

Supplementary Tables and Figures

Hilvo et al., *Accumulated metabolites of hydroxybutyric acid serve as diagnostic and prognostic biomarkers of ovarian high-grade serous carcinomas*

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1 CLINICOPATHOLOGICAL DATA

1.1 Supplementary Tables

Supplementary Table S1. Clinicopathological characteristics of the study cohort.

		Serum samples		Tumor samples	
		Cases	%	Cases	%
All patients					
<i>Malignancy</i>	malignant cases	158	61 %	124	-
	non-malignant cases	100	39 %	-	-
<i>CA-125 data measured</i>	malignant cases	145	92 %	-	-
	non-malignant cases	73	73 %	-	-
Patients with malign tumors					
<i>Figo stage</i>	I	3	2 %	3	2 %
	II	5	3 %	4	3 %
	III	101	64 %	83	67 %
	IV	32	20 %	24	19 %
	NA	17	11 %	10	8 %
<i>Residual tumor mass in surgery</i>	complete reduction	91	58 %	75	60 %
	< 0.5 cm	31	20 %	24	19 %
	< 1 cm	21	13 %	18	15 %
	< 2 cm	2	1 %	2	2 %
	> 2 cm	7	4 %	4	3 %
	NA	6	4 %	1	1 %
<i>Location of analyzed tumor sample</i>	ovary	-	-	59	48 %
	intestine	-	-	23	19 %
	peritoneum	-	-	14	11 %
	other	-	-	26	21 %
	NA	-	-	2	2 %
<i>Ascites</i>	1	31	20 %	24	19 %
	2	57	36 %	50	40 %
	3	64	41 %	50	40 %
	NA	6	4 %	0	0 %
<i>Progress-free survival</i>	event	88	58 %	64	52 %
	median follow-up (months)	16	-	16	-
	no event	64	42 %	60	48 %
	median follow-up (months)	25	-	23	-
<i>Overall survival</i>	event	84	55 %	77	62 %
	median follow-up (months)	23	-	22	-
	no event	68	45 %	47	38 %
	median follow-up (months)	48	-	49	-

2 METABOLOMICS RESULTS

2.1 Supplementary Tables

Supplementary Table S2. Results of statistical tests for all metabolites in serum samples (provided as a separate excel file).

Supplementary Table S3. Malignant vs. control group t-test results for the main identified metabolite peaks.

