Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting information

Halofuginone reduces inflammatory responses of DSS-induced colitis through metabolic reprogramming

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Table S1.

Biomarkers identified in the liver and spleen of mice induced by DSS

No.	R.T.	M/z	Ion modes	Tissue	Metabolite	Mass error	Method
1	1.10	532.3594	+	Liver	LysoPC(O-18:0)	27	Amide
2	1.10	544.3495	+	Liver	LysoPC(20:4(5Z,8Z,11Z,14Z))	17	Amide
3	1.11	556.3499	+	Liver	LysoPE(0:0/22:2(13Z,16Z))	22	Amide
4	1.12	768.5413	+	Liver	PE(22:1(13Z)/14:0)	13	Amide
5	1.16	767.4879	+	Liver	PG(18:3(6Z,9Z,12Z)/18:3(6Z,9Z,12Z))	2	Amide
6	1.17	768.4911	+	Liver	PS(15:0/20:5(5Z,8Z,11Z,14Z,17Z))	13	Amide
7	1.22	510.3483	+	Liver	LysoPC(17:0)	13	Amide
8	1.38	544.3492	+	Liver	LysoPC(20:4(5Z,8Z,11Z,14Z))	17	Amide
9	1.81	756.5586	+	Liver	PC(18:3(9Z,12Z,15Z)/16:0)	6	Amide
10	2.16	757.5628	+	Liver	PA(18:0/22:2(13Z,16Z))	15	Amide
11	2.16	760.5882	+	Liver	PC(16:0/18:1(11E))	4	Amide
12	2.16	761.5930	+	Liver	PA(18:0/22:0)[U]	16	Amide
13	2.16	762.5962	+	Liver	PC(14:0/20:0)	5	Amide
14	3.53	205.0994	+	Liver	L-Tryptophan	10	Amide
15	6.14	133.0612	+	Liver	L-Asparagine	3	Amide
16	7.00	175.1206	+	Liver	L-Arginine	13	Amide
17	7.70	156.0781	+	Liver	L-Histidine	17	Amide
18	1.33	797.5299	-	Liver	PG(16:0/22:4(7Z,10Z,13Z,16Z))	4	Amide
19	3.64	134.0476	-	Liver	Adenine	2	Amide
20	4.90	268.1043	-	Liver	Asparaginyl-Histidine/Histidinyl-Asparagine	3	Amide
21	7.21	503.1626	-	Liver	Maltotriose	1	Amide
22	1.05	703.4604	+	Spleen	PG(18:3(9Z,12Z,15Z)/13:0)	8	Amide
23	1.05	704.4635	+	Spleen	PS(18:2(9Z,12Z)/12:0)	19	Amide
24	1.06	675.4285	+	Spleen	PG(12:0/15:0)	11	Amide
25	1.15	813.4551	+	Spleen	PI(13:0/18:3(6Z,9Z,12Z))	3	Amide
26	1.19	488.3040	+	Spleen	Glycocholic acid	11	Amide
27	1.20	500.3040	+	Spleen	Taurodeoxycholic acid	0	Amide
28	1.51	769.5944	+	Spleen	PA(P-20:0/22:2(13Z,16Z))	20	Amide
29	2.49	808.5193	+	Spleen	PS(20:5(5Z,8Z,11Z,14Z,17Z)/18:1(9Z))	8	Amide
30	1.21	172.9902	-	Spleen	4-Hydroxybenzenesulfonic acid	7	Amide
31	1.37	202.1066	-	Spleen	Acetylcarnitine	9	Amide
32	2.38	806.4922	-	Spleen	PS(18:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	6	Amide
33	2.41	782.4922	-	Spleen	PS(18:0/18:4(6Z,9Z,12Z,15Z))	7	Amide
34	2.80	566.3417	-	Spleen	PS(21:0/0:0)	8	Amide

Table S2.

