Supplementary material for:

Analysis of mucopolysaccharidosis type VI through integrative functional metabolomics

Abdellah Tebani[1,2,3], Lenaig Abily-Donval[2,4], Isabelle Schmitz-Afonso[3], Monique Piraud[5], Jérôme Ausseil[6], Farid Zerimech[7], Carine Pilon[1], Tony Pereira[8], Stéphane Marret[2,4], Carlos Afonso[3], Soumeya Bekri[1, 2,*]

[1] Department of Metabolic Biochemistry, Rouen University Hospital, Rouen, 76000, France
[2] Normandie Univ, UNIROUEN, CHU Rouen, INSERM U1245, 76000 Rouen, France
[3] Normandie Univ, UNIROUEN, INSA Rouen, CNRS, COBRA, 76000 Rouen, France
[4] Department of Neonatal Pediatrics, Intensive Care and Neuropediatrics, Rouen University Hospital, Rouen, 76031, France
[5] Service de Biochimie et Biologie Moléculaire Grand Est, Unité des Maladies Héréditaires du Métabolisme et Dépistage Néonatal, Centre de Biologie et de Pathologie Est, Hospices Civils de Lyon, France.
[6] INSERM U1088, Laboratoire de Biochimie Métabolique, Centre de Biologie Humaine, CHU Sud, 80054, Amiens Cedex, France.
[7] Laboratoire de Biochimie et Biologie Moléculaire, Université de Lille et Pôle de Biologie Pathologie Génétique du CHRU de Lille, 59000, Lille, France.
[8] Department of Pharmacology, Rouen University Hospital, 76000, Rouen, France.

Corresponding author:
Prof. Soumeya BEKRI
Department of Metabolic Biochemistry,
Rouen University Hospital 76031 ROUEN Cedex France
soumeya.bekri@chu-rouen.fr

Tel	00 33 2 32 88 81 24
Fax	00 33 2 32 88 83 41

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Table S1. The normalized concentrations of amino acids (µM/mM creatinine), carnitine (total and free) and acylcarnitines (µM/mM creatinine) in urine samples of from MPS VI and control groups.

	Control		MPS VI	
	Mean	Range	Mean	Range
Alanine	4.645	0.682 - 101	10.649	3.381 - 68.8
Arginine	0.811	0.095 - 287.736	2.138	0.631 - 18.6
Aspartic acid	0.998	0.198 - 13.6	2.802	1.333 - 14.2
Citrulline	0.189	0.035 - 3.278	0.415	0.187 - 1.3
Glutamic acid	3.727	0.322 - 68.7	9.169	3.183 - 58.2
Glycine	26.339	3.884 - 552	46.758	15.357 - 446
Leucine	1.152	0.154 - 18.4	2.884	1.286 - 14.2
Methionine	0.333	0.064 - 8.09	0.745	0.289 - 4.5
Ornithine	1.378	0.258 - 172.17	2.621	0.945 - 15.5
Phenylalanine	1.051	0.094 - 18.2	2.355	0.844 - 16.8
Proline	0.444	0.119 - 12.335	1.138	0.475 - 9.1
Tyrosine	4.035	0.805 - 76.2	9.124	2.857 - 62.2
Total carnitine	1.214	0.031 - 49.693	2.066	0.173 - 11.384
Free carnitine	9.177	0.27 - 358.277	20.402	2.542 - 120.409
Acetylcarnitine	0.507	0.01 - 15.846	0.961	0.068 - 6.21
Propionylcarnitine	0.034	0 - 32.28	0.043	0.002 - 0.46
Butyrylcarnitine	0.150	0.001 - 3.22	0.308	0.025 - 1.357
Isovalerylcarnitine	0.059	0.001 - 0.8	0.146	0.01 - 0.814
Glutarylcarnitine	0.173	0.01 - 7.13	0.300	0.039 - 2.19
Hexanoylcarnitine	0.012	0.001 - 0.146	0.025	0.003 - 0.115
Octanoylcarnitine	0.020	0.002 - 0.328	0.032	0.005 - 0.192
Decanoylcarnitine	0.005	0 - 0.085	0.010	0.002 - 0.055
Lauroylcarnitine	0.009	0.001 - 0.126	0.020	0.006 - 0.13
Tetradecanoylcarnitine	0.007	0.001 - 0.094	0.018	0.006 - 0.123
Palmitoylcarnitine	0.006	0.001 - 0.099	0.012	0.004 - 0.108
Stearolycarnitine	0.002	0 - 0.018	0.003	0.001 - 0.021

