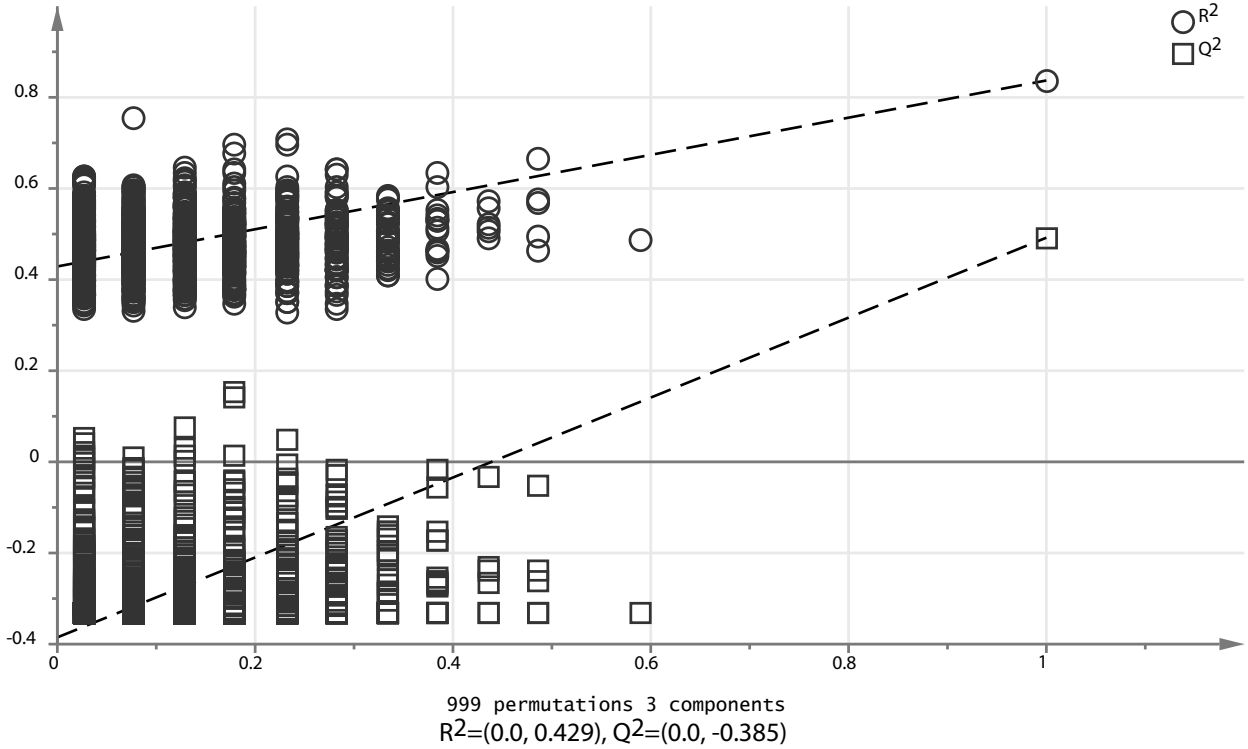
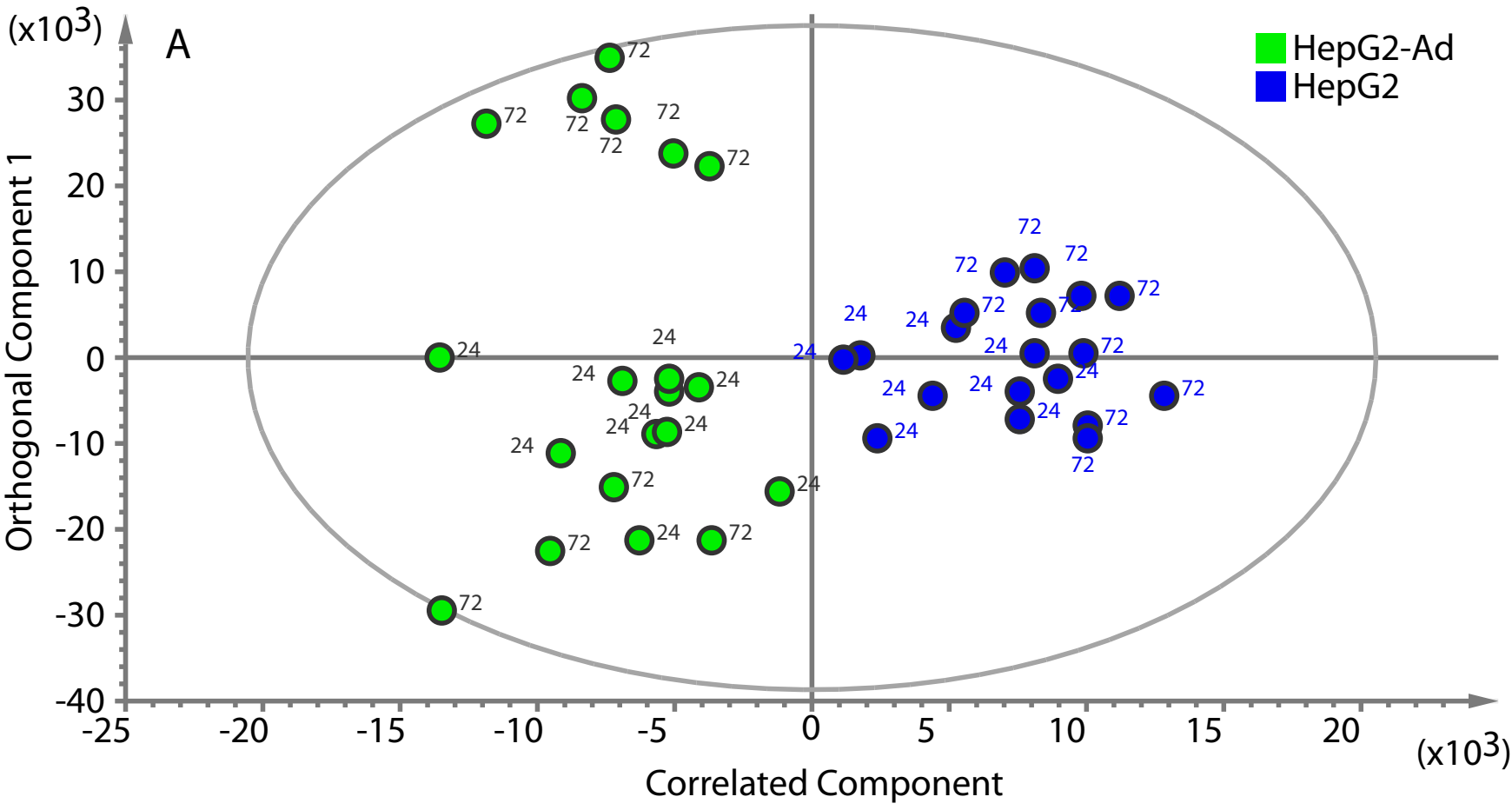

