Supplemental information

Alkaloids of Aconiti Lateralis Radix Praeparata inhibit growth of non-small cell lung cancer by regulating PI3K/Akt-mTOR signaling and glycolysis

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Supplementary Figure

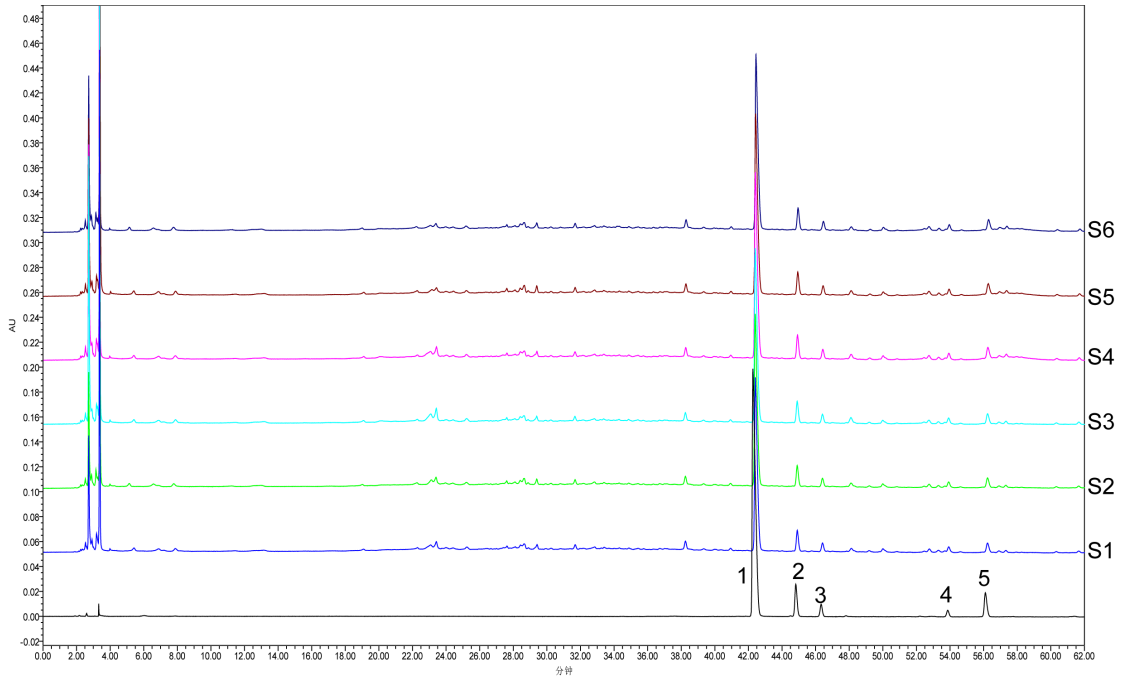

Fig. S1 HPLC chromatograms of different batches of FZA and reference solutions.1. benzoylmesaconitine, 2. benzoylaconitine, 3. benzoylhypaconitine, 4. mesaconitine 5. hypaconitine

