The metabolic responses to hepatitis B virus infection shed new light on pathogenesis and targets for treatment

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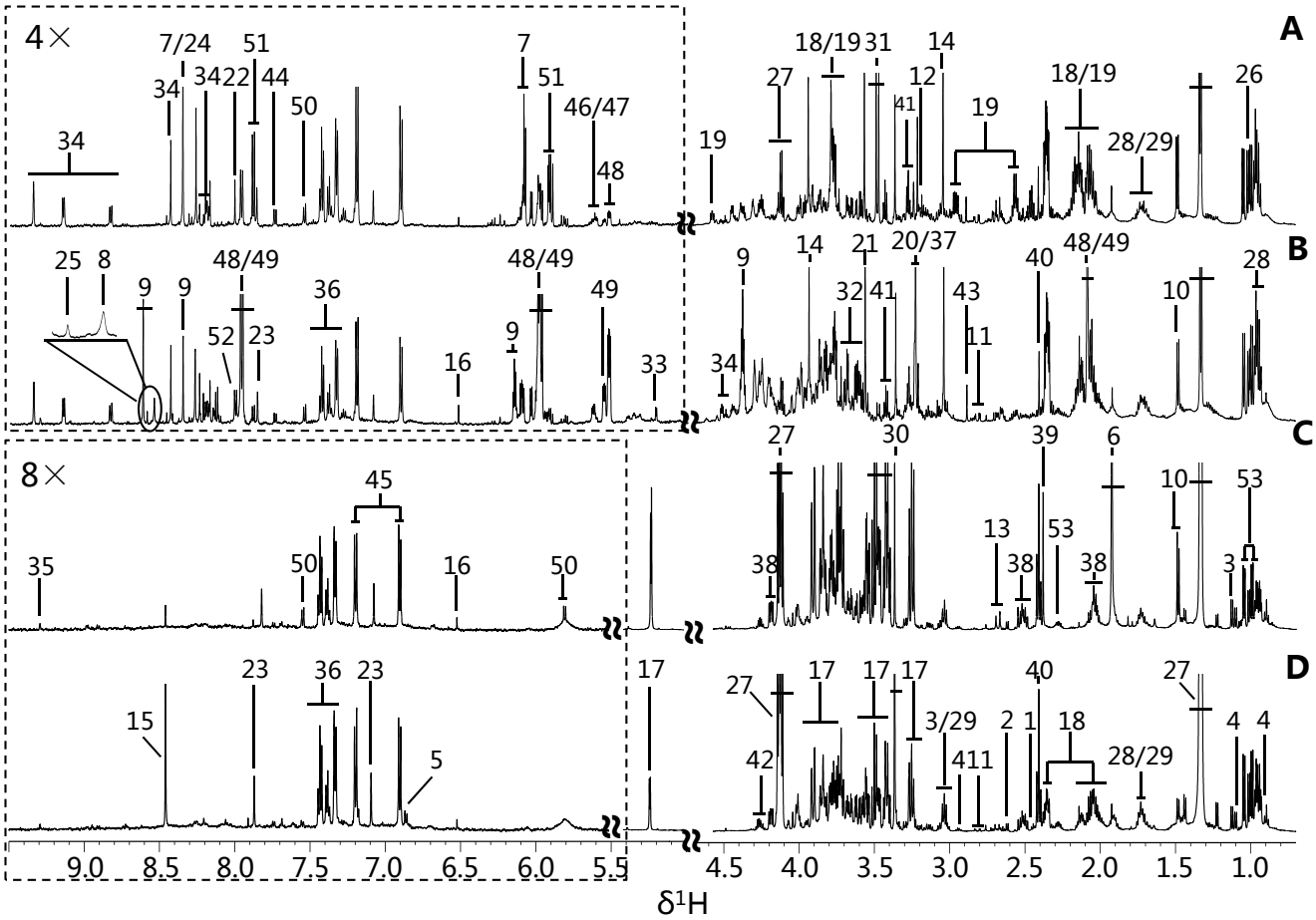