Metabolite name	p-value	q-value	log2 fold	Metabolite name	p-value	q-value	log2 fold
Amino acids				Fatty acid omega oxidation			
Tryptophan	7.91E-25	6.73E-23	-1.64	Adipic acid	1.86E-07	4.53E-07	0.52
Alanine	6.74E-20	9.56E-19	-0.81	Glycolysis & TCA cycle			
Methionine	1.05E-17	8.48E-17	-0.43	Lactic acid	7.90E-12	3.28E-11	-0.54
Threonine	3.59E-16	2.35E-15	-0.62	Malic acid	7.24E-05	1.12E-04	-0.51
Proline	2.90E-05	4.80E-05	-0.30	Glycerol-3-phosphate	2.32E-03	2.49E-03	-0.30
Serine	5.65E-04	7.02E-04	-0.23	Ketone bodies and hydroxybutyric acids			
Phenylalanine	6.13E-04	7.51E-04	-0.25	3,4-Dihydroxybutyric acid	5.65E-27	9.61E-25	0.87
Glycine	1.24E-03	1.41E-03	0.32	3-Hydroxybutyric acid	6.06E-21	1.29E-19	2.53
Tyrosine	1.68E-03	1.85E-03	-0.29	2,3,4-Trihydroxybutyric acid	4.90E-12	2.14E-11	-0.77
Glutamic acid	2.87E-03	3.00E-03	0.38	2-Hydroxybutyric acid	4.32E-10	1.50E-09	0.69
Glutamine	7.02E-03	6.68E-03	0.46	Acetoacetic acid	5.60E-04	7.01E-04	1.21
Valine	8.45E-03	7.77E-03	-0.12	2,4-Dihydroxybutyric acid	1.05E-03	1.22E-03	0.28
Amino acid metabolism				Other			
2-oxo-3-methylpentanoic acid	5.62E-08	1.50E-07	-0.77	Ketoleucine	2.62E-19	2.98E-18	-0.89
Indole-3-acetic acid	1.42E-07	3.56E-07	-1.11	Glycerol	2.44E-13	1.12E-12	0.74
2-Oxoisovaleric acid	3.41E-05	5.47E-05	-0.69	Myo-Inositol	1.34E-07	3.41E-07	0.41
2-Hydroxy-3-methylvaleric acid	3.21E-03	3.31E-03	-0.62	Ethanolamine	8.03E-07	1.87E-06	0.50
4-Hydroxyphenyllactic acid	1.55E-02	1.30E-02	0.22	Cholesterol	1.20E-06	2.65E-06	-0.53
Carbohydrates				2-Aminobutyric acid	1.52E-06	3.32E-06	-1.10
Maltose	1.65E-23	9.38E-22	3.90	3-Hydroxyvaleric acid	7.03E-06	1.38E-05	0.89
Turanose	8.22E-23	3.50E-21	2.59	Erythritol	2.82E-05	4.75E-05	0.33
Melibiose	2.86E-15	1.68E-14	2.87	Maleic acid	1.20E-04	1.79E-04	0.56
Mannonic acid	9.66E-14	4.98E-13	0.68	3-Hydroxyisovaleric acid	1.27E-04	1.89E-04	1.01
Xylitol	4.61E-09	1.51E-08	0.35	Glyceric acid	2.00E-04	2.84E-04	-0.35
Arabinose	1.75E-06	3.78E-06	0.68	3-Hydroxycaproic acid	2.99E-04	3.97E-04	0.59
Glucopyranose	1.14E-02	1.01E-02	0.34				
Mannopyranose	1.75E-02	1.46E-02	0.44				
Xylose	3.74E-02	2.77E-02	0.37				
Free fatty acids							
Linolenic acid (C18:3)	1.33E-05	2.46E-05	0.63				
Palmitelaidic acid (C16:1)	4.20E-04	5.42E-04	0.37				
Oleic acid (C18:1)	7.90E-04	9.52E-04	0.27				
Myristic acid (C14:0)	6.28E-03	6.01E-03	0.27				
Myristoleic acid (C14:1)	1.37E-02	1.17E-02	0.42				
Palmitic acid (C16:0)	1.84E-02	1.52E-02	0.12				
Lauric acid (C12:0)	3.70E-02	2.76E-02	0.22				

Supplementary Table S4. Results of statistical tests regarding serum metabolites and nutrition (provided as a separate excel file).

Supplementary Table S5. Correlation of metabolite concentrations between serum and tissue samples. Identified main metabolite peaks that showed significant results in Pearson correlation analysis when serum and tumor tissue samples were compared.