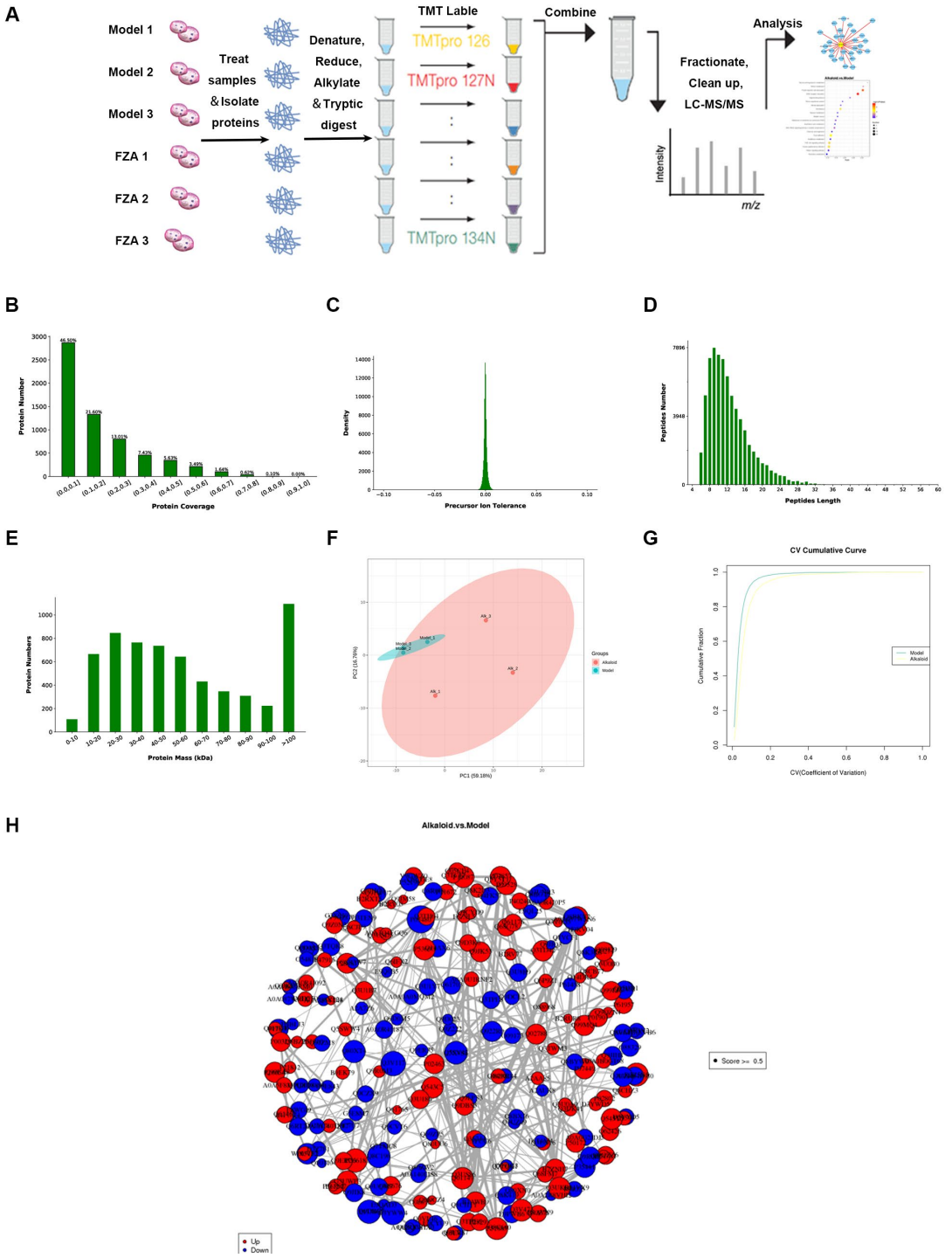

Fig. S2 Proteins of the mice after intervention by FZA was identified by TMT-based proteomics. (A) Schematic illustration of the proteomic study. Proteins were extracted from the tumor tissues of mice treated with saline or FZA, followed by digestion with trypsin. The resulted peptides were labeled with TMT-10 plex reagents, followed by a high-pH reverse-phase fraction and LC-MS/MS analysis. Bioinformatics analysis of differently expressed proteins was performed using the STRING database. (B-E) Quality control diagram of protein qualitative data, such as peptides length, precursor ion tolerance, unique peptide number, protein coverage, protein mass. (F) Principal component analysis between model group and FZA group. (G) Repetitive CV analysis of each group. (H) Differential protein network interaction analysis by TMT-based proteomics.