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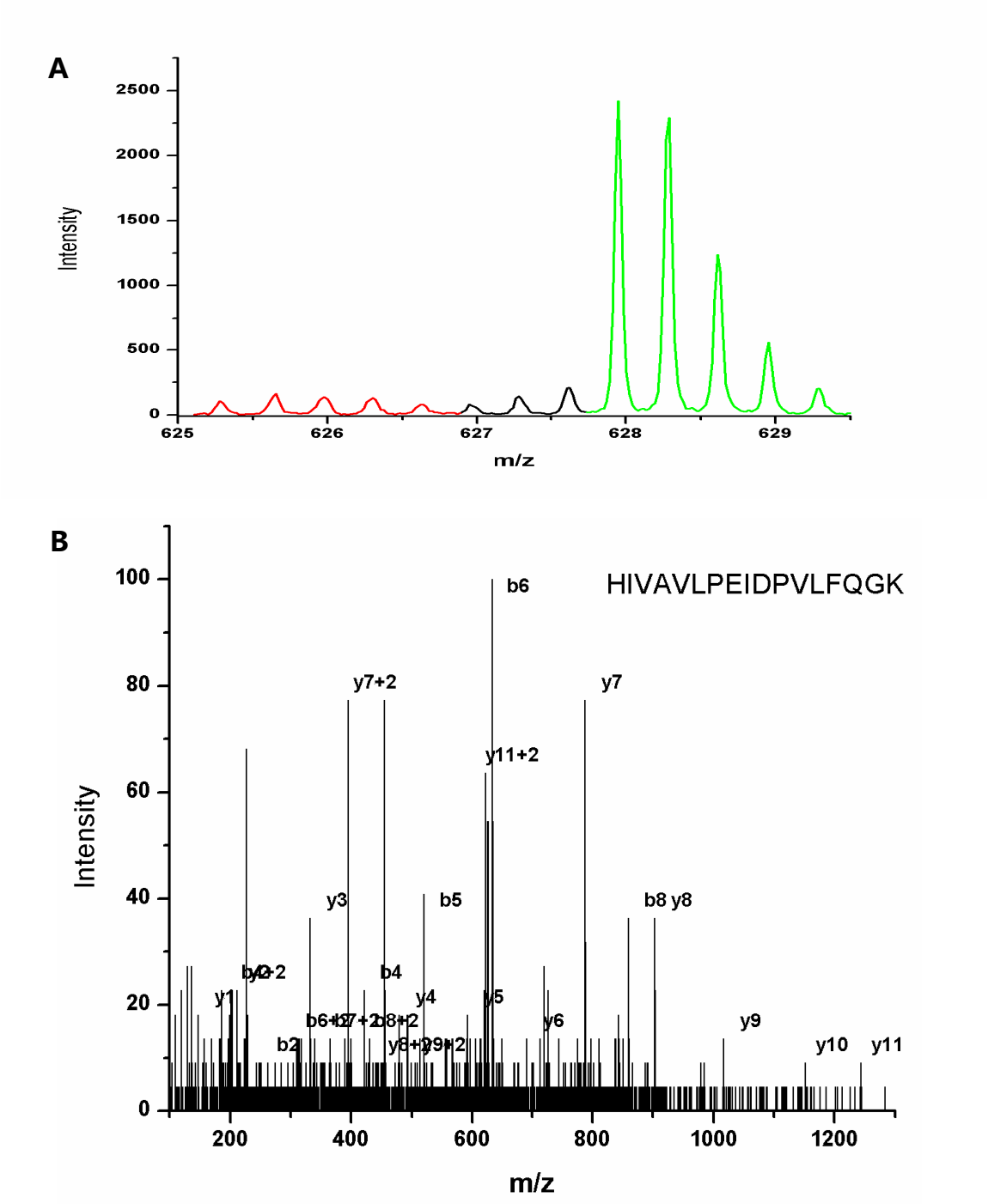

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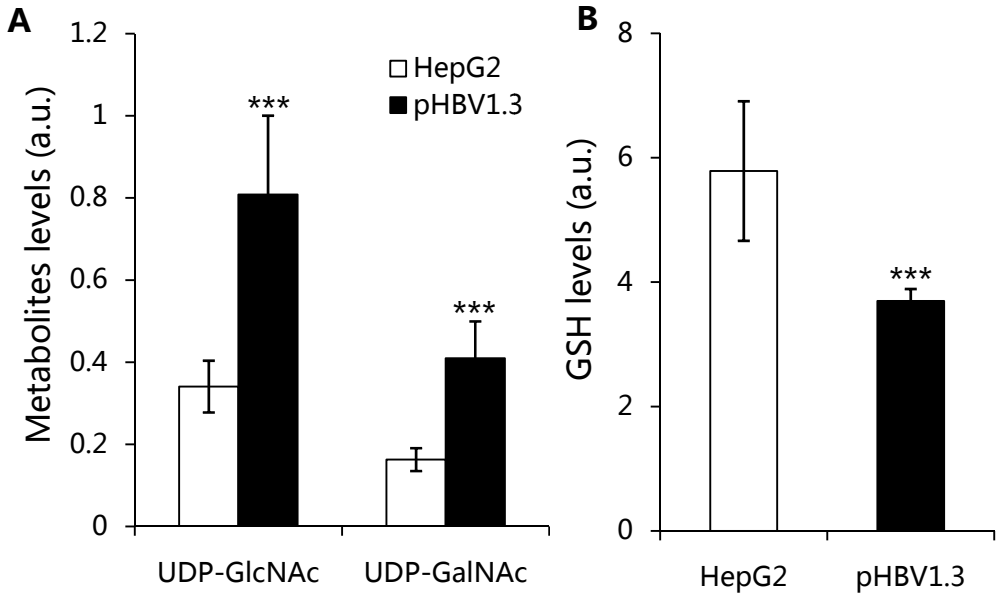