Significant changes of metabolome in liver and spleen tissue of mice induced by DSS

Metabolite	CON	DSS	HF	POS	Fold-change [a]	T-test [a]	Fold-change [b]	T-test [b]
LysoPC(O-18:0)	1.37±0.13	0.93±0.08	1.07±0.12	1.15±0.15	-0.32	0.01	0.16	0.33
LysoPC(20:4(5Z,8Z,11Z,14Z))	2.33±0.12	1.96±0.09	2.21±0.12	2.25±0.14	-0.16	0.02	0.13	0.11
LysoPE(0:0/22:2(13Z,16Z))	0.84±0.11	0.54±0.04	0.63±0.03	0.70±0.07	-0.36	0.02	0.15	0.14
PE(22:1(13Z)/14:0)	1.31±0.09	1.60±0.07	0.11±0.01	0.14±0.01	0.22	0.02	-0.93	0.00
PG(18:3(6Z,9Z,12Z)/18:3(6Z,9Z,12Z))	3.01±0.21	3.75±0.17	0.24±0.03	0.27±0.01	0.25	0.02	-0.94	0.00
PS(15:0/20:5(5Z,8Z,11Z,14Z,17Z))	1.33±0.09	1.65±0.08	0.12±0.01	0.14±0.01	0.24	0.02	-0.92	0.00
LysoPC(17:0)	0.32±0.06	0.63±0.10	0.24±0.03	0.21±0.04	0.99	0.02	-0.62	0.00
LysoPC(20:4(5Z,8Z,11Z,14Z))	1.20±0.08	0.98±0.04	1.16±0.08	1.10±0.08	-0.18	0.02	0.18	0.06
PC(18:3(9Z,12Z,15Z)/16:0)	15.29±0.67	12.62±0.99	14.11±0.74	10.30±0.61	-0.17	0.05	0.12	0.25
PA(18:0/22:2(13Z,16Z))	2.51±0.10	1.99±0.08	2.33±0.12	1.65±0.04	-0.20	0.00	0.17	0.04
PC(16:0/18:1(11E))	12.06±0.38	10.33±0.31	11.26±0.42	10.71±0.43	-0.14	0.00	0.09	0.10
PA(18:0/22:0)[U]	4.03±0.16	3.44±0.16	3.89±0.17	3.76±0.19	-0.14	0.02	0.13	0.08
PC(14:0/20:0)	1.03±0.04	0.87±0.05	1.02±0.04	0.98±0.05	-0.15	0.03	0.16	0.05
L-Tryptophan	5.33±0.36	6.49±0.21	5.86±0.24	4.86±0.38	0.22	0.01	-0.10	0.07
L-Asparagine	1.62±0.08	2.09±0.09	1.68±0.11	2.47±0.18	0.29	0.00	-0.19	0.01
L-Arginine	0.67±0.06	0.97±0.06	0.72±0.15	0.66±0.05	0.44	0.00	-0.26	0.14
L-Histidine	5.35±0.85	8.01±0.26	6.65±0.14	8.36±0.39	0.50	0.01	-0.17	0.00
PG(16:0/22:4(7Z,10Z,13Z,16Z))	0.47±0.05	1.01±0.18	0.82±0.12	0.67±0.04	1.15	0.02	-0.19	0.40
Adenine	1.17±0.14	1.74±0.15	1.25±0.18	1.20±0.14	0.49	0.01	-0.28	0.05
Asparaginyl-Histidine/Histidinyl-Asparagine	20.79±4.70	43.85±1.76	28.54±2.58	39.88±2.66	1.11	0.00	-0.35	0.00
Maltotriose	29.35±1.22	18.50±4.27	22.84±3.05	13.20±3.01	-0.37	0.04	0.23	0.42
PG(18:3(9Z,12Z,15Z)/13:0)	0.15±0.06	0.63±0.10	0.38±0.08	0.78±0.10	3.15	0.00	-0.40	0.06
PS(18:2(9Z,12Z)/12:0)	18.53±2.23	6.79±1.74	12.16±2.36	5.18±2.20	-0.63	0.00	0.79	0.09
PG(12:0/15:0)	0.74±0.19	0.06±0.03	0.20±0.07	0.08±0.06	-0.92	0.00	2.48	0.08
PI(13:0/18:3(6Z,9Z,12Z))	4.12±0.36	5.99±0.30	5.23±0.29	5.87±1.25	0.46	0.00	-0.13	0.09
Glycocholic acid	1.86±0.16	2.68±0.13	2.35±0.13	2.63±0.56	0.44	0.00	-0.12	0.10
Taurodeoxycholic acid	0.81±0.07	1.17±0.06	1.02±0.06	1.15±0.25	0.45	0.00	-0.13	0.09
PA(P-20:0/22:2(13Z,16Z))	1.58±0.24	2.65±0.13	2.28±0.20	1.35±0.11	0.68	0.00	-0.14	0.14
PS(20:5(5Z,8Z,11Z,14Z,17Z)/18:1(9Z))	1.92±0.19	2.90±0.13	2.58±0.09	1.79±0.13	0.51	0.00	-0.11	0.07
4-Hydroxybenzenesulfonic acid	0.76±0.07	1.11±0.04	1.03±0.03	0.71±0.04	0.46	0.00	-0.08	0.15
Acetylcarnitine	1.84±0.12	1.39±0.06	1.73±0.07	1.47±0.08	-0.25	0.00	0.25	0.00
PS(18:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	0.69±0.03	1.04±0.06	0.95±0.06	1.06±0.04	0.51	0.00	-0.09	0.29
PS(18:0/18:4(6Z,9Z,12Z,15Z))	6.98±0.86	1.59±0.37	2.06±0.39	2.03±0.24	-0.77	0.00	0.29	0.40
PS(21:0/0:0)	0.81±0.08	1.59±0.24	1.35±0.10	2.11±0.17	0.95	0.01	-0.15	0.37

