Supporting information

Supporting Information Text

HPLC methods

The contents of the main compounds in HQT were calculated using the standard curve of isocarthamidin-7-O-β-D-glucuronide, carthamidin-7-O-β-D-glucuronide; scutellarin, apigenin-7-O-β-Dglucopyranside, baicalin, isoscutellarein-8-O-β-D-glucuronide, chrysin-7-O-β-D-glucuronide, wogonoside, and chrysin (all were obtained from the aerial part of *S. baicalensis*). The chromatographic separation of compounds was achieved using a YMC-pack ODS-A analytical column (250 mm × 4.6 mm, 5 μm). The column temperature was maintained at 30°C. The mobile phase consists of methyl alcohol (A), acetonitrile (B), and 0.1% formic acid (C). Elution was performed at a flow rate of 1.0 mL/min. The program of linearity gradient elution: 0-60 min, A-B-C (15:5:80) to A-B-C (80:10:10) and followed by 15 min column re-equilibration. The detection wavelength was set at 278 nm. Data acquisition and analysis were achieved using Agilent Chemstation software workstation (C.01.06).

Metabolomic Profiling of Fecal Samples by UPLC-Q-TOF-MS

TOF mass spectrometer parameters were set using both the positive and negative ion modes. The cone and capillary voltage were 40 V and 3,500 V, respectively; cone gas rate was 50 L/h; desolvation gas rate was 900 L/h at a temperature of 350 °C, and the source temperature was 100 °C. The data acquisition rate was 0.15 s. Leucine-enkephalin at a concentration of 0.5 μg/mL with a flow rate of 5 μL/min was used as the lock mass in all analyses (in the positive ion mode $[M + H]^+$ = 556.2771 and in negative ion mode $[M\text{-}H]^-$ = 554.2615). Data were acquired in the centroid mode. The mass ranges from m/z 100–1200 was scanned.

Supporting Information for Figures

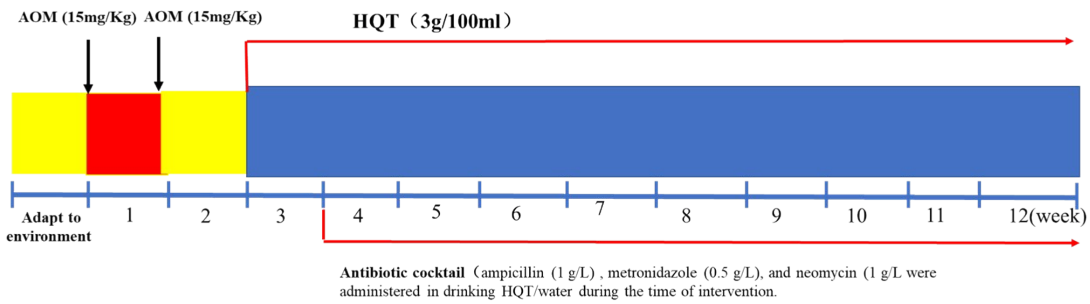

Figure S1. Induction of ACFs and schematic protocol of the animal treatments.