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Table S1. NMR data for the metabolites assignment[#]

Key	Metabolite	Assignment	δ^{1}H(multiplicity[a])	δ^{13}C	Sample[c]
1	2-Oxoglutarate (2-OG)	γCH_2	2.45(t)	33.7	M
		βCH_2	3.01(t)	39	
		2-C		207.8	
2	2-Oxoleucine (2-O-Leu)	CH_3	0.94(d)	24.4	M
		CH_2	2.618(d)	51.2	
		CH	2.12(m)	30.1	
3	2-Oxoisovalerate (2-O-Val)	CH_3	1.12(d)	19.4	M
		CH	3.02(m)	39.9	
4	2-Oxoisoleucine (2-O-Ile)	α-CH_3	1.10(d)	16.7	M
		γ-CH_3	0.89(t)	13.6	
		CH	2.94(m)	46.7	
		CH_2	1.70/1.45(m)	27.4	
5	4-Hydroxyphenylpyruvate	CH	6.86(d)	118.9	M
	(4HPPA)	CH	7.18(d)	134.0	
		CH_2	4.01(s)	b	
6	Acetate	CH_3	1.92(s)	26.1	M
		COO^-		184.2	
7	Adenosine	CH-ring	8.34(s)	143.2	C
		CH-ring	8.178(s)	155.4	
		C1H-ribose	6.04(d)	89.5	
		C2H-ribose	4.77(dd)	76.7	
		C4H-ribose	4.28(dt)	88.4	
		C3H-ribose	4.42(dd)	73	
		C5H-ribose	3.85/3.9(dd)	64.1	
8	Adenosine 5'-diphosphate	CH-ring	8.54(s)	142.4	C
	(ADP)	CH-ring	8.27(s)	155.9	
		C1H-ribose	6.14(d)	89.9	
		C2H-ribose	4.61(m)	72.8	
		C3H-ribose	4.51(m)	73.1	
		C4H-ribose	4.38(m)	86.8	
		C5H-ribose	4.01(m)	66.2	
9	Adenosine 5'-monophosphate	CH-ring	8.61(s)	143.3	C
	(AMP)	CH-ring	8.27(s)	155.9	
		C1H-ribose	6.14(d)	89.9	
		C2H-ribose	4.77(m)	76.8	
		C3H-ribose	4.51(m)	73.1	
		C4H-ribose	4.37(m)	86.77	
		C5H-ribose	4.01(m)	66.2	
10	Alanine (Ala)	α-CH	3.78(q)	53.1	C, M

		β-CH_3	1.48(d)	18.9	
		COO(H)		178.5	
11	Aspartate (Asp)	α-CH	3.90(m)	54.8	C, M
		β-CH	2.69(dd)	39.2	
		β'-CH	2.81(dd)		
		γ COO(H)		180.6	
		COO(H)		177.4	
12	Choline	α-CH_2	3.99(m)	70.0	C
		β-CH_2	3.56(m)	58.6	
		N-CH_3	3.20(s)	56.6	
13	Citrate	α, γ-CH	2.68(dd)	47.1	M
		α', γ'-CH	2.52(dd)	47.1	
		β C		77.4	
		C1,5		180.6	
		C6		182.5	
14	Creatine	CH_3	3.04(s)	39.6	C
		CH_2	3.93(s)	56.5	
		N=C		159.6	
		COO(H)		177.3	
15	Formate	H COO^-	8.46(s)	b	C, M
16	Fumarate	C2,3H	6.52(s)	138.2	C, M
		COO^-		b	
17	Glucose	αC1H	5.24(d)	95.5	M
		βC1H	4.65(d)	99.3	
			3.25(t)	77.5	
			3.39-3.55		
			3.69-3.93		
18	Glutamate (Glu)	α-CH	3.76(m)	57.3	C, M
		β-CH_2	2.08(m)	29.8	
		γ-CH_2	2.35(m)	36.2	
		δCO		183.9	
		COO(H)		177.4	
19	Glutathione (GSH)	Glu α	3.78(t)	56.8	C
		Glu β	2.16(m)	29.2	
		Glu γ	2.56(m)	34.1	
		Cys α	4.57(dd)	58.6	
		Cys β	2.94(m)	28.3	
		Gly α	b	b	
		C=O		174.5	
				177.2	
20	Glycerolphosphocholine	1-CH_2	3.60(dd)	b	C

