

## Supplementary Information

# **Rhynchophylline alleviates neuroinflammation and regulates metabolic disorders in a mouse model of Parkinson's disease**

Chunxia Zhang,<sup>a,b†</sup> Zhen Xue,<sup>a,b†</sup> Lingmeng Zhu,<sup>a,b</sup> Lingfei Han,<sup>a,b</sup> Jiayu Zhou,<sup>a,b</sup>  
Lingxin Zhuo,<sup>a,b</sup> Jiayi Zhang,<sup>a,b</sup> Xinchun Zhang,<sup>a,b</sup> Wenyuan Liu,<sup>\*a,c</sup> Wenting Liao<sup>\*a,b</sup>

<sup>a</sup> *Key Laboratory of Drug Quality Control and Pharmacovigilance, China Pharmaceutical University, Ministry of Education, Nanjing 210009, China*

<sup>b</sup> *Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 210009, China*

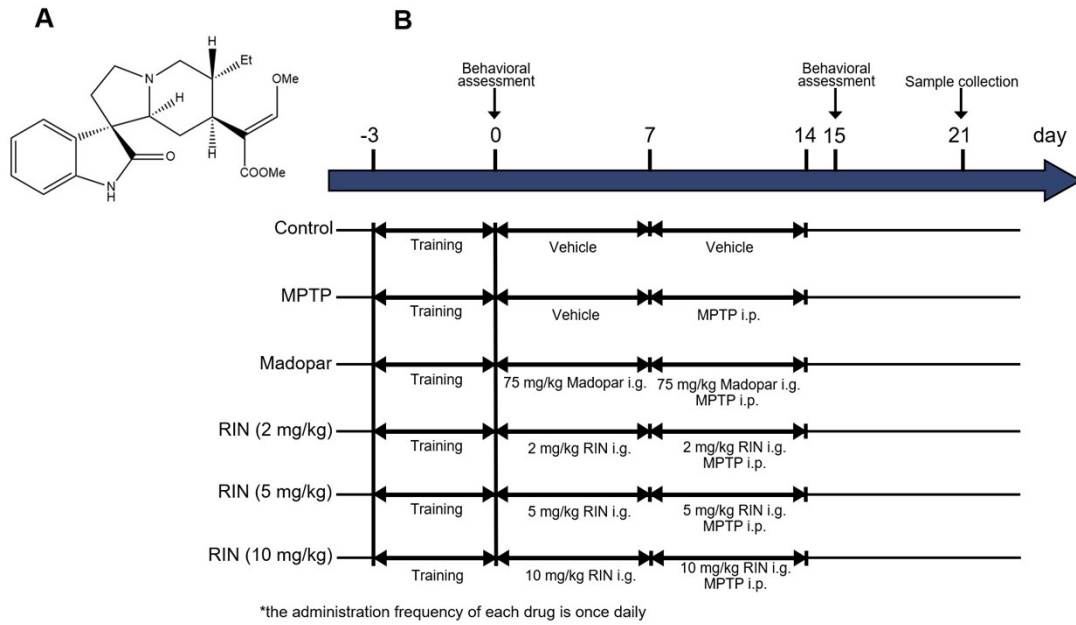
<sup>c</sup> *Zhejiang Center for safety study of drug substances (Industrial Technology Innovation Platform), Hangzhou, 310018, China*

†Chunxia Zhang and Zhen Xue made equal contributions to the research.