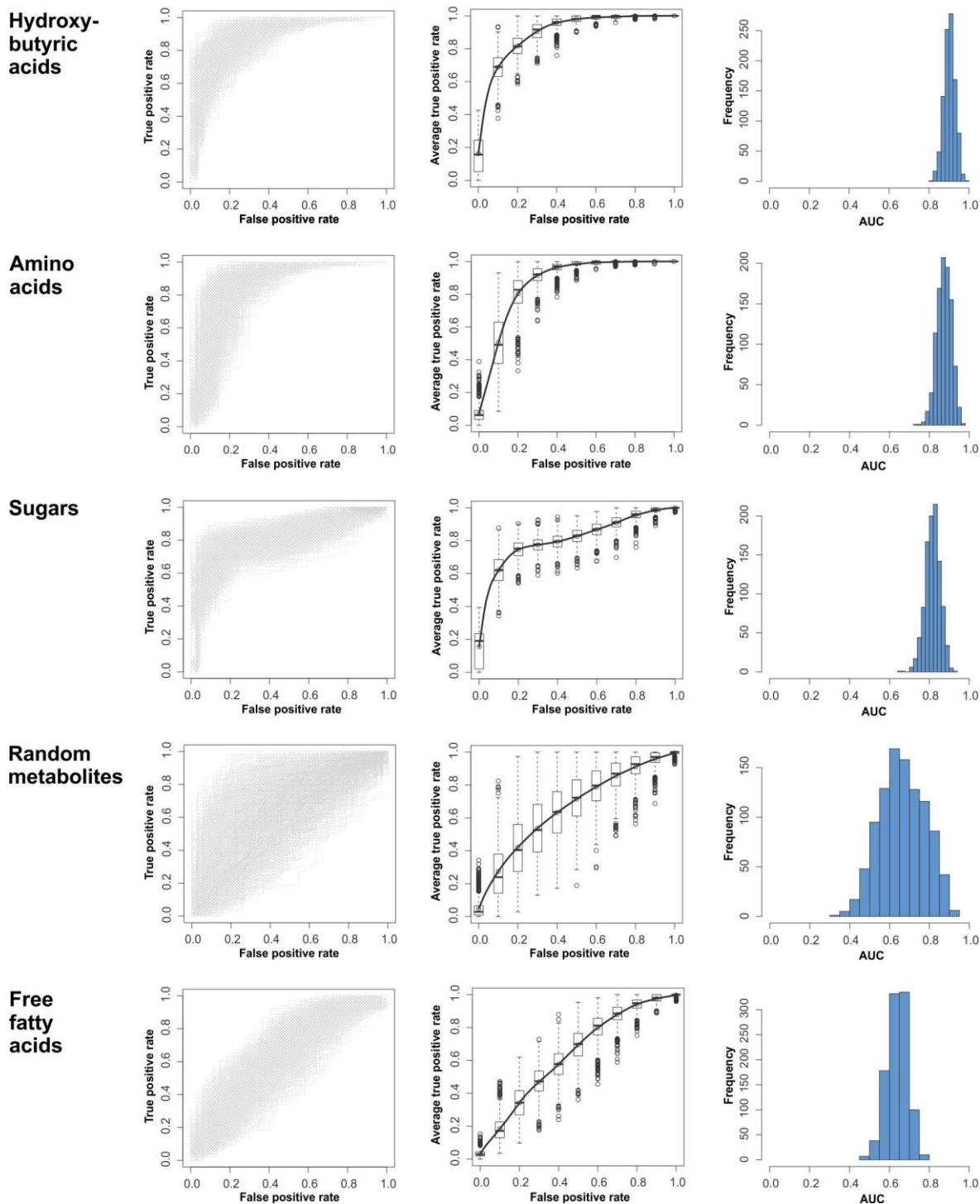
Metabolite	Metabolite class	p-value	R2
3-Hydroxybutyric acid	Ketone bodies and hydroxybutyric acids	<2.4E-13	0.722
2-Hydroxybutyric acid	Ketone bodies and hydroxybutyric acids	2.4E-13	0.627
Erythrotetrofuranose	Sugars and sugar alcohols	3.2E-12	0.603
Erythritol	Sugars and sugar alcohols	1.3E-09	0.538
3,4-Dihydroxybutyric acid	Ketone bodies and hydroxybutyric acids	0.001	0.317
Isoleucine	Amino acids	0.003	0.285
4-Hydroxyphenyllactic acid	Amino acid metabolism	0.005	0.263
2,3-Dihydroxybutyric acid	Ketone bodies and hydroxybutyric acids	0.010	0.243
Leucine	Amino acids	0.012	0.238
Valine	Amino acids	0.014	0.233
Maleic acid	Other	0.018	-0.226
3-Hydroxyisovaleric acid	Other	0.025	0.214
2-Phenyllactic acid	Amino acid metabolism	0.025	0.214
Adipic acid	Fatty acid omega oxidation	0.027	0.211
Palmitelaidic acid (C16:1)	Fatty acids	0.029	0.208
2-Monopalmitin	Other	0.029	0.208
Glutamic acid	Amino acids	0.037	0.199
2,4-Dihydroxybutyric acid	Ketone bodies and hydroxybutyric acids	0.043	0.193

Supplementary Table S6. Cox regression models with age, tumor reduction and figo stage incorporated into the model. The upper and lower parts show a model with and without age, respectively.