R2
Q2
0.8
0.6
0.4
0.2
0
-0.2
-0.4
0
0.2
0.4
0.6
0.8
1
999 permutations 3 components
R2=(0.0, 0.429), Q2=(0.0, -0.385)

Figure S1


A
HepG2-Ad
HepG2
(x10^3)
Orthogonal Component 1
Correlated Component
(x10^3)

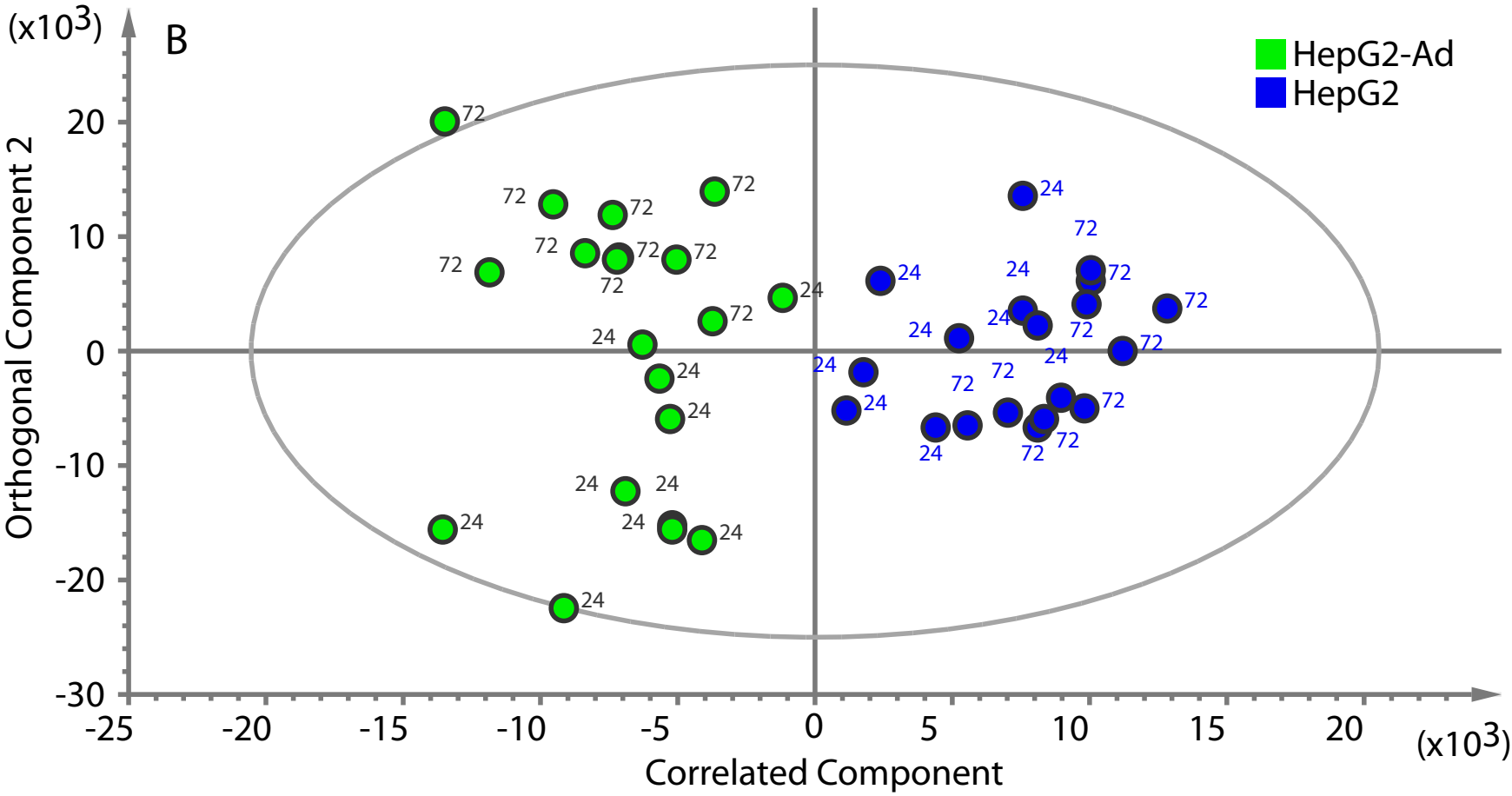

B
HepG2-Ad
HepG2
(x10^3)
Orthogonal Component 2
Correlated Component
(x10^3)

Figure S2

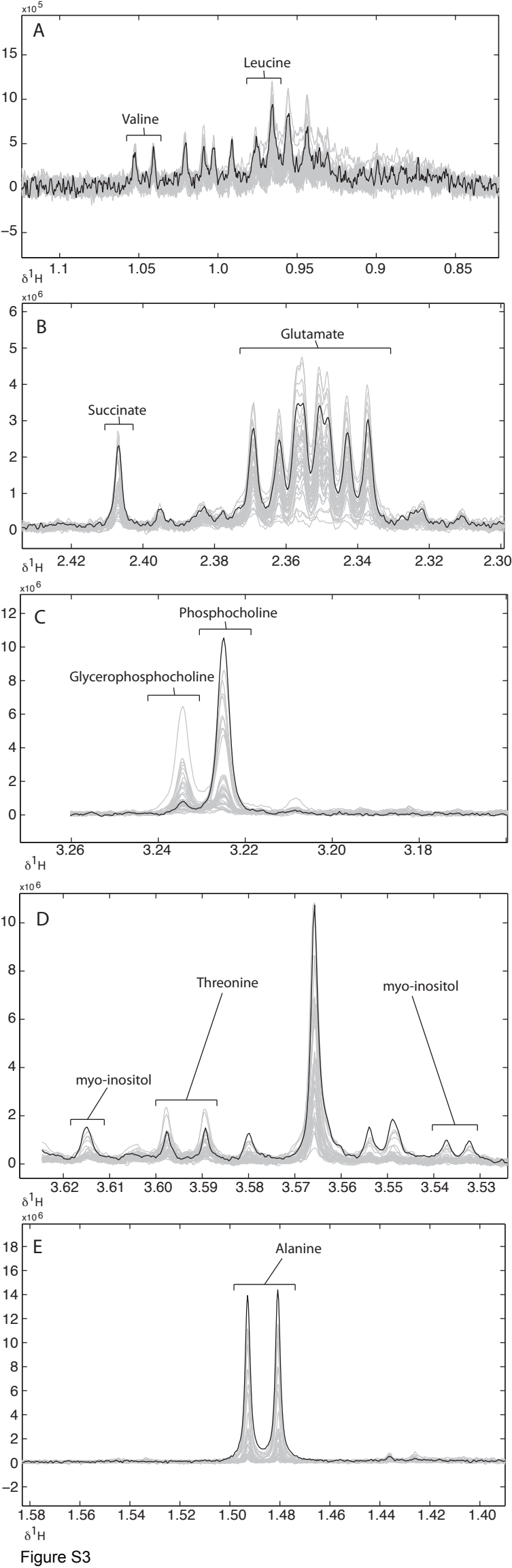

A
Leucine
Valine
x10^5
15
10
5
0
−5
1.1
1.05
1.0
0.95
0.9
0.85
δ1H
B
Glutamate
Succinate
x10^6
6
5
4
3
2
1
0
2.42
2.40
2.38
2.36
2.34
2.32
2.30
δ1H
C
Phosphocholine
Glycerophosphocholine
x10^6
12
10
8
6
4
2
0
3.26
3.24
3.22
3.20
3.18
δ1H
D
Threonine
myo-inositol
myo-inositol
x10^6
10
8
6
4
2
0
3.62
3.61
3.60
3.59
3.58
3.57
3.56
3.55
3.54
3.53
δ1H
E
Alanine
x10^6
18
16
14
12
10
8
6
4
2
0
−2
1.58
1.56
1.54
1.52
1.50
1.48
1.46
1.44
1.42
1.40
δ1H