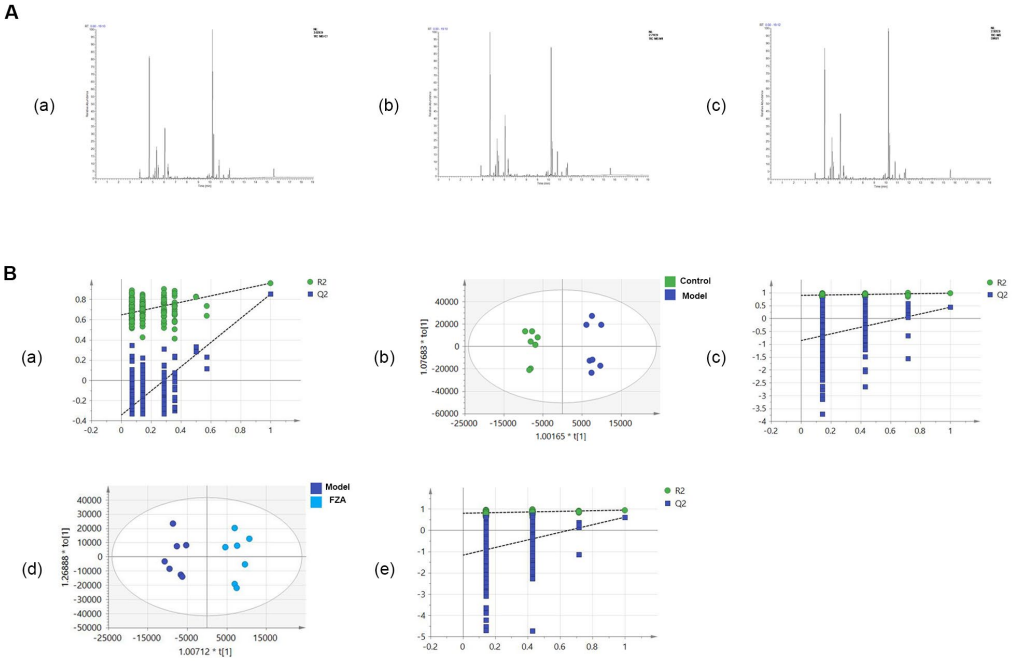

Fig. S3 (A) TICs of serum samples of the mice, (a) control group, (b) model group, and (c) FZA group. (B) The trends in serum metabolites in hormonal nude mice analyzed by using PLS-DA. (a) OPLS-DA results of serum samples between the control, model, and FZA groups. (b-c) OPLS-DA results of control and model groups. (d-e) OPLS-DA results of FZA and model groups.

Fig.S4

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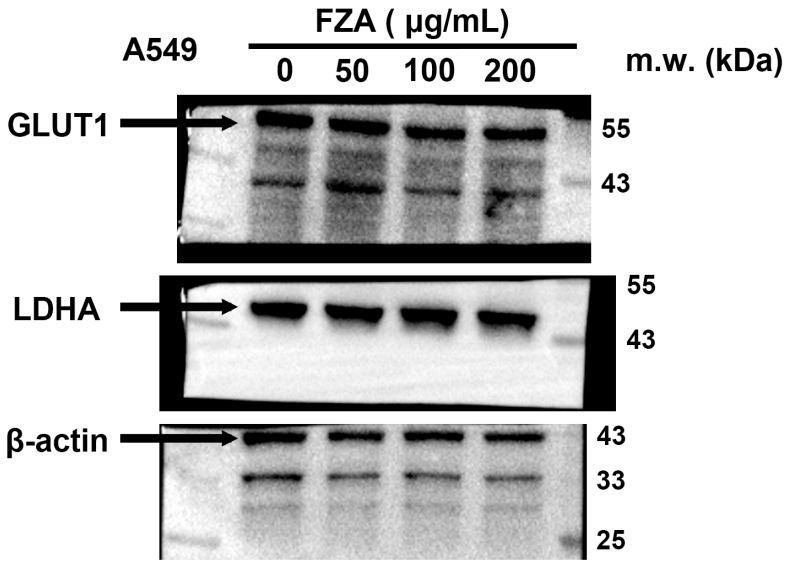

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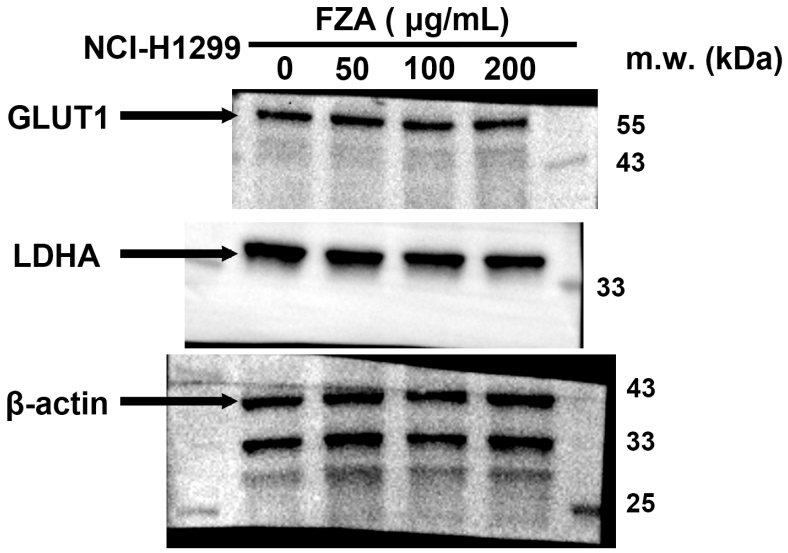