Intensity of metabolite is expressed as mean ± SEM (n=8/group).

Superscript: a, Fold-change and T-test value is calculated after comparison between DSS group and CON group; b, Fold-change and T-test value is calculated after comparison between HF group and DSS group.

Table S3.

Scoring system to calculate the disease activity index (DAI)[a]

Score	Weight loss	Stool consistency	Visible blood in feces
0	None	normal	none
1	1-5%		
2	6-10%	loose	Slight bleeding
3	11-15%		
4	>15%	diarrhea	Gross bleeding

[a]DAI value is calculated as the sum of the scores for weight loss, stool consistency and blood in feces.

Table S4.

Histological scoring system for DSS-induced colitis[a]

Feature	Score	Description
Severity of inflammation	0	none
	1	mild
	2	moderate
	3	severe
Extent of inflammation	0	none
	1	mucosa
	2	Mucosa and submucosa
	3	transmural
Crypt damage	0	none
	1	1/3 damaged
	2	2/3 damaged
	3	Crypt lost, surface epithelium present
	4	Crypt lost, surface epithelium lost

[a]Scores were calculated by adding the score for the three parameters giving a maximum score of 10.

Table S5.

Primer sequences for RT-PCR

Protein (Mus)	**Gene**	**Sequence, forward/reverse**
Hypoxia-inducible factor 1-alpha	HIF1A(MOUSE)	5' TGGAAGGTATGTGGCATTTATTTGG 3'
		5' ACCAACAGGGTGGGCAGAAC3'
Tumor necrosis factor-alpha	TNFA(MOUSE)	5' GTCCCCAAAGGGATGAGAAGT 3'
		5' TTTGCTACGACGTGGGCTAC 3'
β-Actin	ACTB	5' CTGTCGAGTCGCGTCCACCC 3'
□	□	5' GCTTTGCACATGCCGGAGCC 3'

Figure S1.