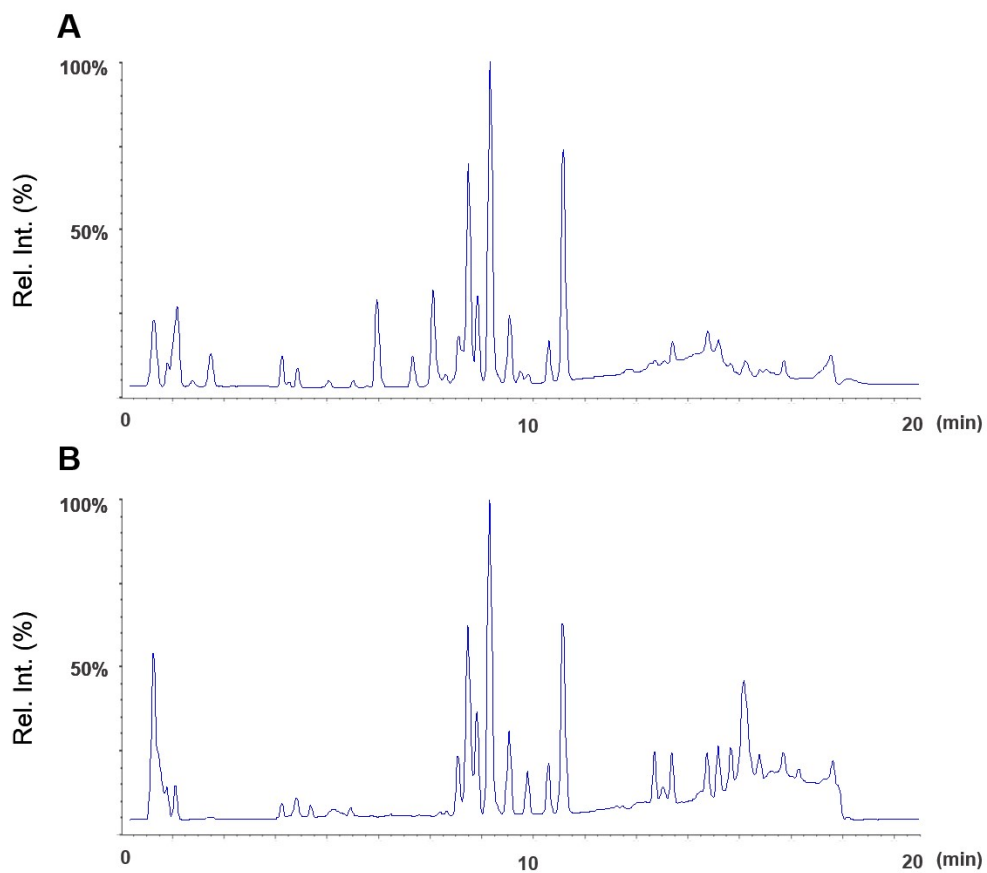
\*Corresponding authors: Wenting Liao, Email: lwting84@163.com, Tel: +86-25-83271038.

Wenyuan Liu, Email: liuwenyuan8506@163.com, Tel: +86-25-83271038.