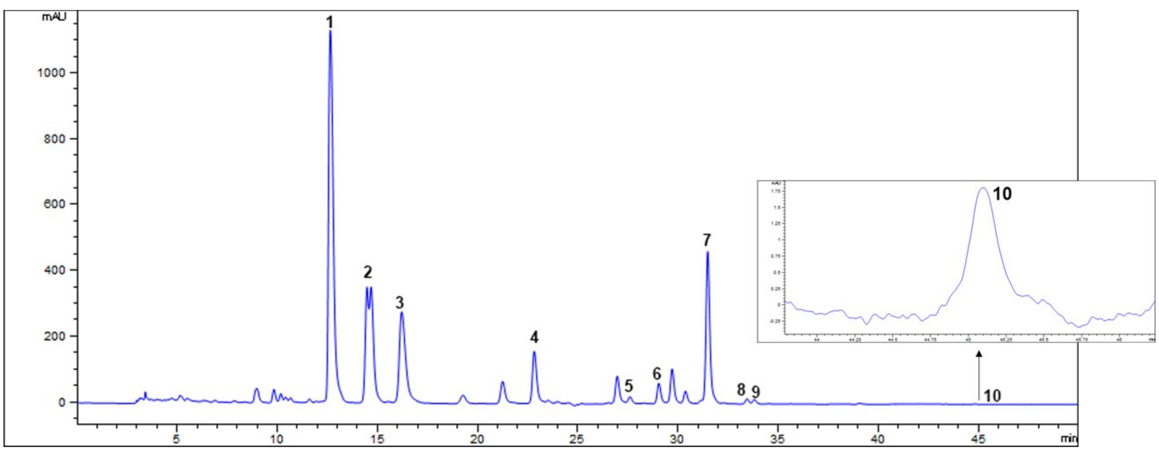

Figure S2. The chromatogram of 10 flavonoids in Huang-qin tea (**1**: isocarthamidin-7-*O*-β-*D*-glucuronide; **2**: carthamidin-7-*O*-β-*D*-glucuronide (a mixture of C (2)-R and -S configuration compounds at the ratio of 1:1); **3**: scutellarin; **4**: apigenin-7-*O*-β-*D*-glucopyranside; **5**: baicalin; **6**: Isoscutellarein-8-*O*-β-*D*-glucuronide; **7**: chrysin-7-*O*-β-*D*-glucuronide; **8**: luteolin; **9**: wogonoside; **10**: chrysin)

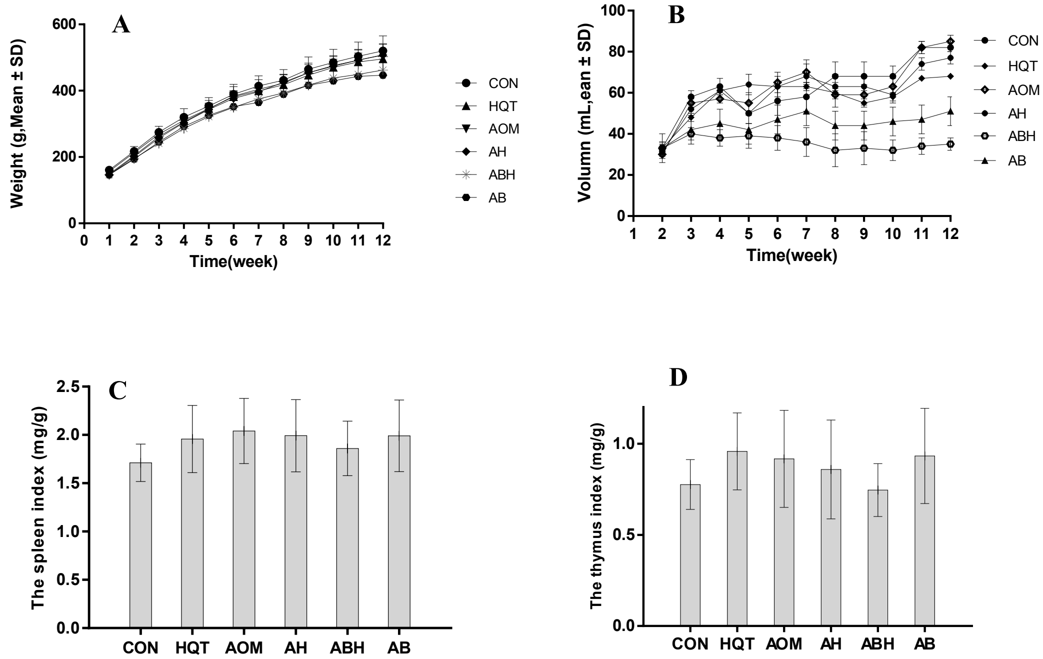

Figure S3. The mean body, consumption of water and HQT infusion, and the weight of spleen and thymus for all groups. Weight change in the rats (1–12 week) (A), consumption of water and HQT infusion by rats (B), the weight of the rat spleen (C) and thymus (D) in different groups (*p*> 0.05, vs Control).