	(GPC)	2-CH	3.89(m)	b	
		3-CH_2	3.72(dd)	b	
		α-CH_2	4.32(t)	b	
		β-CH_2	3.68(t)	b	
		N-CH_3	3.23(s)	56.7	
21	Glycine (Gly)	α-CH_2	3.56(s)	44.1	C
		COO(H)		175.1	
22	Guanosine	CH-ring	8.0(s)	140.9	C
		C1H-ribose	5.92(d)	91.7	
		C3H-ribose	4.41(dd)	73.4	
		C4H-ribose	4.24(dt)	88.21	
		C5H-ribose	3.84	64	
23	Histidine (His)	C4H,ring	7.08(s)	119.8	C, M
		C2H,ring	7.85(s)	137.0	
		COO(H)		176.8	
24	Inosine	CH-ring	8.35(s)	143.2	C
		CH-ring	8.24(s)	155.6	
		C1H-ribose	6.10(d)	91.2	
		C2H-ribose	4.78(dd)	76.65	
		C3H-ribose	4.44(dd)	b	
		C4H-ribose	4.28(dt)	b	
		C5H-ribose	3.87(dd)	b	
25	Inosine-5'-monophosphate	CH-ring	8.59(s)	142.5	C
	(IMP)	CH-ring	8.24(s)	149.1	
		C1H-ribose	6.15(d)	89.9	
		C2H-ribose	4.77	77.1	
		C3H-ribose	4.51(dd)	73.2	
		C4H-ribose	4.37(dt)	87.5	
		C5H-ribose	4.03(m)	66.2	
26	Isoleucine (Ile)	α-CH	3.67(m)	62.3	C, M
		β-CH	1.98(m)	38.6	
		γ-CH	1.27(m)	27.8	
		γ'-CH	1.47(m)		
		δ-CH_3	0.94(t)	13.9	
		3- CH_3	1.01(d)	17.3	
		COO(H)		176.4	
27	Lactate	α-CH	4.11(q)	71.2	C, M
		β-CH_3	1.33(d)	22.7	
		COO^-		185.2	
28	Leucine (Leu)	α-CH	3.73(t)	56.0	C, M
		β-CH_2	1.72(m)	42.6	

		γ-CH	1.69(m)	26.8	
		δ-CH_3	0.97(d)	24.7	
		δ'-CH_3	0.96(d)	23.7	
		COO(H)		177.3	
29	Lysine (Lys)	α-CH	3.74(t)	56.0	C, M
		β-CH_2	1.90(m)	32.1	
		γ-CH_2	1.46(m)	24.3	
		δ-CH_2	1.72(m)	29.3	
		ε- CH_2	3.01(t)	41.6	
		COO(H)		177.3	
30	Methanol	CH_3	3.36(s)	51.6	C, M
31	Monomethyl phosphate	CH_3	3.47(d)	54.2	C
32	*myo*-Inositol	C1,3H	3.54(dd)	73.31	C
		C2H	4.06(t)	73.1	
		C5H	3.26(t)	75.23	
		C4,6H	3.62(t)	72.01	
33	*N*-acetyl-glucosamine	α-C1H	5.21(d)	93.66	C
	(GlcNAc)	α-C2,5,6H	3.85 (m)		
		α-C3H	3.76	73.46	
		α-C4H	3.46	72.72	
		β-C1H	4.71	97.78	
		β-C2H	3.67	59.51	
		β-C3H	3.53	76.73	
		β-C4,5H	3.46	72.72	
		NA-H	2.05 (s)	24.75	
34	NAD	N5ring	8.20(m)	131.2	C
		N4ring	8.83(d)	148.7	
		N2ring	9.34(s)	142.9	
		N6ring	9.15(d)	145.2	
		A2Hring	8.18(s)	155.6	
		A8H ring	8.43(s)	142.5	
		N1'H	6.09(d)	102.8	
		N2'H	4.48	80.4	
		N5'H2	4.23/4.36	67.6	
		N3'H	4.427	73.3	
		N4'H	4.546	89.7	
		A1'H	6.04(d)	89.4	
		A2'H	4.37	86.5	
		A5'H2	4.21/4.24	68.0	
		A3'H	4.51	73.17	
		A4'H	4.77	76.7	