Metabolite name	Metabolite		Age		Tumor reduction		Figo (III and IV)	
	p-value	hazard ratio	p-value	hazard ratio	p-value	hazard ratio	p-value	hazard ratio
2,4-Dihydroxybutyric acid	0.703	0.956	0.002	1.037	0.007	0.637	4.6E-05	3.045
3,4-Dihydroxybutyric acid	0.138	1.192	0.004	1.032	0.012	0.654	3.3E-05	3.140
3-Hydroxyisovaleric acid	0.003	1.317	0.003	1.032	0.005	0.623	2.0E-05	3.249
4-Hydroxyphenyllactic acid	0.094	1.186	0.006	1.031	0.010	0.646	8.8E-05	2.944
Adipic acid	0.094	1.224	0.004	1.032	0.004	0.608	8.2E-05	2.952
Erythritol	0.883	1.018	0.005	1.034	0.007	0.635	4.7E-05	3.047
2,4-Dihydroxybutyric acid	0.341	1.108	-	-	0.004	0.617	0.000	2.760
3,4-Dihydroxybutyric acid	0.025	1.263	-	-	0.007	0.634	0.000	2.832
3-Hydroxyisovaleric acid	0.001	1.334	-	-	0.006	0.625	0.000	2.950
4-Hydroxyphenyllactic acid	0.009	1.287	-	-	0.011	0.644	0.001	2.532
Adipic acid	0.021	1.306	-	-	0.002	0.588	0.000	2.645
Erythritol	0.099	1.194	-	-	0.005	0.622	0.000	2.732

2.2 Supplementary Figures

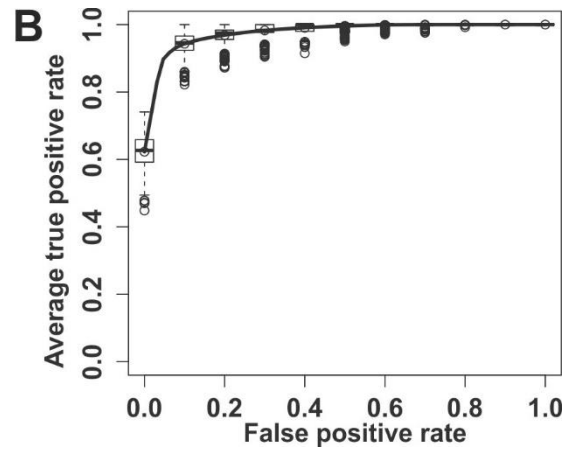
Metabolite class	AUC MEAN	95% LOW	95% HIGH	Metabolites in the model
Hydroxybutyric acids	0.90	0.86	0.95	3,4-Dihydroxybutyric acid, 3-Hydroxybutyric acid, 2-Hydroxybutyric acid
Amino acids	0.87	0.81	0.93	Tryptophan, Alanine, Methionine
Sugars	0.82	0.75	0.88	Maltose, Turanose, Melibiose
Random metabolites	0.66	0.49	0.85	3 Random metabolites selected 1000 times
Free fatty acids	0.64	0.55	0.64	Linolenic acid (C18:3), Palmitelaidic acid (C16:1), Oleic acid (C18:1)