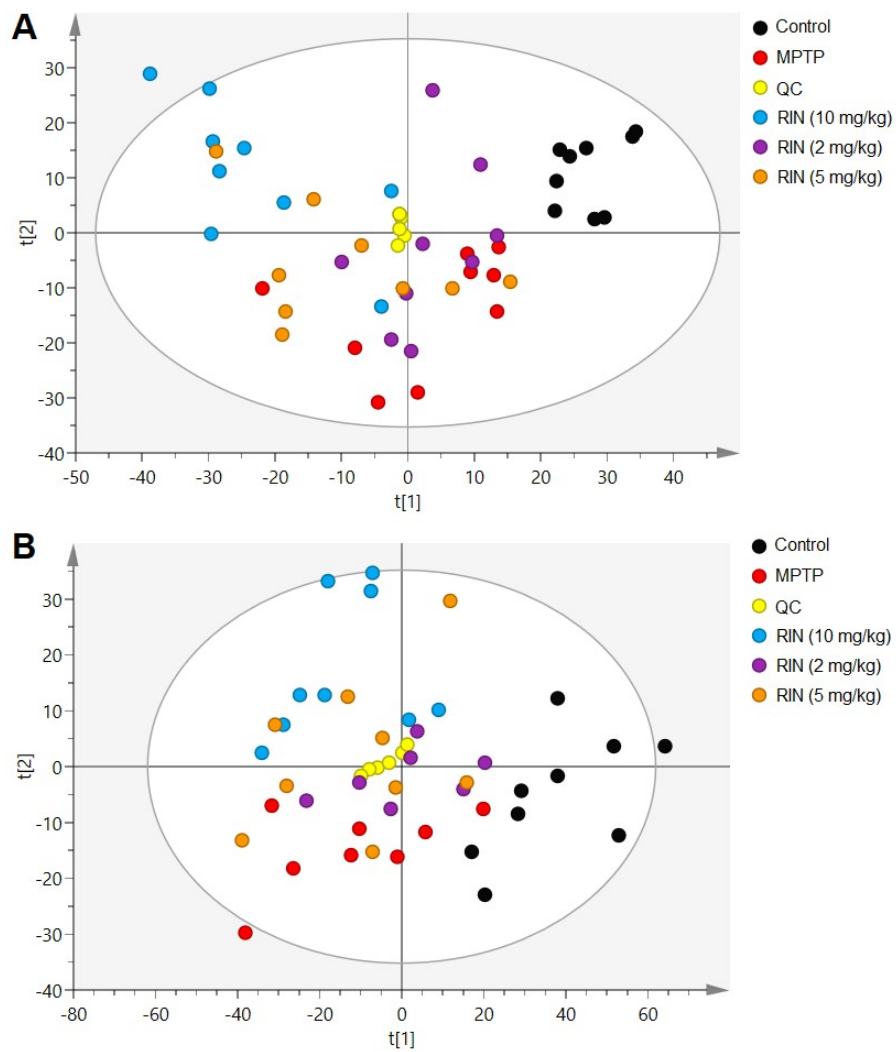
## Supplementary Figures



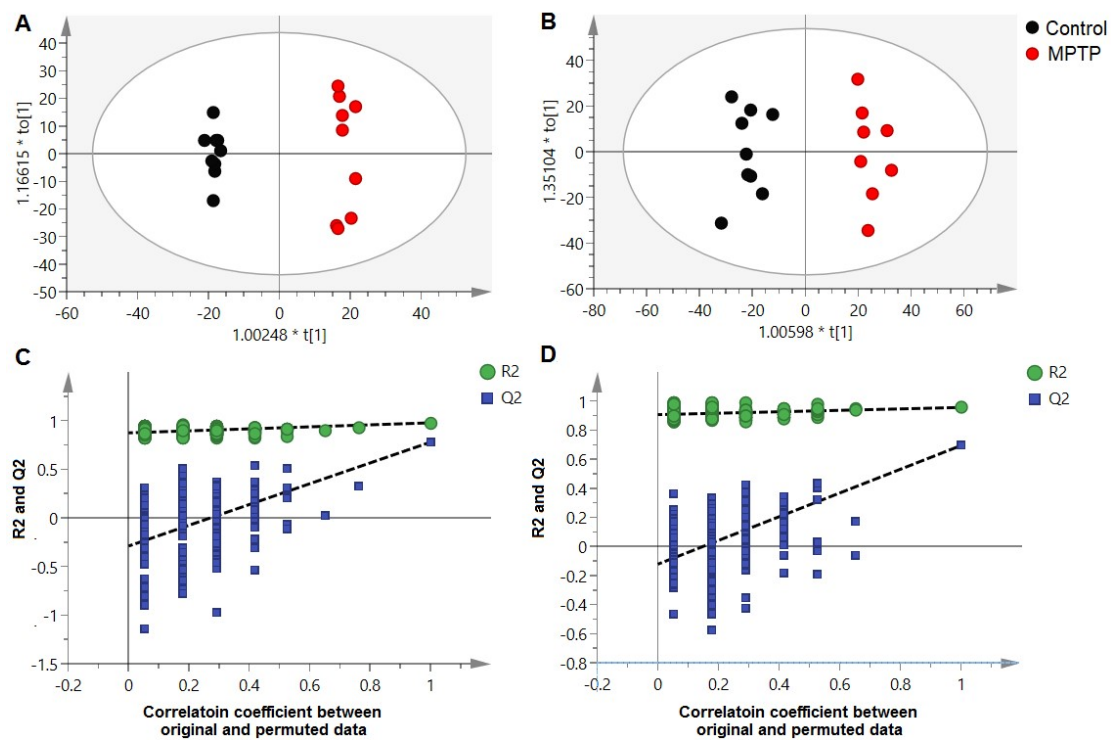
**Fig. S1.** The chemical structure of RIN (A) and the flow chart of animal experiments (B).