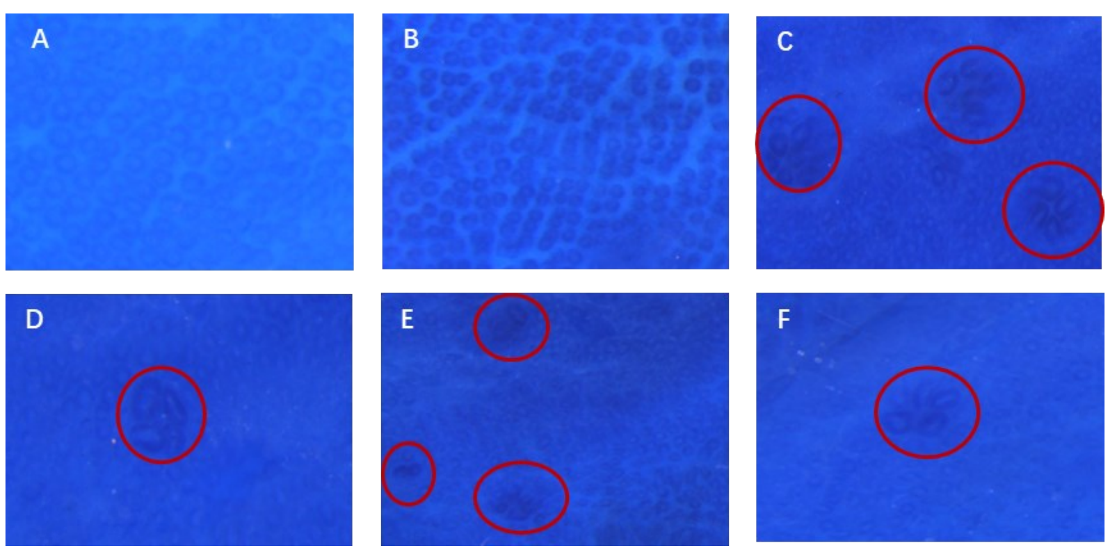

Figure S4. Pathological observation of rat colon tissue. (A) Control group; (B) HQT group; (C) AOM group; (D) AH group; (E) AB group; (F) ABH group; Red circles show ACF (aberrant crypt foci), and AC (aberrant crypts) are shown inside the circles.