Supplementary Figure S1

A

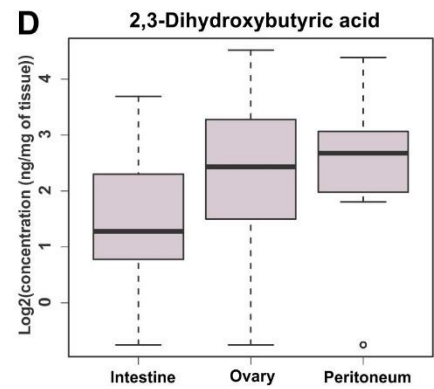
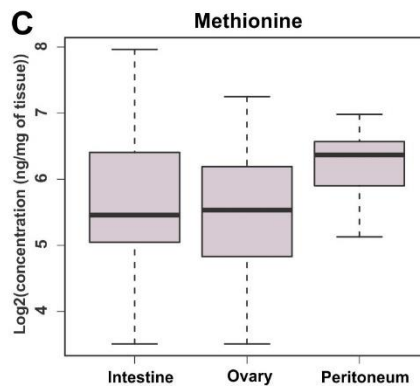
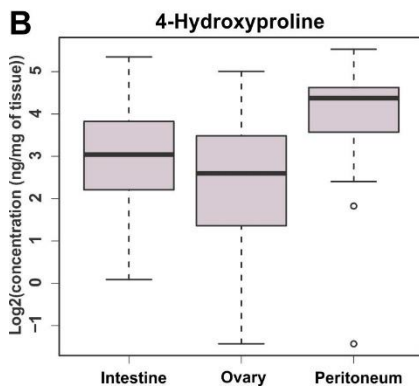
CA-125	3,4-DHBA	3-HBA	3 random metabolites	AUC
X	X	X		0.98 (0.96, 1.00)
X		X		0.97 (0.95, 0.99)
X	X			0.97 (0.95, 0.99)
X				0.96 (0.92, 0.99)
	X	X		0.91 (0.86, 0.95)
	X			0.86 (0.80, 0.91)
		X		0.84 (0.77, 0.90)
			X	0.66 (0.48, 0.85)