Figure S3

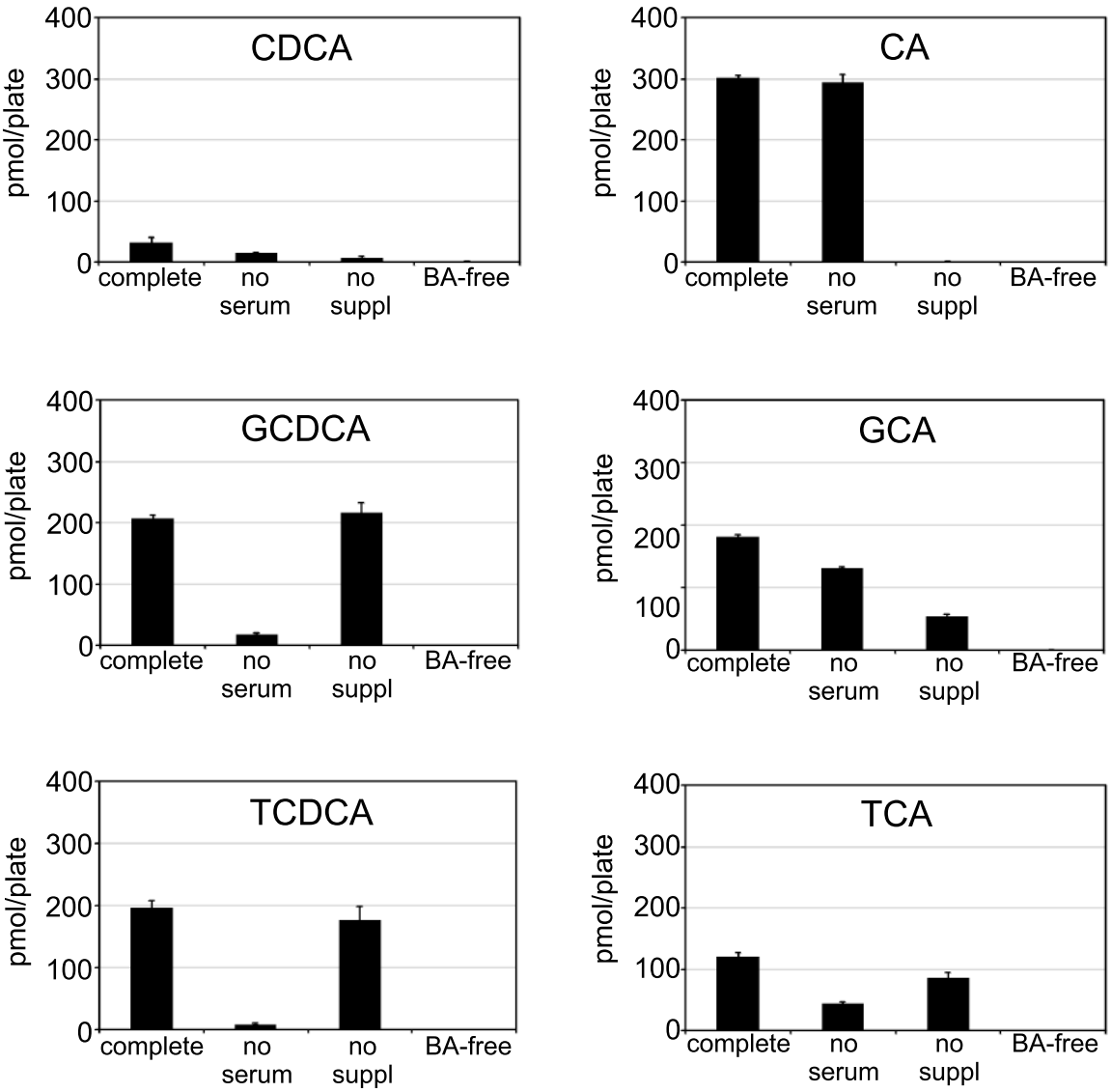

CDCA
CA
GCDCA
GCA
TCDCA
TCA
pmol/plate
400
300
200
100
0
complete
no serum
no suppl
BA-free

