
Glyceric acid	5.6	Fructose ¹³ C ₆	1.0	0.2	1.1	0.4	5.2E-01	6.4E-01	3.7E-01
Pyruvic acid	7.6	Stearic acid d ₃₅	1.0	0.4	0.9	0.3	2.0E-01	2.9E-01	3.7E-01
Cholesterol	6.6	Fructose ¹³ C ₆	1.0	0.2	1.1	0.3	6.8E-01	7.1E-01	3.7E-01
Dodecanoic acid	19.2	Fructose ¹³ C ₆	1.0	0.1	0.9	0.3	1.2E-01	1.8E-01	3.3E-01
Lysine	11.6	Tryptophan d ₅	1.0	0.3	1.1	0.5	5.2E-01	6.4E-01	3.1E-01
Myristic acid	10.2	Stearic acid d ₃₅	1.0	0.2	0.9	0.2	5.2E-01	6.4E-01	3.1E-01
Scyllo-Inositol	2.6	Fructose ¹³ C ₆	1.0	0.5	1.2	0.7	1.0E+00	1.0E+00	3.1E-01
Hypotaurine	9.6	Alanine d ₇	1.0	0.4	1.1	0.4	6.5E-01	7.0E-01	2.8E-01
Fumaric acid	9.7	Alanine d ₇	1.0	0.4	1.1	0.4	4.4E-01	5.8E-01	2.8E-01
Oleic acid	19.1	Stearic acid d ₃₅	1.0	0.2	1.0	0.2	6.2E-01	6.8E-01	2.4E-01
Arachidic acid	4.7	Alanine d ₇	1.0	0.2	1.0	0.2	6.2E-01	6.8E-01	2.3E-01
Gluconic acid	6.3	Fructose ¹³ C ₆	1.0	0.3	0.9	0.3	6.2E-01	6.8E-01	2.3E-01
Hexadecanoic acid	5.0	Stearic acid d ₃₅	1.0	0.2	1.0	0.2	6.2E-01	6.8E-01	1.5E-01
3-Hydroxybutyric acid	8.0	Benzoic acid d ₅	1.0	1.0	0.9	0.7	8.6E-01	8.7E-01	1.5E-01
Palmitoleic acid	11.7	Stearic acid d ₃₅	1.0	0.3	1.0	0.4	6.8E-01	7.1E-01	1.4E-01
Myo-inositol	1.6	Fructose ¹³ C ₆	1.0	0.4	1.0	0.3	5.9E-01	6.8E-01	1.4E-01
Docosahexaenoic acid	30.8	Glycine d ₅	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Asparagine	31.4	Fructose ¹³ C ₆	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
D-a-Tocopherol	33.3	Stearic acid d ₃₅	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Lignoceric acid	34.5	Glycine d ₅	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Pentadecanoic acid	39.0	Stearic acid d ₃₅	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Boric acid	43.1	Glycine d ₅	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Glutamine	45.5	Fructose ¹³ C ₆	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

The metabolite intensity is expressed as mean and stdev (Standard deviation) after normalization with average value of variables in control subjects. Statistically significant values are shown in red color. RSD: Relative Standard Deviation, FDR: False Discovery Rate, VIP: Variable Importance Projection, N.A.: Not assessed.