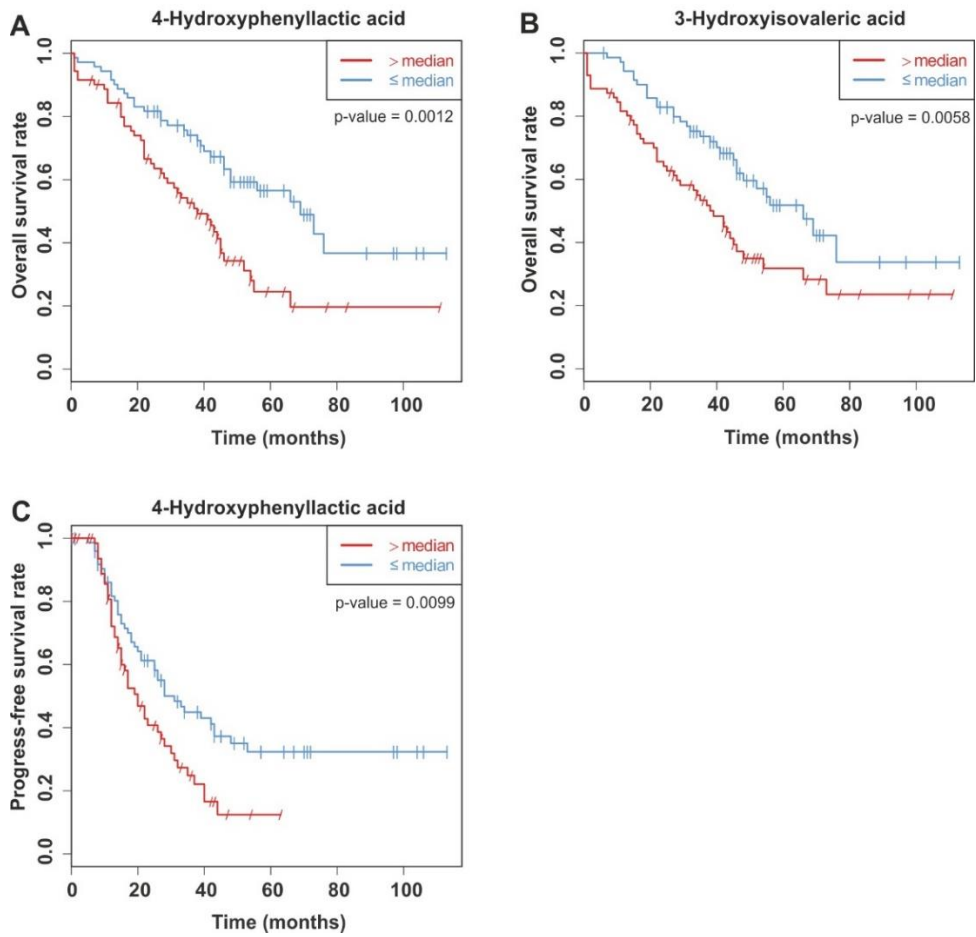
Supplementary Figure S2

A

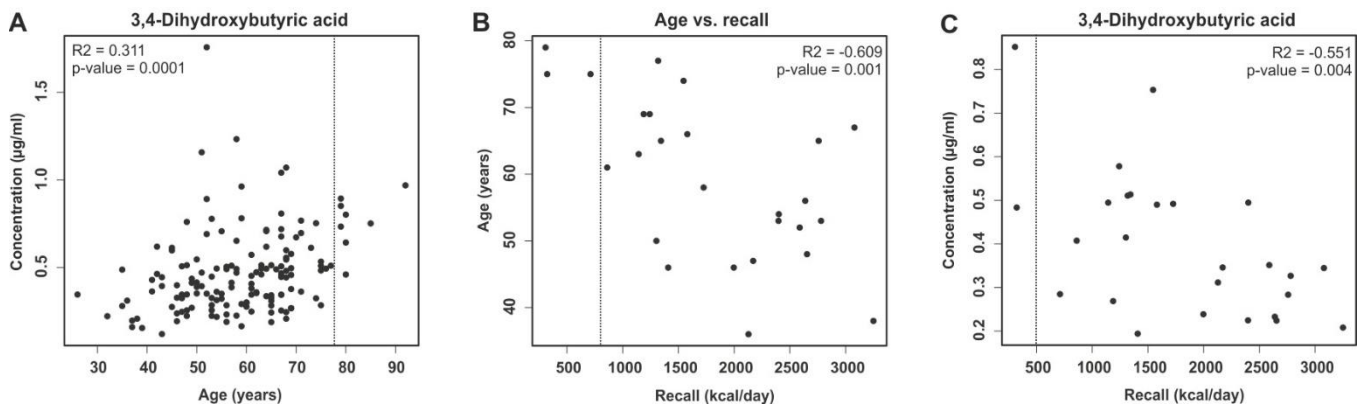
Metabolite name	Metabolite class	p-value	q-value
4-Hydroxyproline	Amino acid metabolism	0.004	0.226
Methionine	Amino acids	0.013	0.287
Glycylglycine	Amino acid metabolism	0.019	0.287
Ribonic acid	Sugars and sugar alcohols	0.025	0.299
2,3-Dihydroxybutyric acid	Ketone bodies and hydroxybutyric acids	0.027	0.299
Myristic acid (C14:0)	Fatty acids	0.028	0.299
Tyrosine	Amino acids	0.033	0.299
Citric acid	Glycolysis and TCA cycle	0.038	0.307