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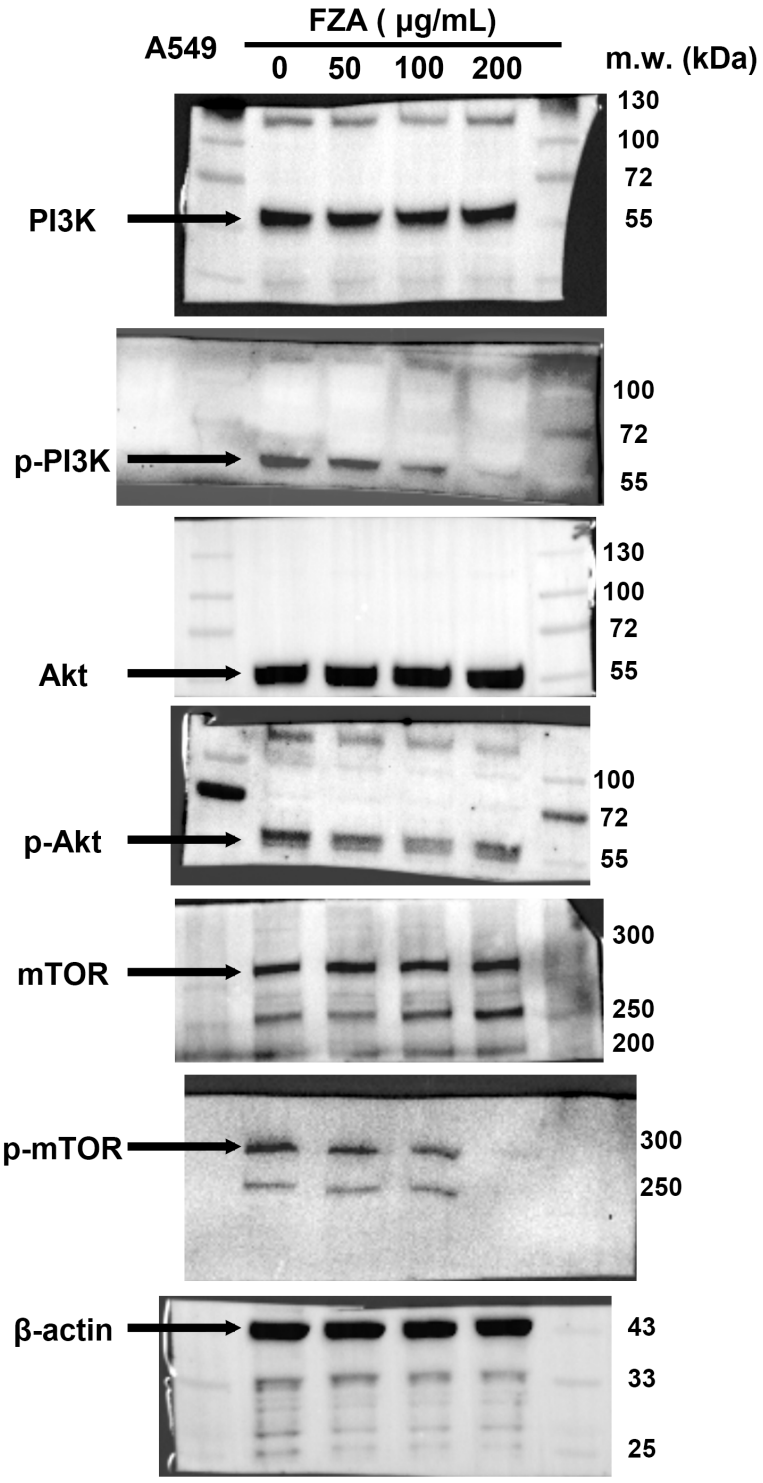
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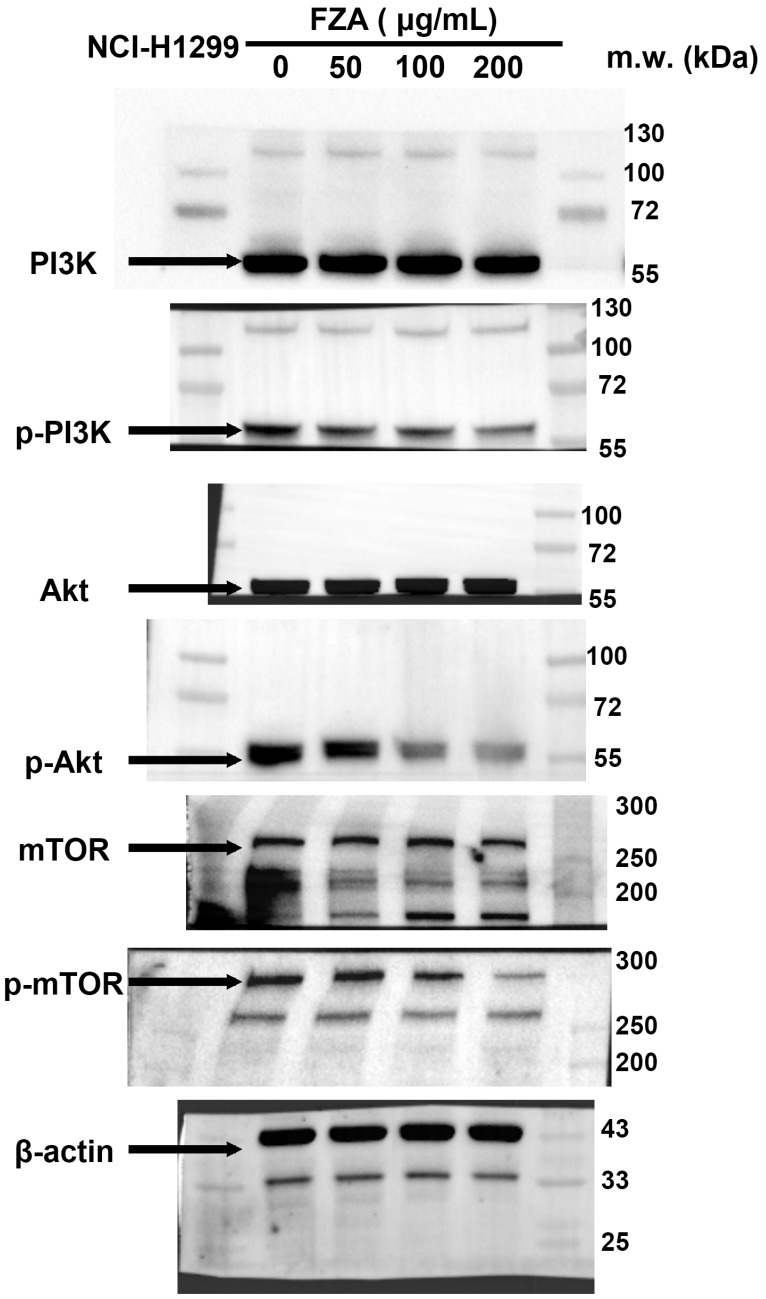