FIGURE S4

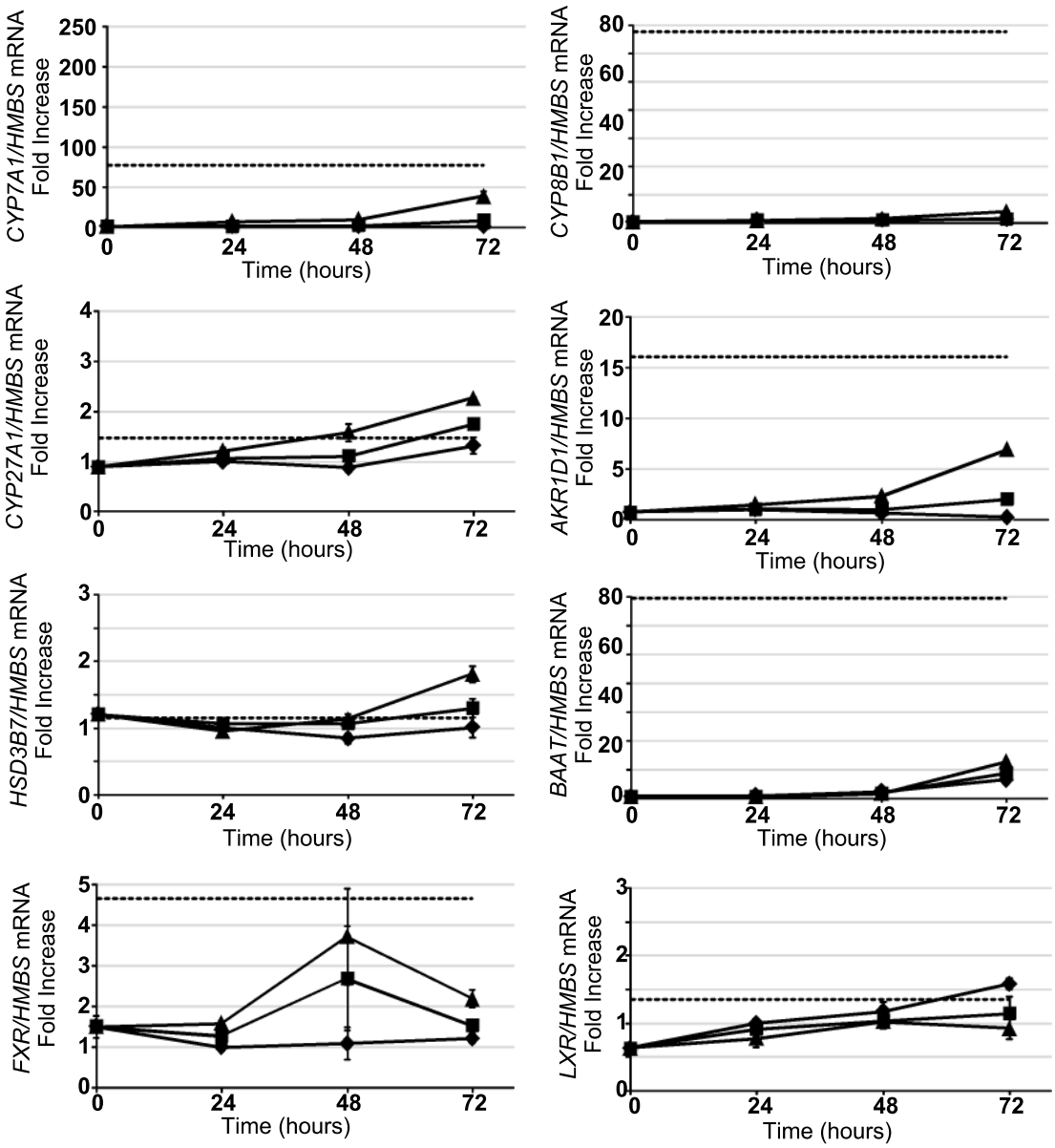

CYP7A1/HMBS mRNA Fold Increase
250
200
150
100
50
0
0
24
48
72
Time (hours)
CYP8B1/HMBS mRNA Fold Increase
80
60
40
20
0
0
24
48
72
Time (hours)
CYP27A1/HMBS mRNA Fold Increase
4
3
2
1
0
0
24
48
72
Time (hours)
AKR1D1/HMBS mRNA Fold Increase
20
15
10
5
0
0
24
48
72
Time (hours)
HSD3B7/HMBS mRNA Fold Increase
3
2
1
0
0
24
48
72
Time (hours)
BAAT/HMBS mRNA Fold Increase
80
60
40
20
0
0
24
48
72
Time (hours)
FXR/HMBS mRNA Fold Increase
5
4
3
2
1
0
0
24
48
72
Time (hours)
LXR/HMBS mRNA Fold Increase
3
2
1
0
0
24
48
72
Time (hours)