35	Niacinamide	2-CH	8.94(dd)	b	M
		4-CH	8.25(d)	b	
		5-CH	7.60(dd)	b	
		6-CH	8.71(d)	b	
36	Phenylalanine (Phe)	α-CH	3.99(dd)	58.8	C, M
		β-CH	3.13(dd)	38.9	
		β'-CH	3.27(dd)	38.9	
		C1,ring		137.9	
		C2,6,ring	7.33(m)	132.0	
		C3,5,ring	7.42(m)	131.5	
		C4,ring	7.38(m)	130.5	
		COO(H)		176.7	
37	Phosphocholine (PCho)	α-CH_2	3.60(m)	b	C
		β-CH_2	4.17(m)	69.5	
		N-CH_3	3.22(s)	56.7	
38	Pyroglutamate	5-CH	4.18	61.6	M
		4-CH_2	2.51/2.04(m)	28.4	
		3-CH_2	2.41(m)	32.6	
		COOH		183.5	
		C=O		184.6	
39	Pyruvate	β-CH_3	2.38(s)	b	M
40	Succinate	CH_2	2.40(s)	35.9	C, M
		COO^-		183.6	
41	Taurine	N-CH_2	3.27(t)	50.3	C
		S-CH_2	3.42(t)	38.2	
42	Threonine (Thr)	α-CH	3.59(d)	63.0	C, M
		β-CH_2	4.25(m)	68.6	
		γ-CH_3	1.33(d)	20.8	
		COO(H)		175.4	
43	Trimethylamine (TMA)	CH_3	2.88(s)	47.5	C
44	Tryptophan (Trp)	C4H,ring	7.74(d)	b	C
		C5H,ring	7.20(t)	b	
		C6H,ring	7.29(t)	b	
		C7H,ring	7.54(d)	114.8	
45	Tyrosine (Tyr)	C3,5H,ring	6.90(d)	118.6	C, M
		C2,6H,ring	7.19(d)	133.3	
		C1,ring		129.5	
		α-CH	3.94(dd)	58.9	
		β-CH_2	3.06	39.0	
		C4,ring		157.7	
		COO(H)		177.1	

46	UDP-glucose (UDP-Glc)	G1-H	5.61	98.65	C
		C6,ring	7.96(d)	144.4	
		C5,ring	5.98(d)	105.4	
		C1'H,ribose	5.99(d)	91.3	
		C2'3'H,ribose	4.38(m)	72.2/76.5	
		C4',ribose	4.29(m)	85.9	
		C5'H,ribose	4.26/4.21(m)	67.8	
		G2-H	3.90	73.1	
		G6-H	3.86/3.78	63.36	
		G3-H	3.77	74.7	
		G4-H	3.54	74	
		G5-H	3.47	72	
47	UDP-glucuronate (UDP-GlcA)	C6,ring	7.95(d)	144.5	C
		C1'H,ribose	6.00(d)	91.4	
		C5,ring	5.98(d)	105.4	
		G1-H	5.62	98.1	
		C2'3'H,ribose	4.38(m)	86.6	
		C4'H,ribose	4.29(m)	86.1	
		C5'H,ribose	4.25/4.19	67.8	
		G5-H	4.14(dd)	75.6	
		G2-H	3.79(dd)		
		G4-H	3.59(m)	63.2	
		G3-H	3.51(dd)	72.7	
48	UDP-*N*-acetyl glucosamine	C6,ring	7.96(d)	144.1	C
	(UDP-GlcNAc)	C1'H,ribose	5.98(d)	91.1	
		C5,ring	5.97(d)	105.4	
		C2'3'H,ribose	4.37(m)	72.2/76.5	
		C5'H,ribose	4.25/4.19(m)	67.8	
		C4'H,ribose	4.29(m)	86.0	
		C2,ring		156.6	
		G1-H	5.52(dd)	97.3	
		G2-H	3.99(m)	56.5	
		G3-H	3.82(m)	73.6	
		G4-H	3.55(dd)	72.2	
		G5-H	3.93	75.8	
		G6-H	3.87	63.2	
		NA-H	2.08(s)	24.9	
		NA-C=O		177.4	
49	UDP-*N*-Acetyl Galactosamine	G1-H	5.55(dd)	97.4	C
	(UDP-GalNAc)	G2-H	4.05(m)	71.2	
		G3-H	3.97(dd)	70.3	