Table S2. Data for Venn diagram of the significant pathways retrieved from metabolomics approaches and *in silico* systems biology approach from Salazar DA et al [1].

| Data	N°	Pathways
MPS VI Targeted *vs* MPS VI *in silico vs* MPS VI untargeted	3	Glutathione metabolism Histidine metabolism Arginine and proline metabolism
MPS VI Targeted *vs* MPS VI untargeted	2	Selenoamino acid metabolism Purine metabolism
MPS VI Targeted *vs* MPS VI *in silico*	8	Urea cycle Glutamate metabolism Cysteine metabolism Tyrosine metabolism Tryptophan metabolism Folate metabolism Beta-alanine metabolism Propanoate metabolism
MPS VI *in silico vs* MPS VI untargeted	8	Pyruvate metabolism Lysine metabolism Glycine, serine, alanine and threonine metabolism Glycosphingolipid metabolism Alanine and aspartate metabolism CoA catabolism Glycerophospholipid metabolism Methionine and cysteine metabolism
MPS VI targeted	19	Thyroid hormone synthesis Aspartate metabolism Catecholamine biosynthesis Nicotinate and nicotinamide metabolism Methionine metabolism Fatty acid metabolism Amino sugar metabolism Glycine and serine metabolism Ammonia recycling Lysine degradation Glucose-alanine cycle Betaine metabolism Warburg effect Alanine metabolism Spermidine and spermine biosynthesis Malate-aspartate shuttle Phenylalanine and tyrosine metabolism Valine, leucine and isoleucine degradation Arachidonic acid metabolism
MPS VI untargeted	12	Linoleate metabolism Urea cycle/Amino group metabolism Porphyrin metabolism Vitamin B5 - coa biosynthesis from pantothenate Pyrimidine metabolism Vitamin B9 (folate) metabolism Amino-sugar metabolism Vitamin B3 (nicotinate and nicotinamide) metabolism Aspartate and asparagine metabolism Vitamin E metabolism Electron transport chain Carnitine shuttle
MPS VI *in silico*	62	Transport, peroxisomal; Keratan sulfate degradation; Oxidative phosphorylation; Vitamin D metabolism; Purine catabolism; Starch and sucrose metabolism; Pyrimidine catabolism; Amino sugar metabolism; Eicosanoid metabolism; Transport, endoplasmic reticular; Transport, Golgi apparatus; Transport, extracellular; Biotin metabolism; Vitamin C metabolism; Pyrimidine synthesis; Citric acid cycle; CoA synthesis; Transport, mitochondrial; Pentose phosphate pathway; NAD metabolism; Triacylglycerol synthesis; ROS detoxification; D-alanine metabolism; Vitamin A metabolism; Taurine and hypotaurine metabolism; Cholesterol metabolism; Steroid metabolism; Glyoxylate and dicarboxylate metabolism; Thiamine metabolism; Nucleotide interconversion; Androgen and estrogen synthesis and metabolism; Fructose and mannose metabolism; Heme synthesis; Dietary fiber binding; Tetrahydrobiopterin metabolism Valine, leucine, and isoleucine metabolism; O-glycan synthesis; Butanoate metabolism; Sphingolipid metabolism; Vitamin B6 metabolism; Fatty acid synthesis; Vitamin B2 metabolism; Squalene and cholesterol synthesis transport, Nuclear phosphatidylinositol phosphate metabolism; C5-branched dibasic acid metabolism; Glycolysis/gluconeogenesis; Transport, lysosomal; Phenylalanine metabolism; Fatty acid oxidation; N-glycan degradation; Inositol phosphate metabolism; Purine synthesis; N-glycan synthesis; R group synthesis; Keratan sulfate synthesis; Heparan sulfate degradation; Bile acid synthesis; Galactose metabolism; Miscellaneous; exchange/demand reaction

Table S3. MRM transitions for free carnitine and acylcarnitines and the corresponding internal standard.