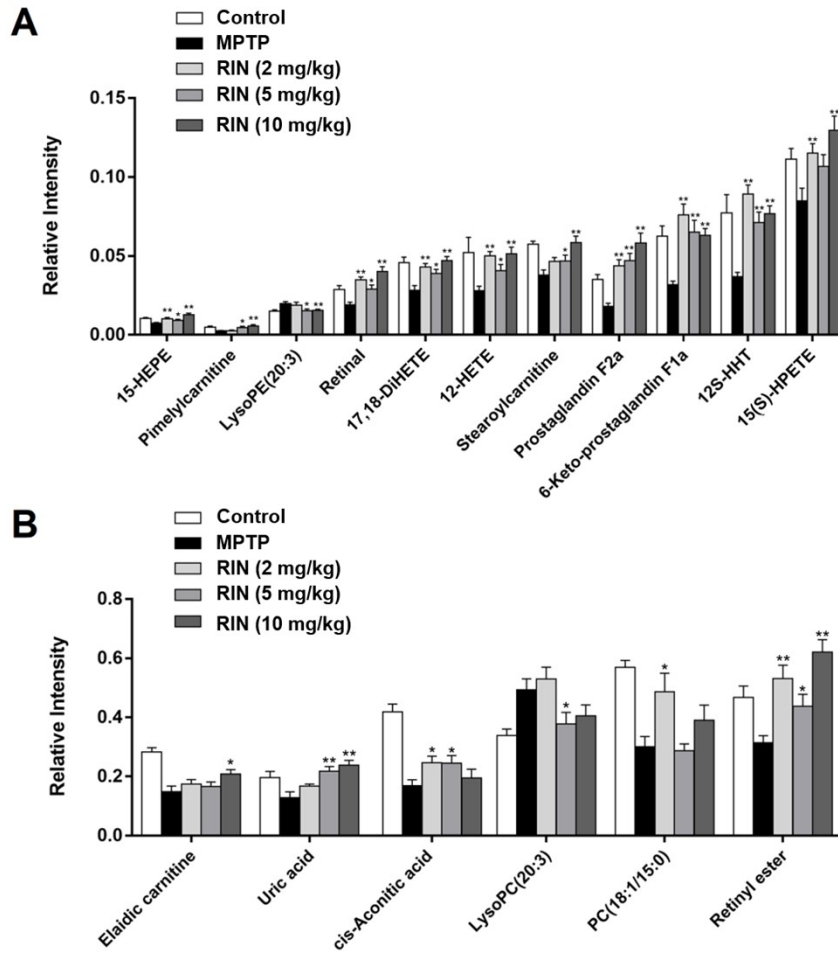
**Fig. S2.** Base peak ion (BPI) chromatograms of RIN as analyzed by UHPLC-QTOFMS. (A) BPI of UHPLC-QTOFMS in positive ion mode. (B) BPI of UHPLC-QTOFMS in negative ion mode.



**Fig. S3.** Quality control of LC-MS methodologies. (A) PCA score plot of real samples and QC samples from positive ion mode LC-MS dataset. (B) PCA score plot of real samples and QC samples from negative ion mode LC-MS dataset.



**Fig. S4.** The OPLS-DA scores plot from positive ion mode LC-MS (A) and negative ion mode LC-MS (B) dataset of control and MPTP groups. The quality was validated by 200 permutation tests (C) for positive ion mode LC-MS and (D) for negative ion mode LC-MS, respectively.



**Fig. S5.** Relative intensity of metabolites regulated by RIN. (Values are shown in Mean  $\pm$  SEM.

\* $p < 0.05$ , \*\* $p < 0.01$  vs MPTP group.)

## Supplemental Tables

**Table S1.** Sequences of primers used for quantitative real-time PCR.

Gene	Primer	Sequence
NLRP3	forward	ATTACCCGCCCGAGAAAGG
	reverse	TCGCAGCAAAGATCCACACAG
TLR4	forward	ATGGCATGGCTTACACCACC
	reverse	GAGGCCAATTTTGTCTCCACA
COX2	forward	TGCACTATGGTTACAAAAGCTGG
	reverse	TCAGGAAGCTCCTTATTTCCCTT
$\beta$ -actin	forward	GGCTGTATTCCCCTCCATCG
	reverse	CCAGTTGGTAACAATGCCATGT