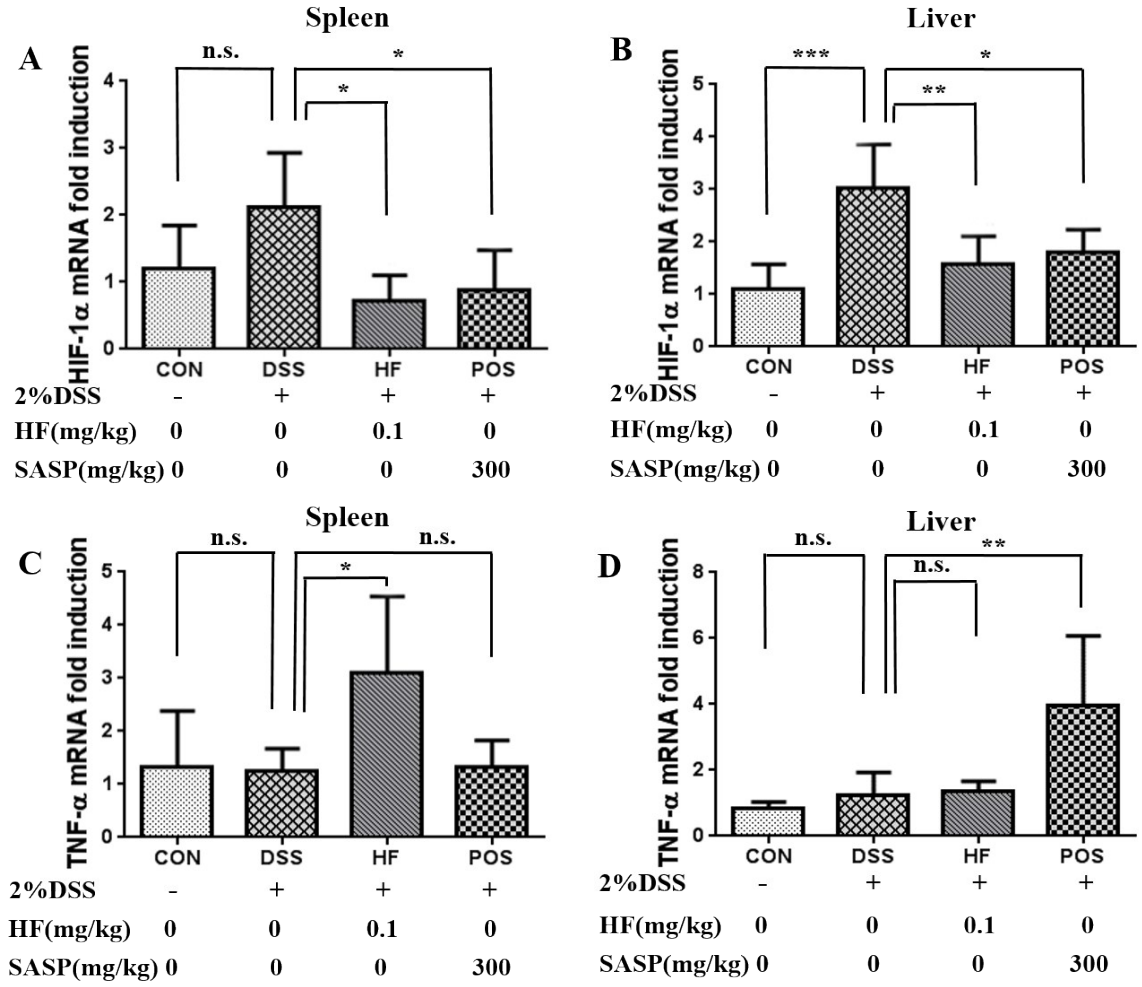

A
Spleen
HIF-1α mRNA fold induction
n.s.
*
*
CON DSS HF POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300
B
Liver
HIF-1α mRNA fold induction
***
**
*
CON DSS HF POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300
C
Spleen
TNF-α mRNA fold induction
n.s.
*
n.s.
CON DSS HF POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300
D
Liver
TNF-α mRNA fold induction
n.s.
n.s.
**
CON DSS HF POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300

Figure S2.