FIGURE S5

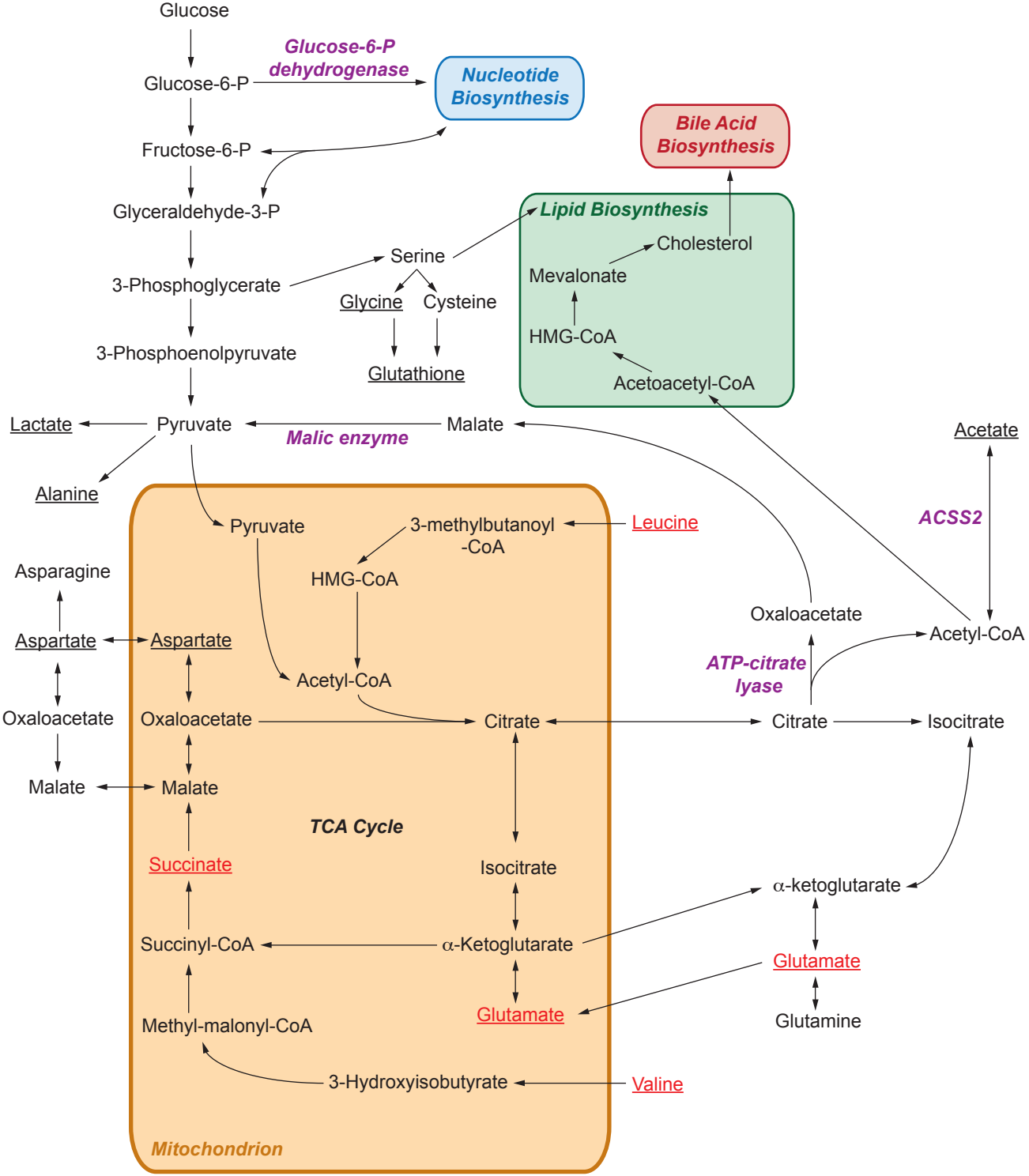

Glucose
Glucose-6-P
Glucose-6-P dehydrogenase
Nucleotide Biosynthesis
Fructose-6-P
Glyceraldehyde-3-P
Bile Acid Biosynthesis
Lipid Biosynthesis
Cholesterol
Serine
Mevalonate
3-Phosphoglycerate
Glycine
Cysteine
HMG-CoA
3-Phosphoenolpyruvate
Glutathione
Acetoacetyl-CoA
Lactate
Pyruvate
Malate
Malic enzyme
Acetate
Alanine
ACSS2
Pyruvate
3-methylbutanoyl -CoA
Leucine
Asparagine
HMG-CoA
Oxaloacetate
Aspartate
Aspartate
Acetyl-CoA
ATP-citrate lyase
Oxaloacetate
Oxaloacetate
Citrate
Citrate
Isocitrate
Malate
Malate
TCA Cycle
Succinate
Isocitrate
α-ketoglutarate
Succinyl-CoA
α-Ketoglutarate
Glutamate
Glutamate
Glutamine
Methyl-malonyl-CoA
3-Hydroxyisobutyrate
Valine
Mitochondrion