		G4-H	3.76(m)	57.4	
		G5-H	3.79	63.6	
		G6-H	3.78	63.6	
		C2'3'H,ribose	4.37(m)	76.6	
		C4',ribose	4.29(m)	85.9	
		C5'H,ribose	4.25/4.19(m)	67.8	
		NA-H	2.089(s)	24.9	
		C1'H,ribose	5.99(d)	91.1	
		C5,ring	5.97(d)	105.4	
		C6,ring	7.96(d)	144.1	
50	Uracil	C5H	5.80(d)	b	C, M
		C6H	7.54(d)	b	
51	Uridine	C6,ring	7.87(d)	144.1	C
		C1'H,ribose	5.92(d)	92.0	
		C5,ring	5.90(d)	105.1	
		C3'H,ribose	4.34(t)	72.2	
		C5'H,ribose	4.12(q)	66.2	
		C4'H,ribose	4.23(t)	86.0	
		C2,ring		156.6	
52	Uridine 5'-diphosphate (UDP)	CH-ring	7.99(d)	b	C
		CH-ring	5.97(d)	b	
		C1H-ribose	5.96(broad, s)	b	
		C3H-ribose	4.43(t)	b	
		C2H-ribose	4.39(t)	b	
		C4H-ribose	4.27(m)	b	
		C5H-ribose	4.23(c)	b	
53	Valine (Val)	α-CH	3.61(d)	63.1	C, M
		β-CH	2.28(m)	31.9	
		γ-CH_3	0.99(d)	19.5	
		γ'-CH_3	1.04(d)	20.7	
		COO(H)		176.8	

[#]The assignment was accomplished with the assistance of a series of two dimensional NMR spectra including $^{1}H-^{1}H$ COSY, $^{1}H-^{1}H$ TOCSY, ^{1}H *J*-resolved, $^{1}H-^{13}C$ HSQC and $^{1}H-^{13}C$ HMBC; Small signals were confirmed by standard compounds as well.

[a]Multiplicity: singlet(s), doublet(d), triplet(t), quartet(q), doublet of doublets(dd), doublet of triplets (dt), multiplet(m)

[b]The signals or the multiplicities were not determined.

[c]C, cell extract; M, medium

Table S2. Coefficients of metabolites with significant difference between HepG2.2.15 cells and HepG2 cells.

Key	Metabolite	δ^1H (ppm)	OPLS-DA coefficient (r)
7	Adenosine	6.086	−0.89
8	Adenosine 5'-diphosphate (ADP)	8.542	+0.88
9	Adenosine 5'-monophosphate (AMP)	8.626	+0.92
11	Aspartate (Asp)	2.802	−0.76
12	Choline	3.210	−0.89
16	Fumarate	6.522	+0.88
19	Reduced glutathione (GSH)	4.570	−0.95
22	Guanosine	8.006	−0.79
24	Inosine	8.242	−0.87
25	Inosine 5'-monophosphate (IMP)	8.590	+0.67
27	Lactate	1.330	−0.82
33	*N*-acetyl-glucosamine (GlcNAc)	5.206	+0.98
37	Phosphocholine (PCho)	3.226	+0.98
47	UDP-glucuronate (UDP-GlcA)	5.622	+0.92
48	UDP-*N*-acetyl galactosamine (UDP-GalNAc)	5.558	+0.98
49	UDP-*N*-acetyl glucosamine (UDP-GlcNAc)	5.522	+0.98
51	Uridine	5.922	−0.98
52	Uridine 5'-diphosphate (UDP)	7.998	+0.98

Positive (+) and negative (−) signs indicate the level of metabolite is higher or lower in HepG2.2.15 compared with HepG2 cells. The cutoff value of |r| is 0.602, n = 10, P < 0.05. The numbers of the metabolites are the same as Table S1.

Table S3. Comparative total fatty acid profiles in HepG2 cells and HepG2.2.15 cells.