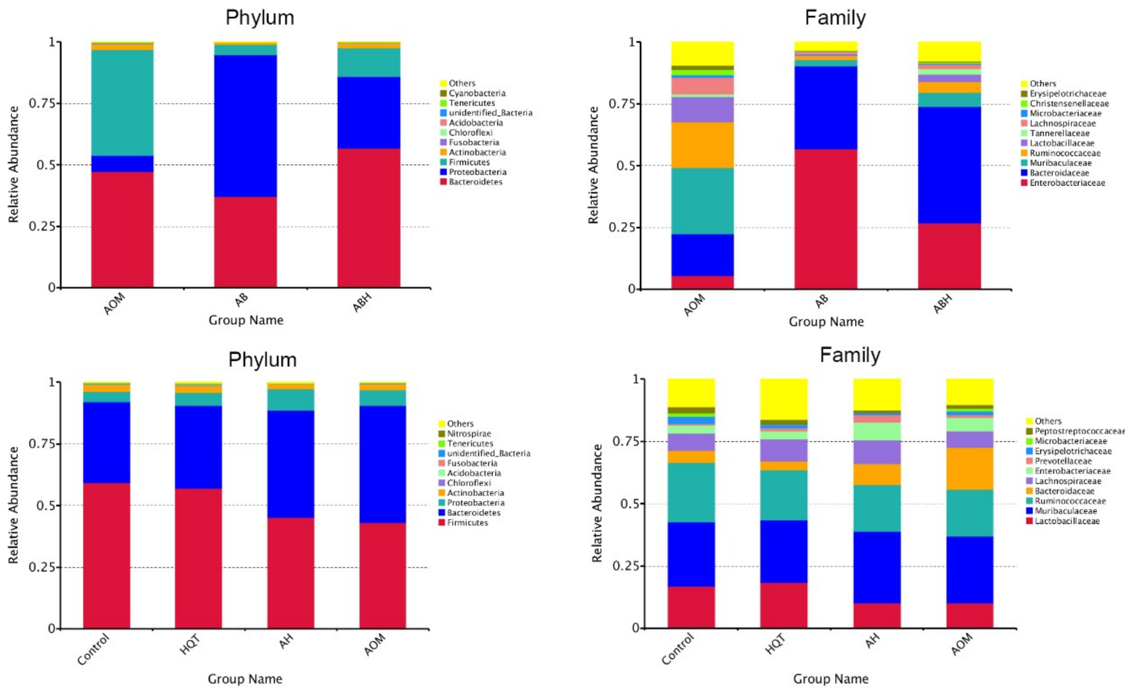

Figure S5. The top 10 relative abundances of bacteria at phylum, family levels in a different group.

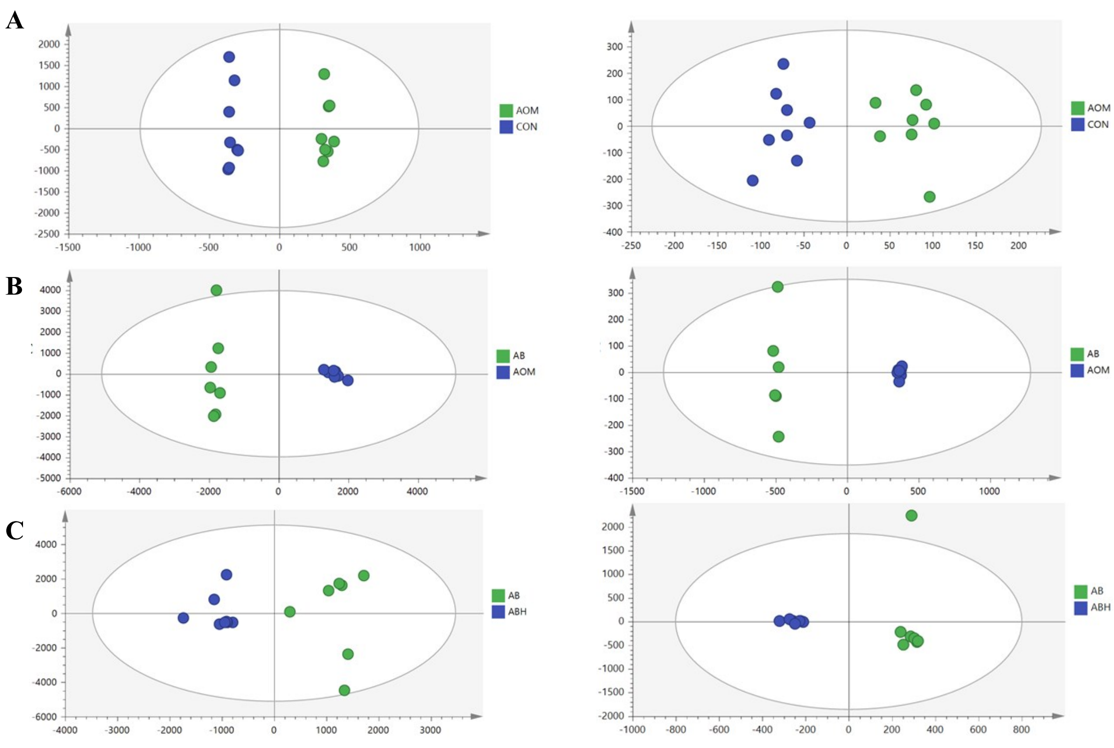

Figure S6. OPLS-DA score plots based on the feces metabolite profiling in the negative (left) and positive (right) ion modes of CON/AOM (A), AOM/AB (B), AB/ABH (C)

Supporting Information for Tables

Table S1. Contents of 9 compounds in HQT (mean ±SD, mg /g).