		Transition	
Analyte	Analyte	Q1 (*m/z*)	Q2 (*m/z*)
Free carnitine	Carnitine	218	85
	Carnitine-D9	227	85
Acetylcarnitine	C2-Carnitine	260	85
	C2-Carnitine-D3	263	85
Propionylcarnitine	C3-Carnitine	274	85
	C3-Carnitine-D3	277	85
Butyrylcarnitine	C4-Carnitine	288	85
	C4-Carnitine-D3	291	85
Isovalerylcarnitine	C5-Carnitine	302	85
	C5-Carnitine-D9	311	85
Glutarylcarnitine	C5DC-Carnitine	388	85
	C5DC-Carnitine-D6	394	85
Hexanoylcarnitine	C6-Carnitine	316	85
	C6-Carnitine-D3	319	85
Octanoylcarnitine	C8-Carnitine	344	85
	C8-Carnitine-D3	347	85
Decanoylcarnitine	C10-Carnitine	372	85
	C10-Carnitine-D3	375	85
Lauroylcarnitine	C12-Carnitine	400	85
	C12-Carnitine-D3	403	85
Tetradecanoylcarnitine	C14-Carnitine	428	85
	C14-Carnitine-D3	431	85
Palmitoylcarnitine	C16-Carnitine	456	85
	C16-Carnitine-D3	459	85
Stearolycarnitine	C18-Carnitine	484	85
	C18-Carnitine-D3	487	85

Table S4. MRM transitions for amino acids and the corresponding internal standard.

Analyte	Transition	
	Q1 (*m/z*)	Q2 (*m/z*)
Alanine	146	44
Alanine-D4	150	48
Aspartic acid	246	144
Aspartic acid -D3	249	147
Glutamic acid	260	158
Glutamic acid -D5	265	163
Leucine	188	86
Leucine-D3	191	89
Methionine	206	104
Methionine-D3	209	107
Phenylalanine	222	120
Phenylalanine-D5	227	125
Tyrosine	238	136
Tyrosine-D4	242	140
Valine	174	72
Valine-D8	182	80
Arginine	231	70
Arginine-D7	238	77
Citrulline	232	113
Citrulline-D2	234	115
Glycine	132	76
Glycine-13C2-15N	135	79
Ornithine	189	70
Ornithine-D6	195	76
Proline	172	116
Proline-D7	179	123

Figure S1. OPLSDA model validation including the two groups: MPS VI and Controls. Below the figure, model parameters and CV-ANOVA results are presented.
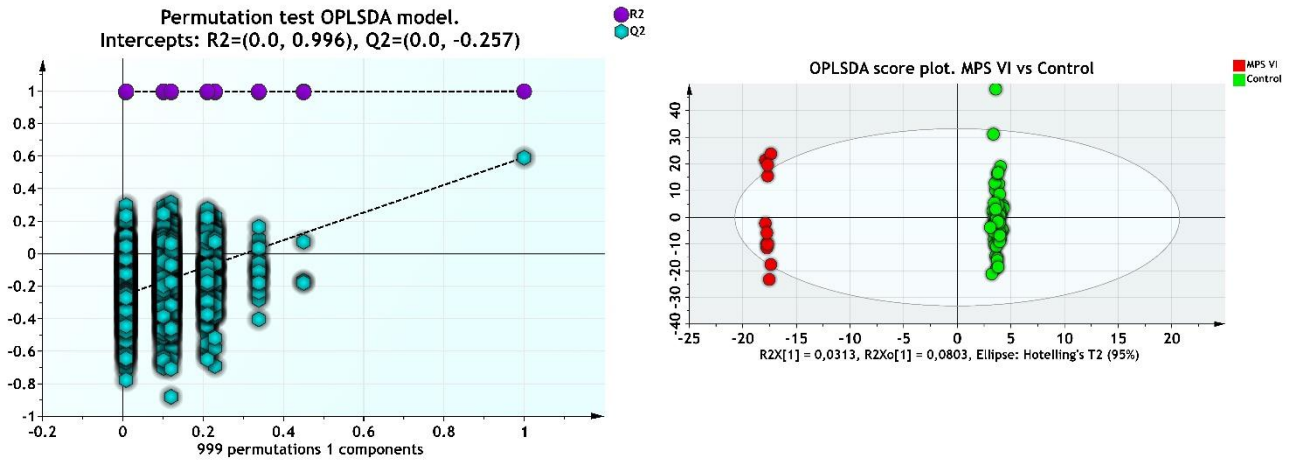
### MPS VI *vs* Controls - OPLSDA model parameters.