Fatty acid	HepG2	HepG2.2.15	*P* value
C14:0	108.74 ± 8.03	217.73 ± 7.63	1.57×10^{-4}
C16:0	1572.17 ± 110.28	1989.49 ± 80.65	1.57×10^{-4}
C18:0	657.56 ± 44.71	878.19 ± 51.19	5.96×10^{-9}
C20:0	6.52 ± 0.42	16.05 ± 0.81	1.49×10^{-17}
C22:0	8.27 ± 0.52	11.55 ±0.46	1.27×10^{-11}
C24:0	7.15 ± 0.51	9.42 ± 0.37	1.57×10^{-4}
Total SFA	2360.41 ± 161.88	3122.44 ± 124.65	6.66×10^{-10}
C16:1n7	373.47 ± 28.45	709.74 ± 43.36	1.57×10^{-4}
C16:1n9	44.72 ± 7.89	184.54 ± 18.47	3.52×10^{-11}
C18:1n7	751.86 ± 70.75	920.67 ± 63.64	3.81×10^{-4}
C18:1n9	1624.46 ± 114.12	2191.01 ± 97.68	5.57×10^{-10}
C20:1n7	46.42 ± 3.42	68.64 ± 2.83	1.57×10^{-4}
C20:1n9	38.30 ± 2.44	76.61 ± 2.97	3.38×10^{-17}
C22:1n9	12.25 ± 0.82	15.76 ± 0.74	8.44×10^{-9}
C24:1n9	9.57 ± 0.67	15.37 ± 0.76	1.57×10^{-4}
Total MUFA	2901.05 ± 215.98	4182.35 ± 181.20	1.57×10^{-4}
C18:3n3	15.37 ± 0.92	10.58 ± 0.29	1.57×10^{-4}
C20:3n3	3.69 ± 0.30	3.18 ± 0.24	6.18×10^{-4}
C20:5n3	57.65 ± 3.93	97.71 ± 3.76	6.89×10^{-15}
C22:6n3	184.72 ± 13.68	252.73 ± 9.31	1.38×10^{-10}
Total n3	261.43 ± 18.68	364.20 ±13.20	1.57×10^{-4}
C18:2n6	212.81 ± 14.12	135.99 ± 4.43	5.87×10^{-9}
C18:3n6	2.47 ± 0.21	7.58 ± 0.30	9.36×10^{-20}
C20:2n6	8.04 ± 0.59	3.21 ± 0.59	4.80×10^{-3}
C20:3n6	39.14 ± 2.77	93.48 ± 3.09	1.57×10^{-4}
C20:4n6	375.53 ± 27.24	443.85 ± 15.21	2.85×10^{-4}
C22:2n6	4.86 ± 0.42	18.79 ± 0.67	1.24×10^{-21}
Total n6	642.85 ± 45.10	702.90 ± 22.31	0.007
C18:2n7	4.78 ± 0.92	72.53 ± 3.60	3.82×10^{-14}
C20:2n7	13.04 ± 0.89	47.84 ± 2.47	1.57×10^{-4}
C20:3n7	9.64 ± 0.67	18.46 ± 0.80	1.57×10^{-4}
C20:3n9	18.00 ± 1.27	280.03 ± 11.76	1.57×10^{-4}
Total PUFA	949.73 ± 67.15	1485.96 ± 50.95	1.57×10^{-4}
Total UFA	3850.78 ± 282.83	5668.31 ± 230.68	1.57×10^{-4}
Total	6211.19 ± 440.83	8790.75 ± 331.68	1.57×10^{-4}

Data are shown as mean ± s.d. (μg fatty acid/g cell), $n = 10$. The significance is tested using *t*-test if the distribution is normal, if not the Mann-Whitney Test is performed. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table S5. List of the primers used for quantitative RT-PCR

Gene Name	Primer sequence (F: forward; R: reverse, 5'-3')
GCLM	F: GGCACAGGTAAAACCAAATAGTAAC
	R: CAAATTGTTTAGCAAATGCAGTCA
GSS	F: GCCCCTAGCCGGTTTGTGCT
	R: CACTGGACCACTCGGGCAGG
GCLC	F: CTGTTGCAGGAAGGCATTGAT
	R: TTCAAACAGTGTCAGTGGGTCTCT
GFAT1	F: ATCTCTCTCGTGTGGACAGC
	R: TGACGCGATTGGTGTGTTCTA
CHKA	F: TGGGCCAAAACTCTATGGCA
	R: ATGTAGCCATTTTCTCGGCG
CHPT1	F: GCTCGTGCTCATCTCCTACTG
	R: CTTCTGGCTTGTTTCCCATCA
CEPT1	F: TGATGGGAAACAGGCAAGAAGA
	R: AATCAGGGTTTGTCCCCAGC
PHOSPHO1	F: CTCCAAACTCAGCCGGGACA
	R: GTCGGTGCATTACCGTGAGC
PCYT1A	F: ATCATCACCCGAATTGTGCG
	R: TTTGTCAACCCTCTCCTGCAA
ACTB	F: TGTGTTGGCGTACAGGTCTTTG
	R: GGGAAATCGTGCGTGACATTAAG
HBV	F: GTTGCCCGTTTGTCCTCTAATTC
	R: GGAGGGATACATAGAGGTTCCTT

Supplementary Methods

Metabolites assignment

The metabolite assignment was accomplished with the assistance of two dimensional NMR spectra including $^{1}H-^{1}H$ COSY, $^{1}H-^{1}H$ TOCSY, ^{1}H *J*-resolved, $^{1}H-^{13}C$ HSQC and $^{1}H-^{13}C$ HMBC[1], and HMDB database[2]. Some metabolites were also confirmed by standard compounds.