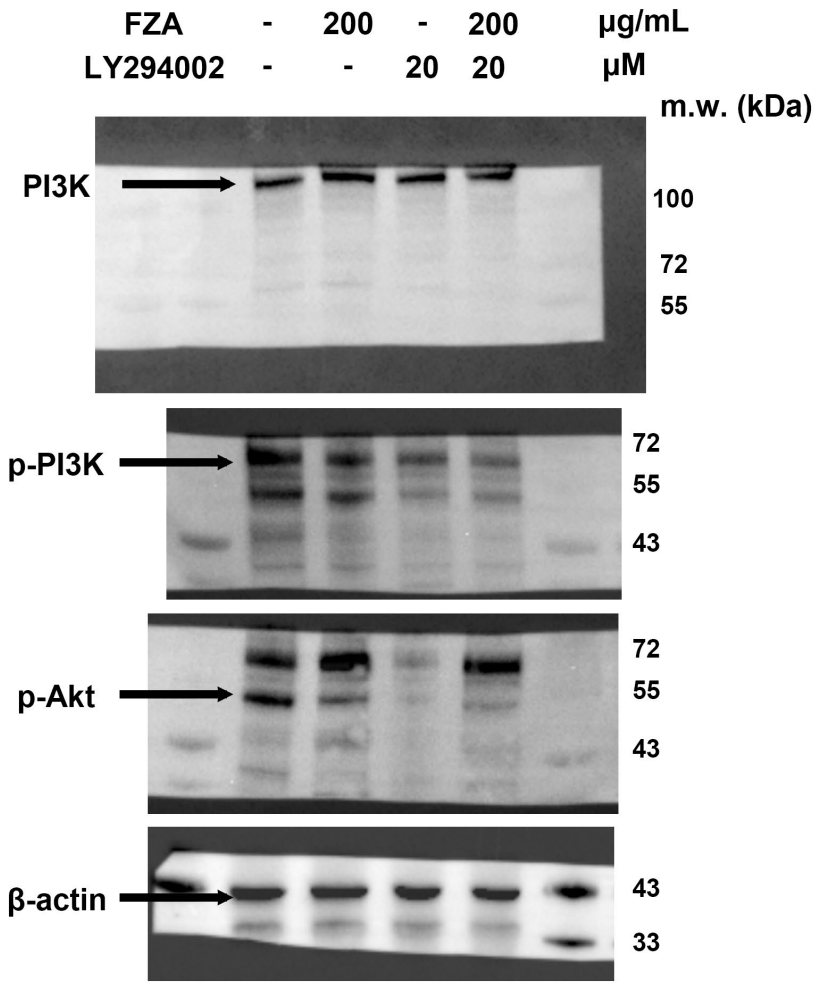
FZA (μg/mL)
0 50 100 200
m.w. (kDa)
PI3K
130
100
72
55
p-PI3K
100
72
55
Akt
130
100
72
55
p-Akt
100
72
55
mTOR
300
250
200
p-mTOR
300
250
β-actin
43
33
25