Supplementary Figure S3



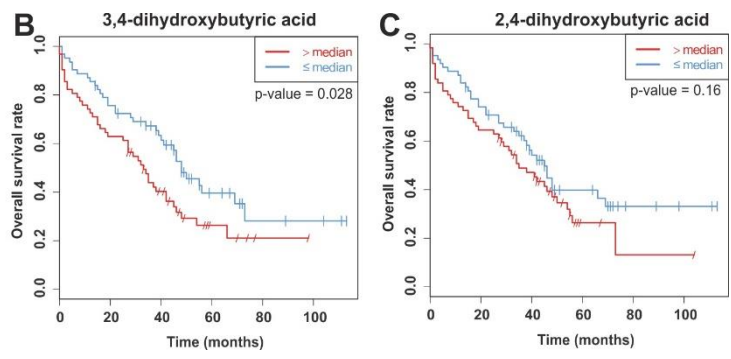
Supplementary Figure S4



Supplementary Figure S5

A

Metabolite	KM	Cox regression	
	p-value	p-value	hazard ratio
3,4-Dihydroxybutyric acid	0.028	0.058	1.22
Adipic acid	0.409	0.305	1.12
Erythritol	0.709	0.021	1.35
3-Hydroxyisovaleric acid	0.048	0.017	1.22
2,4-Dihydroxybutyric acid	0.162	0.001	1.50
4-Hydroxyphenyllactic acid	0.954	0.275	0.88

**Supplementary Figure S6**

3 GENE EXPRESSION AND COPY NUMBER SURVIVAL ANALYSES

3.1 Supplementary Tables

Supplementary Table S7. Results of survival analyses in the TCGA data set. In this table the results are summarized only for those genes whose low/high gene expression or gain/loss of copy number was associated with worse survival. The results for all analyzed genes (n=69) are shown in the **Supplementary Statistical Code**.

Gene	Enzyme	Expression quartiles		CNA			
		p-value	Worse survival	p-value	Worse survival	Percent	Deficiency / pathway related to
ALDH5A1	Succinic semialdehyde dehydrogenase	4.1E-04	Low	0.025	Loss	17.0 %	SSADH deficiency
DLD	Dihydrolipoamide dehydrogenase	4.6E-04	High	0.120	-	-	Leucine catabolism
PEX6	Peroxisomal biogenesis factor 6	8.6E-04	Low	0.235	-	-	Peroxisomal biogenesis, Zellweger syndrome
HMGCL	3-hydroxymethyl-3-methylglutaryl-CoA lyase	0.004	High	0.560	-	-	Ketogenesis, leucine catabolism
ACSL3	Acyl-CoA Synthetase Long-Chain Family Member 3	0.009	High	0.137	-	-	Activation of long-chain fatty acids for synthesis and oxidation
ADH1B	Alcohol dehydrogenase 1B	0.027	High	0.641	-	-	Omega oxidation
HLCS	Holocarboxylase synthetase	0.029	Low	0.388	-	-	Leucine catabolism
AUH	3-methylglutaconyl hydratase	0.033	High	0.093	-	-	Leucine catabolism
ALDH7A1	Antiquitin	0.029	-	0.022	Gain	12.8 %	Pipecolic acidemia, Zellweger syndrome
MCCC2	Methylcrotonoyl-CoA carboxylase 2 (beta)	0.887	-	1.7E-04	Gain	2.9 %	Leucine catabolism
PEX12	Peroxisomal biogenesis factor 12	0.428	-	3.1E-03	Gain	3.2 %	Peroxisomal biogenesis, Zellweger syndrome
OXCT1	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1	0.178	-	0.004	Loss	10.3 %	Ketogenesis
ALDH1B1	Aldehyde dehydrogenase X, mitochondrial	0.217	-	0.005	Loss	39.4 %	Omega oxidation
EHHADH	Peroxisomal bifunctional enzyme	0.063	-	0.010	Loss	3.8 %	Peroxisomal oxidation of fatty acids, Zellweger syndrome
HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1	0.082	-	0.012	Loss	11.2 %	Ketogenesis, leucine catabolism
FABP4	Fatty acid binding protein 4	0.059	-	0.018	Gain	50.9 %	Fatty acid transport for oxidation
ECI1	Enoyl-CoA delta isomerase 1	0.355	-	0.025	WT/loss	82.7 %	Fatty acid beta oxidation of unsaturated fatty acids
HADHA	Mitochondrial trifunctional protein	0.043	-	0.031	Loss	13.1 %	Mitochondrial beta oxidation of long chain fatty acids
CPT1B	Carnitine palmitoyltransferase I	0.088	-	0.040	Gain	5.1 %	Fatty acid beta oxidation