Enzyme expression analysis

The levels of enzymes were detected using SILAC coupled with LC-MS/MS. The SILAC labeling, subcellular fractionation, and SCX-coupled RPLC-MS/MS were all carried out as described previously[3]. Tandem mass spectra were extracted by Analyst version 2.0. The raw MS spectra analysis was performed with ProteinPilot 4.0 (AB SCIEX, USA) using the Paragon algorithm[4]. The ipi_HUMAN_v3_77 database was used. The data analysis parameters were as follows: Sample type: SILAC (Lys+8, Arg+10); Cys Alkylation: Iodoacetamide; Digestion: Trypsin; Instrument: QSTAR Elite ESI; Special Factors: Urea denaturation; Search Effort: Thorough ID；FDR Analysis: Yes; Detected Protein Threshold [Unused ProtScore (Conf)]: 1.3 (95.0%); the others are default parameters in the software.

Proteins with corrected assigned peptides (identified with Unused ProtScore greater than 1.3 for 95% confidence) were considered as identified. The false discovery rates of peptide-spectra matches determined by decoy database search were < 5%. Protein ratio *P*-value less than 0.05 indicated significant

change.

Fatty acids analysis

The methylesterification of fatty acids was accomplished as described previously[5] with a little modification, namely the residue from 10 mg cell pellets aqueous extraction was dissolved in 1 mL methanol/hexane solution (4:1, v/v) containing 20 μL internal standard hexane solution (1 mg/mL heptadecanoate-methyl ester, 0.5 mg/mL tricosanoate-methyl ester and 2 mg/mL 2,6-di-tert-butyl-4-methyl phenol, Sigma-Aldrich, USA), then 100 μL acetylchloride was added in an ice bath after vigorous vortex. The reaction was kept for 24 h at room temperature in the dark. Then, 2 mL 6% K_2CO_3 was added slowly to stop the reaction and neutralize the mixture. The solution was extracted 3 times with 200 μL hexane per time. All the supernatants were mixed together and evaporated until dry, then kept for GC analysis.

The prepared sample was redissolved with 20 μL hexane. The analysis was performed with a Shimadzu GC-MS 2010 plus chromatography system (Shimadzu Scientific Instruments, Japan) equipped with a flame ionization detector and a DB-225 column (cut to 10 m, 0.1 mm ID, 0.1 μm film thickness) (Agilent, USA). The injection volume was 1 μL and the split ratio was 60:1. The injection port and detector temperatures were 230 ^{o}C. The column temperature program was as follows: temperature was held at 55 ^{o}C for 0.5 min, increased to 205 ^{o}C at the speed of 30 ^{o}C/min, held at 205 ^{o}C for 3 min, increased to 230 ^{o}C at

5 ^{o}C /min, held at 230 ^{o}C for 2.5 min. The identification of fatty acids was made by comparison with standard fatty acid-methyl esters according to the retention time and also confirmed by MS analysis. The quantity of fatty acids was determined by the internal standard.

Plasmid transfection

HBV 1.3-fold length plasmid (pHBV1.3, GenBank accession No. U95551, ayw)[6] was transfected into HepG2 cells by electroporation using the method recommended by Bio-Rad Laboratories (www.bio-rad.com).

Supplementary References

1. Dai, H. *et al.* Combined NMR and LC-DAD-MS analysis reveals comprehensive metabonomic variations for three phenotypic cultivars of salvia miltiorrhiza bunge. *J Proteome Res* **9**, 1565-1578 (2010).
2. Wishart, D. S. *et al.* HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res* **37**, D603-D610 (2009).
3. Zhang, L.-K. *et al.* Identification of host proteins involved in Japanese encephalitis virus infection by quantitative proteomics analysis. *J Proteome Res* **12**, 2666-2678 (2013).
4. Shilov, I. V. *et al.* The paragon algorithm, a next generation search engine that uses sequence temperature values and feature probabilities to identify peptides from tandem mass spectra. *Mol Cell Proteomics* **6**, 1638-1655

(2007).

5. Xu, Z. *et al.* An improved method for determining medium- and long-chain FAMEs using gas chromatography. *Lipids* **45**, 199-208 (2010).

6. Wu, C. C. *et al.* Amino Acid Substitutions at Positions 122 and 145 of Hepatitis B Virus Surface Antigen (HBsAg) Determine the Antigenicity and Immunogenicity of HBsAg and Influence In Vivo HBsAg Clearance. *J Virol* **86**, 4658-4669 (2012).