NO.	Analyte	HQT(n=5)
1	Isocarthamidin-7-O-β-D-glucuronide	52.19±29.81
2	Carthamidin-7-O-β-D-glucuronide	31.48±6.82
3	Scutellarin	12.77±1.14
4	Apigenin-7-O-β-D-glucopyranside	5.39±0.92
5	Baicalin	1.88±0.48
6	Isoscutellarein-8-O-β-D-glucuronide	2.84±0.60
7	Chrysin-7-O-β-D-glucuronide	10.65±0.40
8	luteolin	0.40±0.01
9	Wogonoside	0.23±0.02
10	Chrysin	0.03±0.01

Table S2. Effect of HQT on rat ACF induced by AOM (Mean ± SD)

Group	Incidence of ACF	Number of ACF	Number of AC	Mean number of Crypts/Focus
CON	0/8	0	0	0
HQT	0/8	0	0	0
AOM	8/8	96.57±24.31 a	335.57±108.65 a	3.92±0.83 a
AH	7/7	44.17±11.55 b***	197.00±60.03 b**	4.23±0.70 a
ABH	8/8	65.33±15.09 c**	223.00±27.65 b*	3.91±0.49 a
AB	7/7	68.38±24.58 c*	228.83±69.36 b*	4.24±1.28 a

Number a/b/c/d, means within the same row with different letter mark among content levels groups were significantly different (*p<0.05, **p<0.01, *** p<0.001)

Table S3. Values of the Statistic Parameters Obtained for Different PLS-DA and OPLS-DA Models on UPLC-QTOF-MS Data (in Negative and positive Model)

Classes	OPLS-DA (Negative Model)		OPLS-DA (Positive Model)	
	R^2Y	Q^2Y	R^2X	Q^2Y
CON vs AOM	0.982	0.703	0.922	0.533
AOM vs AH	0.958	0.792	0.934	0.602
AOM vs AB	0.991	0.969	0.998	0.993
ABH vs AB	0.998	0.812	0.987	0.963

R^2X: the variation displayed by all components in the model; Q^2Y: the accuracy of the predicted class membership by the model.

Table S4. Potential biomarkers and the trends associated with precancerous lesions in colon cancer ("↑," increase in signal; "↓," decrease in signal)

No.	RT-EM	Proposed Molecular Formula	Metabolites	AOM group /Control group		AH group /AOM group		Metabolite pathway	Type of metabolites	Ref.
Negative model					VIP value		VIP value			
1	2.65_141.0780n	$C_6H_6N_4O_2$	1-methylxanthine	↑*	1.6	↓***	1.38	Microbial metabolism in diverse environments	Purine	[1]
2	2.81_239.0915m/z	$C_{12}H_{16}O_5$	3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	↑*	1.63	↓**	1.2	Fatty acid biosynthesis	Fatty acids	[1, 2]
3	2.93_161.0485n	$C_9H_9NO_2$	4-Oxo-1-(3-pyridyl)-1-butanone	-	-	↑**	6.52	Microbial metabolism in diverse environments	Carbonyl compounds	
4	3.44_327.0868m/z	$C_9H_8O_3$	Coumaric acid	↓*	2.22	↑*	1.32		Cinnamic acids	[3]
5	3.50_315.1224m/z	$C_{18}H_{20}O_5$	Unknown 1	-	-	↑***	7.68			
6	3.74_345.2249m/z	$C_{18}H_{34}O_6$	Sorbitan laurate	↑*	7.09	↓	1.83	Fatty acid metabolism	Fatty acid esters	
7	3.95_313.1073m/z	$C_{14}H_{14}N_6O_3$	7, 8-Dihydropteroic acid	↑**	12.11	↓	3.17	Folate biosynthesis	Pterins and derivatives	