Component	R2X	R2X(cum)	Eigenvalue	R2	R2(cum)	Q2	Limit	Q2(cum)	R2Y	R2Y(cum)
Model		0.255			0.999			0.592		1
Predictive		0.0313			0.999			0.592		1
P1	0.0313	0.0313	1.97	0.999	0.999	0.592	0.01	0.592	1	1
Orthogonal in X(OPLS)		0.223			0					
O1	0.0803	0.0803	5.06	0	0					
	0.0574	0.138	3.61	0	0					
O2	0.0266	0.164	1.67	0	0					
	0.0328	0.197	2.07	0	0					
	0.0263	0.223	1.66	0	0					

MPS IV *vs* Controls - OPLSDA model parameters.

	SS	DF	MS	F	*p*	SD
Total corr.	62	62	1			1
Regression	36.7331	12	3.0611	6.05753	2.143e-006	1.7496
Residual	25.2669	50	0.505337			0.710871

Figure S2. A) Boxplots of selected discriminant features and dermatan sulfate in the assessed groups: MPS VI and Control samples. B**)** Correlation plot of aminoacids, dermatane sulfate and selected discriminant features. Correlation is displayed in percentage. The size of the circles is correlated to the correlation coefficients.
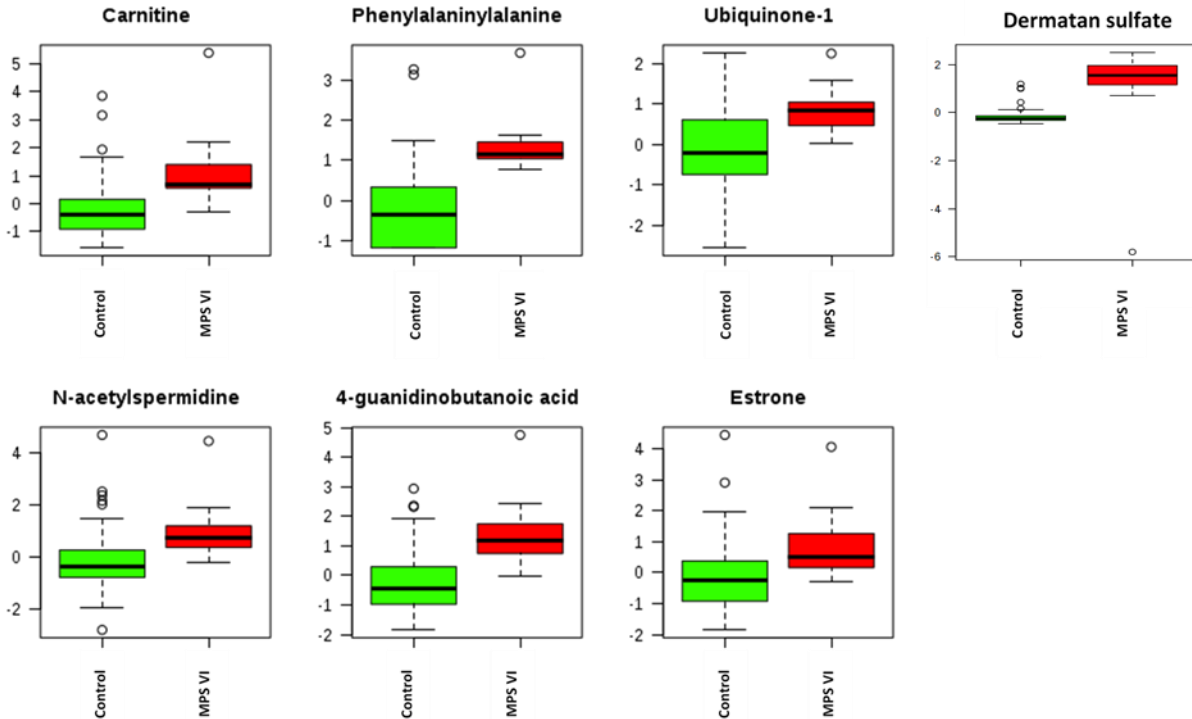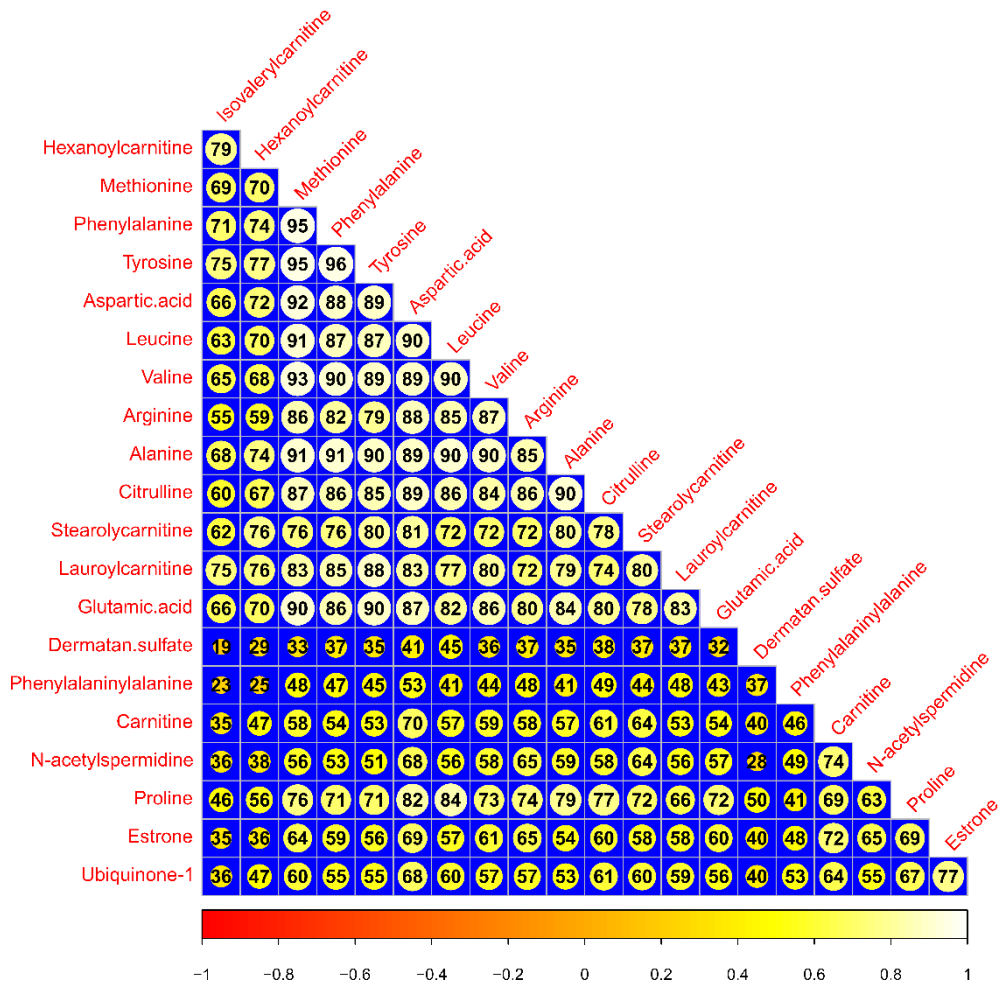