Figure S6

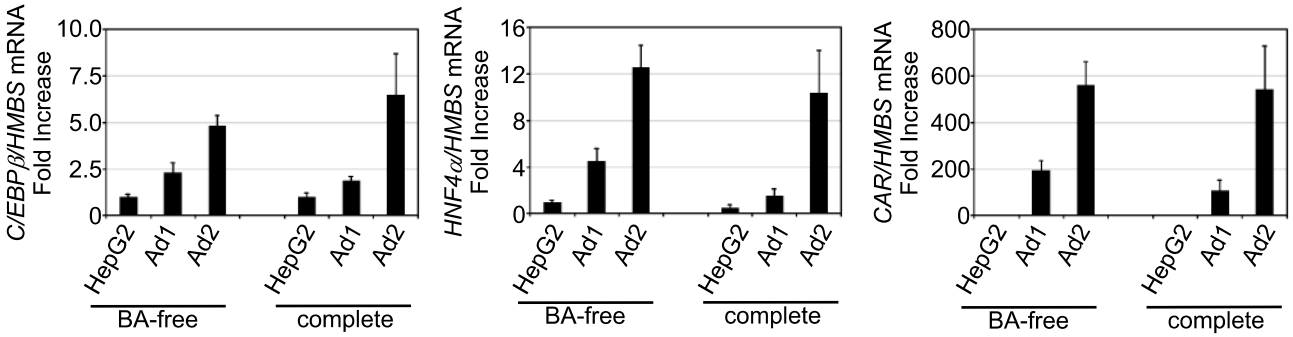

C/EBPβ/HMBS mRNA Fold Increase
10.0
7.5
5.0
2.5
0
HepG2
Ad1
Ad2
HepG2
Ad1
Ad2
BA-free
complete
HNF4α/HMBS mRNA Fold Increase
16
12
8
4
0
HepG2
Ad1
Ad2
HepG2
Ad1
Ad2
BA-free
complete
CAR/HMBS mRNA Fold Increase
800
600
400
200
0
HepG2
Ad1
Ad2
HepG2
Ad1
Ad2
BA-free
complete

FIGURE S7

Text and Figure Legends for Supplementary

Results

OPLS-DA and PLS-DA models are calculated using the same method. The models do produce a different Q^2 but this is due to the cross validation being calculated in a different way. The OPLS-DA model (1 correlated component + 2 orthogonal components) in figure 1 was tested for validity by analysing a PLS-DA model (3 latent variables) of the same data (Figure S1).

The 2D scores plots are shown for the correlated component and the two orthogonal components of the OPLS-DA model (Figure S2). The scores plots further demonstrate a clear separation in the metabolic profiles of the HepG2 and HepG2-Ad cell populations. They also demonstrate the variation within the cell type (HepG2 and HepG2-Ad) along the orthogonal component axis. Figure S2A shows two discrete groups of samples at the 72 hour time point in the HepG2-Ad cells, which could be due to variation in transfection efficiency. Figure S2B shows a clear time related effect in the HepG2-Ad cell population but not in the HepG2 cells.

Supplementary Figure legends

Figure S1. Permutation analysis to test the validity of the multivariate model. The validation plot is the result of permuting the class (Y variable) of the PLS-DA model. The permutation test was carried out in SIMCA and is the result of 1000 permutations.

Figure S2. Two dimensional scores plots of the OPLS-DA model describing the separation of the HepG2 and HepG2-Ad intracellular metabolic profiles. The plots

are coloured by class (HepG2 or HepG2-Ad) and labeled according to time point (24 or 72 hours). $R^2Y(cum)=0.838$, $Q^2(cum)=0.671$. **(A)** Scores plot of the correlated and first orthogonal components. **(B)** Scores plot of correlated and second orthogonal component.

Figure S3. Representative ^{1}H NMR spectra of selected metabolites. Full resolution 600 MHz ^{1}H CPMG spin-echo NMR spectra of aqueous intracellular metabolites. All spectra in the dataset are coloured grey except a single representative spectrum, which is coloured in black (*nb*. It is not necessarily the same spectrum coloured in black in each part of the figure (A-E)).

Figure S4. Levels of conjugated and unconjugated primary bile acids in HepG2 media. Data is expressed as pmol/plate assuming 1,5ml of media per plate.

Figure S5. Levels of selected gene transcripts involved in bile acid metabolism in HepG2 and HepG2-Ad cells cultured in BA-free media. HepG2 (diamonds) cells were infected with 24 (HepG2-Ad1; squares) or 96 (HepG2-Ad2; triangles) MOI of adenoviral vectors expressing C/EBPβ, HNF4α and CAR (1:1:1). Total RNA was extracted at 24, 48 and 72 hours. mRNA levels of selected genes were normalized to hydroxymethylbilane synthase (*HMBS)* levels. Data correspond to the average ± SD from four independent experiments in duplicate relative to control HepG2 cells at 24 hours. Horizontal dashed line corresponds to the average mRNA levels in human liver (n=5).