NCI-H1299:


NCI-H1299
FZA (μg/mL)
0 50 100 200
m.w. (kDa)
PI3K
130
100
72
55
p-PI3K
130
100
72
55
Akt
100
72
55
p-Akt
100
72
55
mTOR
300
250
200
p-mTOR
300
250
200
β-actin
43
33
25

A549:


A549
FZA - 200 - 200 μg/mL
LY294002 - - 20 20 μM
m.w. (kDa)
PI3K
100
72
55
p-PI3K
72
55
43
p-Akt
72
55
43
β-actin
43
33

Supplementary Table

Table S1 Content of main alkaloids in different preparation batches of FZA

No	BMA (μg/g)	BA (μg/g)	BHA (μg/g)	MA (μg/g)	HA (μg/g)
1	814.42	76.31	17.95	29.53	48.49
2	801.42	75.94	18.42	31.86	49.12
3	824.48	79.34	19.21	35.13	56.37
4	886.73	85.86	20.33	36.82	59.30
5	863.54	83.34	19.46	38.10	57.17
6	834.86	80.48	19.52	35.12	53.09
Mean	837.58	80.21	19.15	34.43	53.92
RSD (%)	3.49	4.44	4.05	8.45	7.52

Table S2 Differential metabolites between the model and FZA groups by metabolomics.

No.	name	*P* value（FZA/Model)	Fold Change (FZA/Model)
1	pipecolinic acid	0.0009	4.0434
2	beta-alanine	0.0002	2.8573
3	5,6-dihydrouracil	0.0011	2.6467
4	1-hydroxyanthraquinone	0.0004	0.3929
5	allantoic acid	0.0019	2.2583
6	dodecanol	0.0001	2.2349
7	2,3-dihydroxypyridine	0.0012	2.0807
8	glucose-6-phosphate	0.0052	1.9624
9	glycerol-1-phosphate	0.0001	1.8756
10	aspartic acid	0.0012	1.8505
11	proline	0.0011	1.7914
12	methionine	0.0013	1.7879
13	O-phosphorylethanolamine	0.0002	0.6084
14	4-hydroxybenzoic acid	0.0001	1.6111
15	fumaric acid	0.0019	1.5955
16	ornithine	0.0235	1.5923
17	glycine	0.0016	0.6325
18	L-malic acid	0.0045	1.5797
19	uracil	0.0179	1.5718
20	glucose-1-phosphate	0.0004	1.5681
21	threitol	0.0013	1.5384
22	threonine	0.0055	1.5338
23	lysine	0.0102	1.4803
24	aconitic acid	0.0102	1.4718
25	trans-4-hydroxy-L-proline	0.0009	1.4387
26	glycolic acid	0.0026	1.4318
27	gluconic acid	0.0115	1.4160
28	threo-beta hydroxyaspartate	0.0010	0.7339
29	acetylsalicylic acid	0.0147	1.3518
30	tagatose 2	0.0016	1.3397
31	arabitol	0.0011	1.3258
32	2'-deoxycytidine 5'-triphosphate	0.0001	1.2801