Supplementary Table S8. Genes that were significantly associated with the overall survival of the patients in four different cohorts. The data is from Supplementary Figure S6 from publication in Nature 474, 609-615, 2011 (Integrated genomic analyses of ovarian carcinoma).

Name	Title	TCGA training		Bonome (non-TCGA samples)		Tothill (no stage I)		Dressman	
		Overall survival Cox (cap 60 m) 9/29/09	beta	Overall survival Cox (cap 60m)	beta	Overall survival Cox (cap 60m)	beta	Overall survival Cox (cap 60m)	beta
ALDH5A1	aldehyde dehydrogenase 5 family, member A1	-0.668	2.29E-04	-0.546	2.99E-02	-0.468	6.61E-03	-1.078	8.19E-05
PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	-0.650	1.11E-03	-0.629	1.59E-03	-0.401	1.26E-02	-1.148	1.08E-05
GMPR	guanosine monophosphate reductase	-0.350	1.13E-03	-0.451	6.09E-05	-0.304	9.06E-04	-0.348	2.38E-02

4 GENE SET ENRICHMENT ANALYSES

4.1. Supplementary Tables

Supplementary Table S9. Statistically significant pathways negatively associated with the expression of *ALDH5A1* gene. False discovery rate Q-value less than or equal to 0.1 is used as the significance cut off. SIZE = number of genes in the gene set; NES = normalized enrichment score; Q-value = false discovery rate Q-value. P-values less than 0.0001 are shown as 0.000 in the table. With and FDR Q-value cut off 0.1 there were no pathways significantly positively associated with the expression of the *ALDH5A1* gene.

PATHWAY	SIZE	NES	P-value	Q-value
KEGG ECM RECEPTOR INTERACTION	78	-2.191	0.000	0.002
EMT UP-REGULATED	90	-2.106	0.000	0.003
KEGG FOCAL ADHESION	182	-1.991	0.000	0.018
KEGG HYPERTROPHIC CARDIOMYOPATHY HCM	76	-1.893	0.000	0.042
KEGG TGF BETA SIGNALING PATHWAY	77	-1.901	0.002	0.046
KEGG LEUKOCYTE TRANSENDOTHELIAL MIGRATION	99	-1.858	0.006	0.058
KEGG REGULATION OF ACTIN CYTOSKELETON	181	-1.816	0.000	0.067
KEGG DILATED CARDIOMYOPATHY	81	-1.818	0.000	0.074
KEGG CYTOKINE CYTOKINE RECEPTOR INTERACTION	214	-1.747	0.011	0.096
KEGG GAP JUNCTION	75	-1.769	0.002	0.097

Supplementary Table S10. P-values of the association of EMT signatures with ALDH enzymes. P-values less than 0.0001 are shown as 0.000 in the table.

	Positive association with EMT+	Positive association with EMT-	Negative association with EMT+	Negative association with EMT-
ALDH5A1			0.000	0.510
ALDH1A1	0.059	0.992		
ALDH1A2	0.025			0.097
ALDH1A3	0.000	0.084		
ALDH1B1	0.008	0.030		
ALDH1L1			0.036	0.498
ALDH2	0.264			0.418
ALDH3A1		0.977	0.087	
ALDH3A2			0.874	0.693
ALDH3B1		0.002	0.146	
ALDH3B2		0.000	0.579	
ALDH4A1			0.068	0.965
ALDH6A1			0.143	0.731
ALDH7A1	0.932			0.959
ALDH8A1			0.189	0.981
ALDH9A1			0.380	0.516
ALDH18A1	0.178			1.000