Figure S6. A summary of the key intracellular metabolic pathways involved in cholesterol and bile acid biosynthesis. Metabolites that are underlined were measured in the current study. Those that are underlined and shown in red were at lower levels in the HepG2-Ad cells, when compared to the HepG2 population. Four enzymes are shown in purple (malic enzyme, glucose-6-P dehydrogenase, ACSS2 & ATP-citrate lyase), which are important in either acetyl-CoA or NADPH production.
ACSS2=Acetyl-CoA synthetase 2. TCA=Tricarboxylic acid. HMG-CoA=3-hydroxy-3-methyl-glutaryl-CoA. Figure adapted from Lunt et al 2011 (Lunt and Vander Heiden 2011)

Figure S7. Levels of C/EBPβ, HNF4α and CAR in HepG2 and HepG2-Ad cells cultured in complete and BA-free media. HepG2 cells were infected with 24 (Ad1) or 96 (Ad2) MOI of adenoviral vectors expressing C/EBPβ, HNF4α and CAR (1:1:1). mRNA levels at 48 hours were normalized to hydroxymethylbilane synthase (*HMBS)* levels. Data correspond to the average ± SD relative to control HepG2 cells at 24 hours.

Supplementary Tables

Table S1. Primers for qRT-PCR used in this study

Gene	Sequence	Product (bp)
CYP7A1-F	CTGCTACCGAGTGATGTTTGAA	342
CYP7A1-R	GAAAGTCGCTGGAATGGTGTT	
CYP8B1-F	ATACCCTGAAGATGTCCAGTGG	419
CYP8B1-R	AAGCTCACTCTGTAGGATGC	
CYP27A1-F	GTGCCCCGCTCTTGGAGCAA	124
CYP27A1-R	CCTTCCGTGGTGAACGGCCC	
AKR1D1-F	TGTCCCAGAGATGGTCCGCCC	188
AKR1D1-R	GGCACTCAACCTCCCAAGTGGC	
HSD3B7-F	AAGCAGTGCACAGGCACCCC	151
HSD3B7-R	TACCTGGCCGCCCATCAGGG	
BAAT-F	TGGCCTTGGCTTACCATAAC	197
BAAT-R	CGTGGCTGTGACTTGCTTTA	
FXR-F	AACTTCCGTCTGGGCATTCTG	375
FXR-R	GTGGGGTAAACTTGTGGTCGT	
LXR-F	AAGCGGCAAGAGGAGGAACAG	240
LXR-R	GCTCAGTGAAGTGGGCAAAGC	
MRP2-F	GCCGGTGGTCAGATTATCAT	292
MRP2-R	GATCTTGGATTTCCGAAGCA	
C/EBPβ-F	CTCGCAGGTCAAGAGCAAG	271
C/EBPβ-R	CTAGCAGTGGCCGGAGGAGGCGACC	
HNF4-F	GCCTACCTCAAAGCCATCAT	255
HNF4-R	GACCCTCCCAGCAGCATCTC	
CAR-F	TGCTGCCTCTGGTCACACACTT	385
CAR-R	TCAATCTCATCTCTCTGGGTAAC	
HMBS-F	CGGAAGAAAACAGCCCAAAGA	272
HMBS-R	TGAAGCCAGGAGGAAGCACAGT	

Table S2. The chemical shift regions integrated for confidently assigned metabolites in the NMR spectral data.

Metabolite Assignment	Start ppm	End ppm
leucine	0.96	0.98
valine	1.03	1.06
lactate	1.31	1.33
threonine	1.33	1.335
lactate	1.335	1.34
threonine	1.34	1.36
alanine	1.46	1.51
acetate	1.91	1.93
glutamate	2.027	2.035
glutamate	2.035	2.05
glutamate	2.33	2.375
succinate	2.4	2.42
aspartate	2.78	2.815
aspartate	2.82	2.84
glutathione*	2.93	2.96
creatine	3.035	3.045
Phosphocholine	3.22	3.23
Glycerophosphocholine	3.23	3.24
myo-inositol	3.52	3.54
myo-inositol	3.54	3.557
glycine	3.557	3.575
threonine	3.585	3.6
myo-inositol	3.61	3.62
creatine	3.92	3.94
myo-inositol	4.065	4.085
lactate	4.09	4.1
lactate*	4.1	4.125
lactate*	4.125	4.13
ATP/ADP/AMP*	6.13	6.17
ATP/ADP/AMP*	8.26	8.29

* = metabolites marked with an asterisk were overlapped with resonances that were unassigned to any metabolite/metabolites.

Reference

Lunt, Sophia Y, and Matthew G Vander Heiden. 2011. “Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation.” Annual Review of Cell and Developmental Biology 27: 441–64.