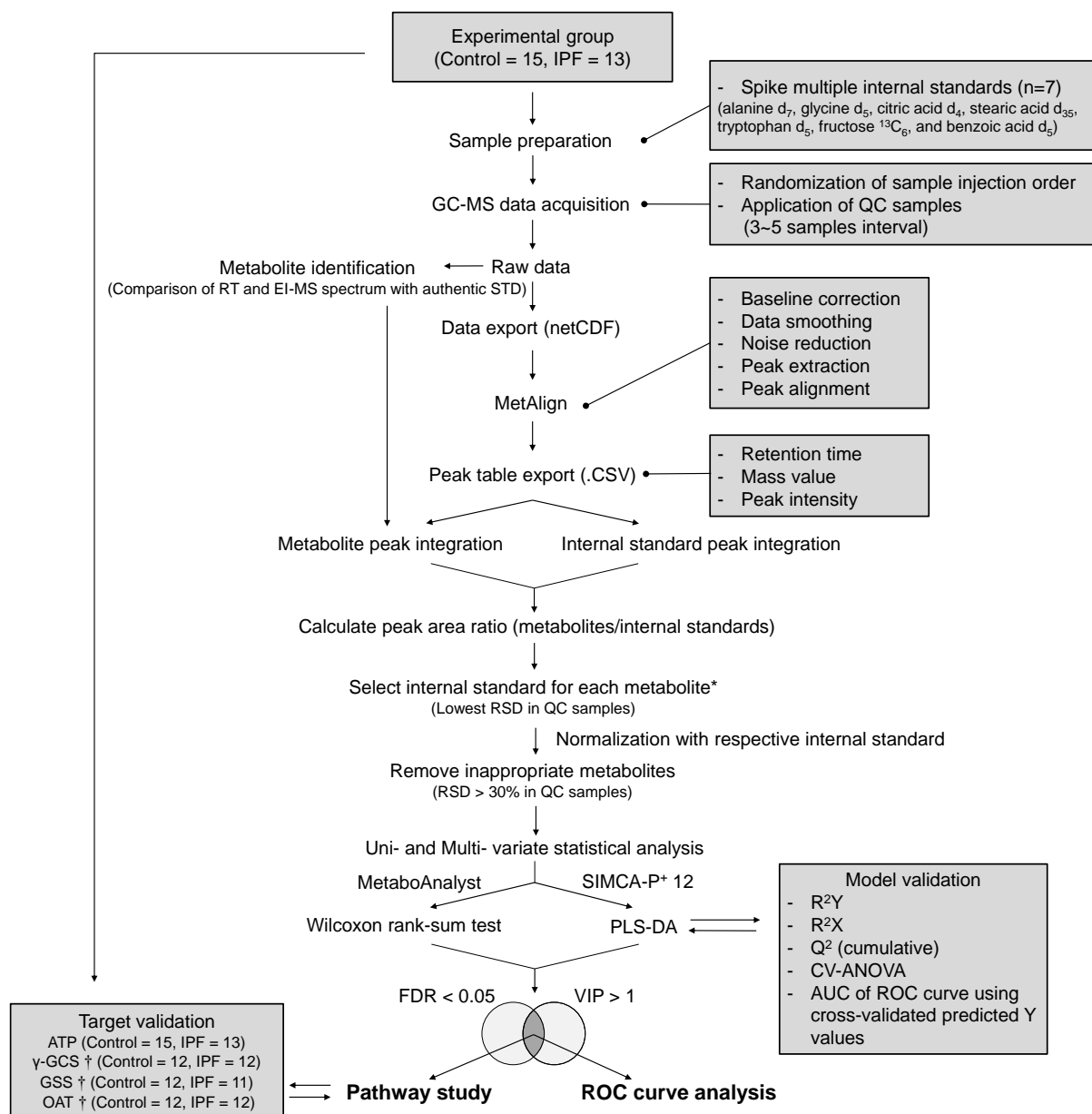


Figure S-1. Workflow of study

*: alanine, glycine, citric acid, stearic acid, and tryptophan were normalized by their isotope-labeled internal standards.

†: these enzymes were measured in the samples which had sufficient amount of tissues for quantification.

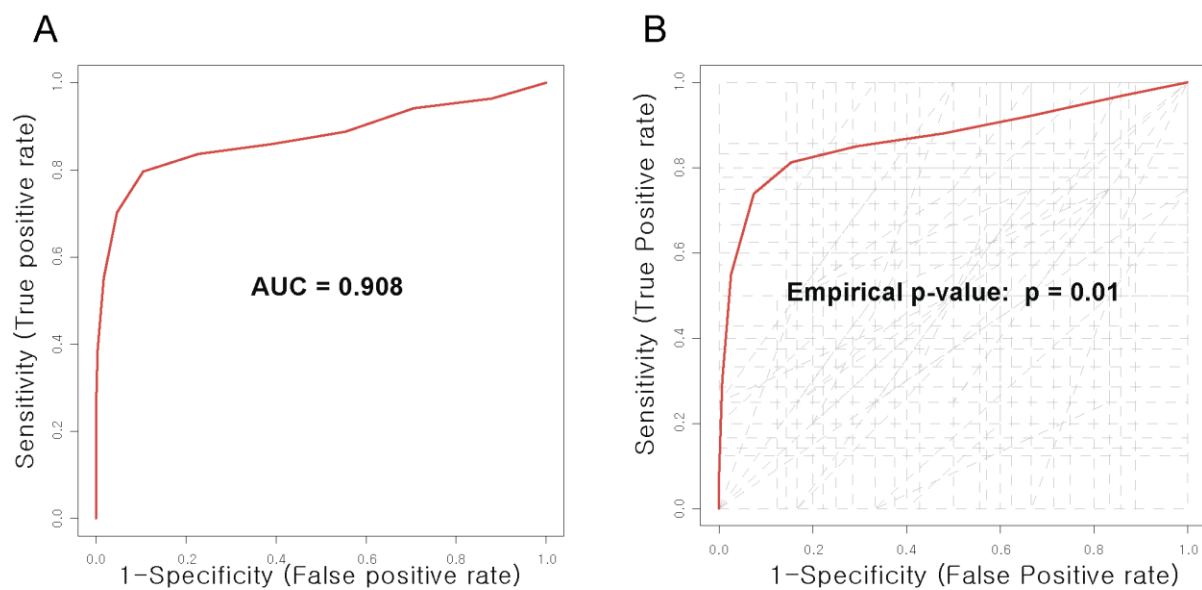


Figure S-2. ROC curve of metabolite signatures. (A) ROC curve generated from 25 metabolite signatures (FDR < 0.05 and VIP > 1) and (B) the 1000-iteration permutation test result.