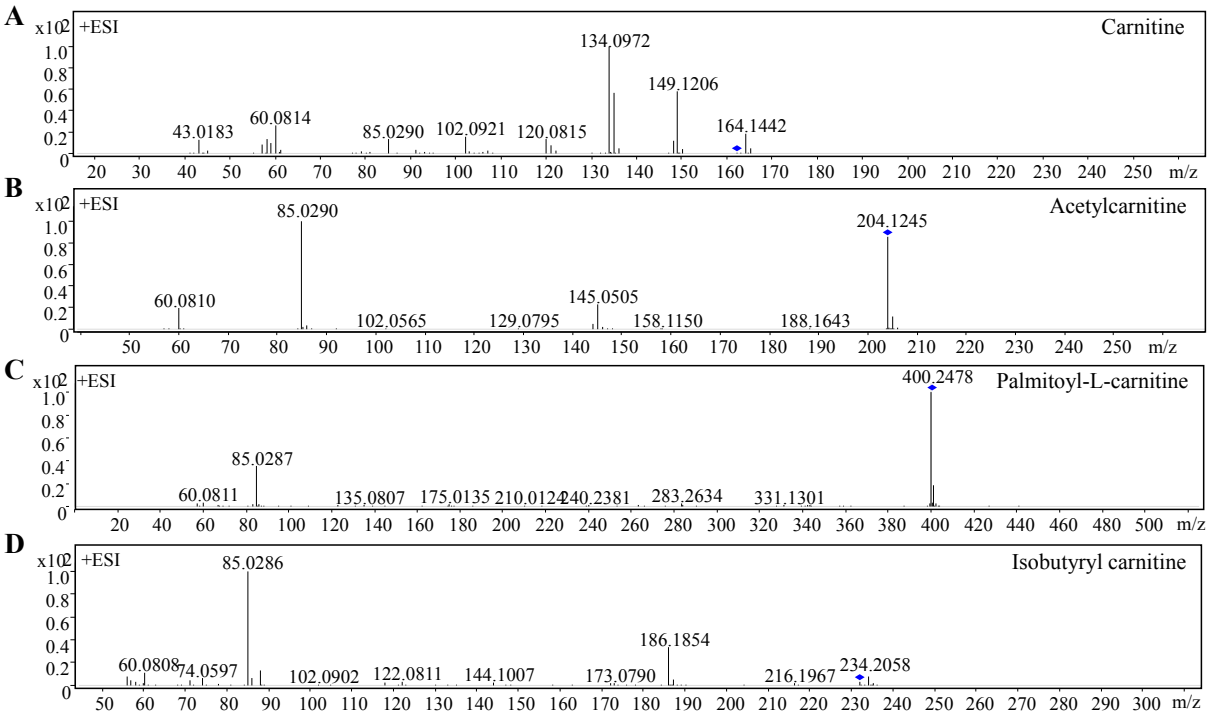

A
+ESI
Carnitine
134.0972
149.1206
164.1442
43.0183
60.0814
85.0290
102.0921
120.0815
B
+ESI
Acetylcarnitine
85.0290
204.1245
60.0810
145.0505
102.0565
129.0795
158.1150
188.1643
C
+ESI
Palmitoyl-L-carnitine
400.2478
85.0287
60.0811
135.0807
175.0135
210.0124
240.2381
283.2634
331.1301
D
+ESI
Isobutyryl carnitine
85.0286
186.1854
234.2058
60.0808
74.0597
102.0902
122.0811
144.1007
173.0790
216.1967
m/z

Figure S3.