**Table S2.** Influence of RIN on neurobehavioral abnormalities in MPTP-treated mice.

	Control	MPTP	Madopar	RIN (2 mg/kg)	RIN (5 mg/kg)	RIN (10 mg/kg)
Total time (s)	8.06±0.64	18.02±2.07 <sup>###</sup>	9.54±0.83 <sup>***</sup>	9.87±0.59 <sup>***#</sup>	9.29±0.92 <sup>***</sup>	8.32±1.84 <sup>***</sup>
Residence time (s)	220.3±21.91	95.78±19.76 <sup>###</sup>	165.22±20.36 <sup>*#</sup>	170.56±27.8 <sup>*</sup>	169.56±22.48 <sup>*</sup>	177.88±29.76 <sup>*</sup>
Total distance (mm)	14586.35±906.04	5385.08±1041.89 <sup>###</sup>	9623.89±1509.63 <sup>###</sup>	10237.1±1177.21 <sup>**###</sup>	10543.42±682.83 <sup>***###</sup>	10085.68±1057.56 <sup>***###</sup>
Central distance (mm)	1660.41±223.94	612.3±189.00 <sup>#</sup>	1074.72±165.64 <sup>#</sup>	1272.15±275.46 <sup>*</sup>	1267.18±130.9 <sup>**</sup>	1314.89±233.44 <sup>*</sup>

Data are mean ± SEM. <sup>#</sup>*p* < 0.05, <sup>##</sup>*p* < 0.01, <sup>###</sup>*p* < 0.001 vs. control group; <sup>\*</sup>*p* < 0.05, <sup>\*\*</sup>*p* < 0.01, <sup>\*\*\*</sup>*p* < 0.001 vs. MPTP group.



**Table S3.** Representative photomicrographs of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra and TH-positive fibers in the striatum.

	Control	MPTP	Madopar	RIN (2 mg/kg)	RIN (5 mg/kg)	RIN (10 mg/kg)
Substantia nigra	56.16±3.63	36.81±2.13 <sup>#</sup>	43.54±1.12 <sup>*#</sup>	41.46±1.08 <sup>#</sup>	44.82±0.88 <sup>*#</sup>	47.12±2.78 <sup>*</sup>
Striatum	47.07±1.82	27.66±1.12 <sup>###</sup>	36.5±3.78	36.06±4.12	37.50±4.95	37.32±3.04 <sup>*</sup>

Data are mean ± SEM. <sup>#</sup>*p* < 0.05, <sup>###</sup>*p* < 0.001 vs. control group; <sup>\*</sup>*p* < 0.05 vs. MPTP group.

**Table S4.** The serum levels of inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-10 and TNF- $\alpha$ ) and oxidative stress indicators (SOD, GSH, LDH and MDA).

	Control	MPTP	Madopar	RIN (2 mg/kg)	RIN (5 mg/kg)	RIN (10 mg/kg)
IL-1 $\beta$ (pg/mL)	46.78 $\pm$ 4.94	70.21 $\pm$ 4.15 <sup>##</sup>	55.53 $\pm$ 2.07 <sup>**</sup>	74.9 $\pm$ 6.11 <sup>##</sup>	54.37 $\pm$ 4.72 <sup>*</sup>	50.06 $\pm$ 5.16 <sup>**</sup>
IL-6 (pg/mL)	55.8 $\pm$ 4.07	80.77 $\pm$ 4.06 <sup>###</sup>	61.99 $\pm$ 4.15 <sup>**</sup>	82.87 $\pm$ 2.33 <sup>###</sup>	66.39 $\pm$ 4.68 <sup>*</sup>	58.42 $\pm$ 3.68 <sup>***</sup>
IL-10 (pg/mL)	276.04 $\pm$ 8.62	204.08 $\pm$ 13.07 <sup>###</sup>	258.76 $\pm$ 12.52 <sup>**</sup>	204.7 $\pm$ 17.67 <sup>##</sup>	217.91 $\pm$ 13 <sup>##</sup>	247.92 $\pm$ 10.89 <sup>*</sup>
TNF- $\alpha$ (pg/mL)	280.12 $\pm$ 22.75	403.57 $\pm$ 19.1 <sup>###</sup>	284.18 $\pm$ 19.41 <sup>***</sup>	408.93 $\pm$ 20.91 <sup>###</sup>	343.19 $\pm$ 20.1 <sup>*</sup>	291.5 $\pm$ 18.65 <sup>***</sup>
SOD (ng/mL)	8.99 $\pm$ 0.28	6.3 $\pm$ 0.45 <sup>###</sup>	7.76 $\pm$ 0.47 <sup>*#</sup>	6.05 $\pm$ 0.46 <sup>###</sup>	7.02 $\pm$ 0.41 <sup>##</sup>	8.84 $\pm$ 0.34 <sup>***</sup>
GSH (ng/mL)	5.3 $\pm$ 0.25	3.19 $\pm$ 0.19 <sup>###</sup>	4.52 $\pm$ 0.26 <sup>***</sup>	3.81 $\pm$ 0.25 <sup>*##</sup>	3.65 $\pm$ 0.18 <sup>###</sup>	4.6 $\pm$ 0.46 <sup>**</sup>
LDH (ng/mL)	9.02 $\pm$ 0.66	13.48 $\pm$ 0.53 <sup>###</sup>	9.38 $\pm$ 0.63 <sup>***</sup>	13.04 $\pm$ 0.86 <sup>##</sup>	10.57 $\pm$ 0.46 <sup>***</sup>	9.93 $\pm$ 0.49 <sup>***</sup>
MDA (nmol/L)	11.19 $\pm$ 0.94	17.47 $\pm$ 0.94 <sup>###</sup>	11.31 $\pm$ 0.47 <sup>***</sup>	16.02 $\pm$ 0.99 <sup>##</sup>	13.46 $\pm$ 0.95 <sup>**</sup>	12.57 $\pm$ 0.94 <sup>**</sup>