8	4.67_297.1113m/z	$C_{11}H_{23}O_7P$	Enterolactone	-	-	↑**	17.16		Furanoid lignans	[1, 3, 4]
9	5.48_253.0497m/z	$C_{15}H_{10}O_4$	Chrysin	-	-	↑**	4.35			
10	5.58_258.1813n	$C_{14}H_{26}O_4$	Tetradecanedioic acid	↓	1.57	↑***	5.73	Fatty acid metabolism	Fatty acids	[3, 4]
11	6.33_425.2515n	$C_{19}H_{40}NO_7P$	LysoPE(14:0/0:0)	↓	5.84	↑*	5.85	Glycerophospholipid metabolism	Glycerophospholipids	[5]
12	6.68_439.2676n	$C_{20}H_{42}NO_7P$	LysoPE(0:0/15:0)	↓	6.01	↑*	14.7	Glycerophospholipid metabolism	Glycerophospholipids	
13	6.71_476.2754m/z	$C_{23}H_{44}NO_7P$	LysoPE(18:2(9Z,12Z)/0:0)	↓*	5.26	↓	1.01	Glycerophospholipid metabolism	Glycerophospholipids	[1]
14	6.80_439.2673n	$C_{20}H_{42}NO_7P$	LysoPE(15:0/0:0)	↓	6.1	↑*	7.82	Glycerophospholipid metabolism	Glycerophospholipids	[5]
15	7.40_483.2704m/z	$C_{22}H_{45}O_9P$	LysoPG(16:0/0:0)	↓	9.42	↑**	12.23	Glycerophospholipid metabolism	Glycerophospholipids	
16	7.63_376.2950n	$C_{24}H_{40}O_3$	Lithocholic acid	↑	2.8	↓**	5.06	Secondary bile acid biosynthesis	Bile acid	[1]
17	7.63_567.1997m/z	$C_{17}H_{16}O_4$	Unknown 2	↑	4.14	↑**	8.24			
18	7.80_493.2910m/z	$C_{24}H_{47}O_8P$	PA(8:0/13:0)	↓	5.91	↑**	20.13	Glycerophospho	Glycerophos	

								lipid metabolism	pholipids	
19	8.42_511.2998m/z	$C_{24}H_{49}O_{9}P$	1-stearoylglycerophosphoglycerol	↓*	11.26	↑	5.57	Glycerophospholipid metabolism	Glycerophospholipids	[1, 4]
20	8.43_495.3053m/z	$C_{22}H_{4}0N_{8}O_{5}$	Postin	↓	5.92	↑*	1.02		Amino acids	
	Positive model									
21	4.98_382.3199n	$C_{27}H_{42}O$	Cholesta-4,6-dien-3-one	↑**	7.03	↑	2.11	Fatty acid metabolism	Cholestane steroids	
22	5.94_390.2773n	$C_{24}H_{38}O_{4}$	(5beta,12beta)-12-Hydroxy-3-oxocholan-24-oic acid	↓	3.02	↑**	6.22	Fatty acid metabolism	Bile acid	
23	6.46_392.2928n	$C_{24}H_{40}O_{4}$	Isodeoxycholic acid	↓	7.24	↑*	6.76		Bile acid	
24	6.89_519.3341n	$C_{26}H_{5}0NO_{7}P$	LysoPC(18:2(9Z,12Z)/0:0)	↓	1.55	↑**	8.12	Glycerophospholipid metabolism	Glycerophospholipids	[6]
25	7.32_495.3330n	$C_{21}H_{44}NO_{7}P$	LysoPE(16:0/0:0)	↑	1.46	↑**	15.19	Glycerophospholipid metabolism	Glycerophospholipids	[1, 5]
26	7.52_521.3490n	$C_{26}H_{52}NO_{7}P$	LysoPC(18:1(11Z)/0:0)	↓	4.23	↑**	7.77	Glycerophospholipid metabolism	Glycerophospholipids	[6]