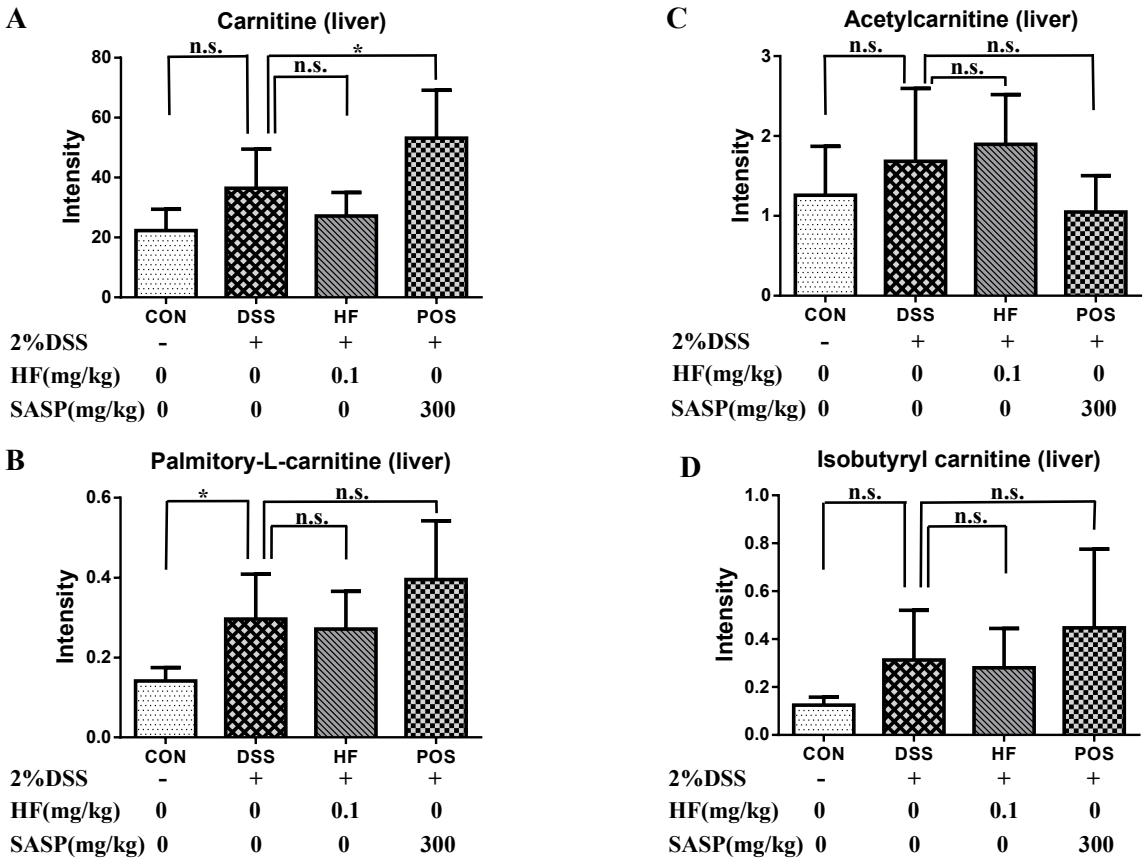
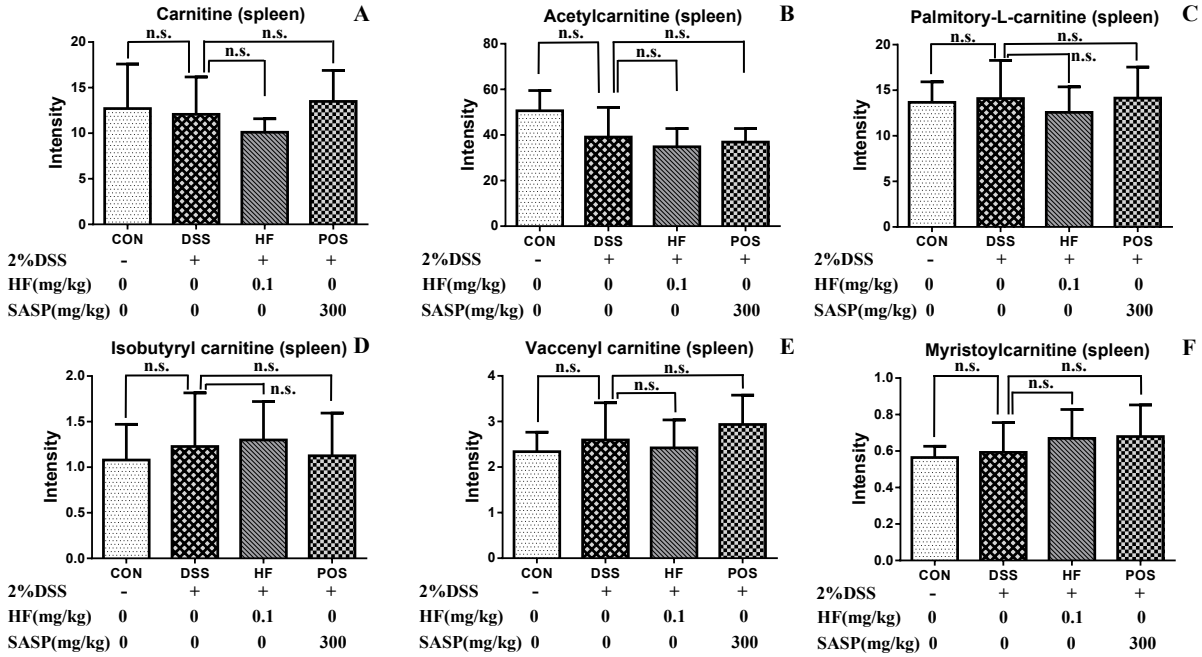

A
Carnitine (liver)
Intensity
n.s.
n.s.
*
CON
DSS
HF
POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300
B
Palmitory-L-carnitine (liver)
Intensity
*
n.s.
n.s.
CON
DSS
HF
POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300
C
Acetylcarnitine (liver)
Intensity
n.s.
n.s.
n.s.
CON
DSS
HF
POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300
D
Isobutyryl carnitine (liver)
Intensity
n.s.
n.s.
n.s.
CON
DSS
HF
POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300

Figure S4.


Carnitine (spleen)
A
Acetylcarnitine (spleen)
B
Palmitory-L-carnitine (spleen)
C
Isobutyryl carnitine (spleen)
D
Vaccenyl carnitine (spleen)
E
Myristoylcarnitine (spleen)
F
Intensity
n.s.
CON DSS HF POS
2%DSS - + + +
HF(mg/kg) 0 0 0.1 0
SASP(mg/kg) 0 0 0 300