Data are mean  $\pm$  SEM. # $p$  < 0.05, ## $p$  < 0.01, ### $p$  < 0.001 vs. control group; \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001 vs. MPTP group.

**Table S5.** Analyzed pathways of metabolomics data differently regulated in serum of MPTP-induced PD mice.

	Total <sup>a</sup>	Hits <sup>b</sup>	Raw p <sup>c</sup>	-LOG <sub>10</sub> (p)	FDR	Impact
Synthesis and degradation of ketone bodies	5	2	3.01E-03	2.52	4.82E-03	0.60
Retinol metabolism	16	2	1.71E-02	1.77	1.95E-02	0.41
Glycine, serine and threonine metabolism	34	1	5.57E-04	3.25	1.48E-03	0.27
Glyoxylate and dicarboxylate metabolism	32	3	2.01E-03	2.70	4.05E-03	0.16
Glycerophospholipid metabolism	36	3	9.34E-06	5.03	5.99E-05	0.16
Citrate cycle (TCA cycle)	20	2	2.03E-03	2.69	4.05E-03	0.14
Butanoate metabolism	15	2	3.01E-03	2.52	4.82E-03	0.11
Glutathione metabolism	28	1	5.57E-04	3.25	1.48E-03	0.09
Phosphatidylinositol signaling system	28	1	7.76E-03	2.11	9.80E-03	0.03
Inositol phosphate metabolism	30	1	7.76E-03	2.11	9.80E-03	0.03
Primary bile acid biosynthesis	46	1	5.57E-04	3.25	1.48E-03	0.02
Arachidonic acid metabolism	36	5	4.19E-06	5.38	5.99E-05	0.02
Purine metabolism	66	1	2.15E-02	1.67	2.24E-02	0.02
Tyrosine metabolism	42	2	1.29E-03	2.89	3.11E-03	0.01

<sup>a</sup> Total is the total number of compounds in the pathway; <sup>b</sup> the hits is the actually matched number from the user uploaded data; <sup>c</sup> the p is the original p value calculated from the enrichment analysis; <sup>d</sup> the impact is the pathway impact value calculated from pathway topology analysis.

**Table S6.** Analyzed pathways of metabolomics data differently regulated in serum of RIN-treated mice compared with model mice.

	Total <sup>a</sup>	Hits <sup>b</sup>	Raw p <sup>c</sup>	-LOG <sub>10</sub> (p)	FDR	Impact
Retinol metabolism	16	2	0.00	4.50	0.00	0.41
Glycerophospholipid metabolism	36	2	0.01	2.01	0.02	0.11
Citrate cycle (TCA cycle)	20	1	0.06	1.20	0.06	0.05
Glyoxylate and dicarboxylate metabolism	32	1	0.06	1.20	0.06	0.02
Arachidonic acid metabolism	36	5	0.00	2.99	0.00	0.02
Purine metabolism	66	1	0.00	3.25	0.00	0.02

<sup>a</sup>Total is the total number of compounds in the pathway; <sup>b</sup> the hits is the actually matched number from the user uploaded data; <sup>c</sup> the p is the original p value calculated from the enrichment analysis; <sup>d</sup> the impact is the pathway impact value calculated from pathway topology analysis.