Table S5. Potential biomarkers and the trends associated with precancerous lesions in colon cancer ("↑", increase in signal; "↓", decrease in signal)

No.	RT-EM	Proposed Molecular Formula	Metabolites	AB group /AOM group		ABH group /AB group		Metabolite pathway	Type of metabolites	Ref.
Negative model					VIP value		VIP value			
27	3.66_349.0015m/z	$C_{15}H_{10}O_{8}S$	Unknown 3	-	-	↑*	8.3			
28	3.77_269.0450m/z	$C_{15}H_{12}O_{6}$	Isocarthamidin	-	-	↑*	8.37		Flavonoids	
29	3.79_301.0708m/z	$C_{16}H_{14}O_{6}$	Hesperetin	↑*	1.20	↑**	9.21		Flavonoids	[4]
30	3.84_333.0071m/z	$C_{15}H10O_{7}S$	Unknown 4	↑*	4.18	↓*	5.81			
31	4.05_285.0404m/z	$C_{15}H_{1}0O_{6}$	Luteolin	-	-	↑**	6.31		Flavonoids	
32	4.18_429.1926m/z	$C_{24}H_{32}O_{8}$	Unknown 5	↑*	5.37	↓	4.06			
33	4.46_269.0452m/z	$C_{15}H_{1}0O_{5}$	Apigenin	-	-	↑**	10.17		Flavonoids	
34	4.57_514.2822m/z	$C_{26}H_{45}NO_{7}S$	Taurocholic acid	↑*	6.31	-	-	Primary bile acid biosynthesis, Taurine and hypotaurine metabolism	Bile acid	[1, 4]
8	4.67_297.1113m/z	$C_{18}H_{18}O_{4}$	Enterolactone	↓***	6.15	-	-		Furanoid lignans	[1]
35	5.35_405.2615m/z	$C_{24}H_{38}O_{5}$	7-ketodeoxycholic acid	↑*	5.95	↓*	8.06		Bile acid	[1, 4]
36	5.37_407.2770m/z	$C_{24}H_{40}O_{5}$	3a,6b,7b-Trihydroxy-5b-cholanoic acid	↑**	6.58	↓*	8.09		Bile acid	[1]
37	5.47_392.2899n	$C_{24}H_{40}O_{4}$	Deoxycholic acid	↓***	11.99	↓**	1.89	Secondary bile acid biosynthesis	Bile acid	[1, 3, 4]

38	5.51_596.3534n	$C_{33}H_{48}N_4O_6$	Stercobilinogen	↓***	10.11	↓	1.08		Bilirubins	[1]
39	5.66_389.2669m/z	$C_{24}H_{38}O_4$	Nutriacholic acid	↓***	5.64	↓	1.01		Bile acid	[1, 4]
23	6.46_392.2903n	$C_{24}H_{40}O_4$	Isodeoxycholic acid	↓***	11.08	↓	3.81		Bile acid	
40	7.30_453.2826n	$C_{21}H_{44}NO_7P$	LysoPE(0:0/16:0)	↓**	5.55	↑*	3.54		Glycerophospholipids	
41	7.52_509.2856m/z	$C_{24}H_{47}O_9P$	LysoPG(18:1(9Z)/0:0)	↓**	5.37	-	-		Glycerophospholipids	
	Positive Model									
42	5.36_408.2882n	$C_{24}H_{40}O_5$	Cholic acid	↑**	5.58	↓*	5.54	Secondary bile acid biosynthesis	Bile acid	[1, 4]
43	6.46_392.2928n	$C_{24}H_{40}O_4$	Ursodeoxycholic acid	↓***	6.32	↓**	1.28		Bile acid	[4, 7]

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