Figure S3. Boxplots of amino acid, carnitine (free and total) and acylcarnitine concentrations.
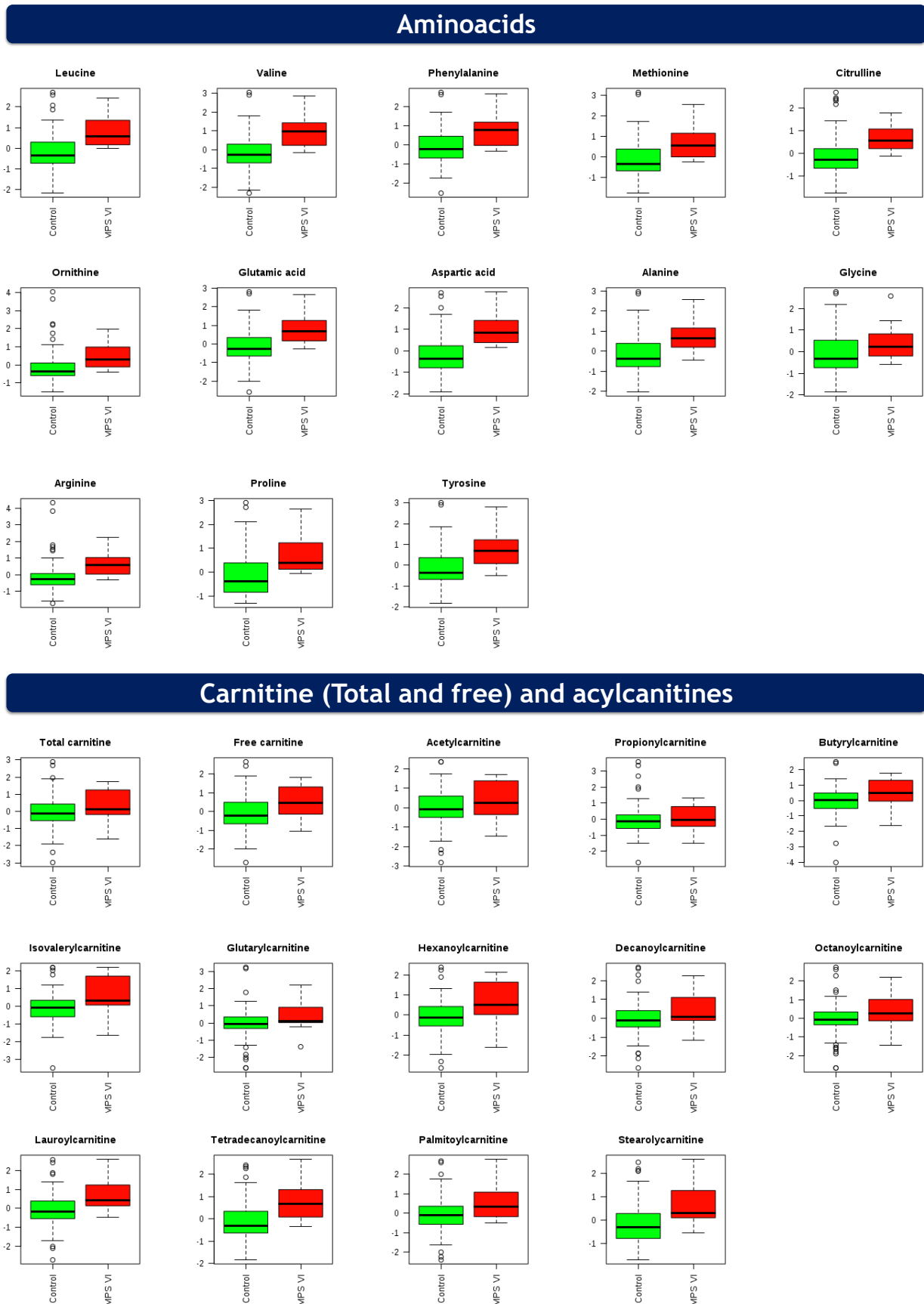
Figure S4. The area under the receiver operating characteristic (ROC) curves, comparing diagnostic performance of the most significant quantified amino acids to differentiate MPS VI from Control samples. A) Aspartic acids, Valine and Arginine. B) A comparison of different combinations of seven most significant metabolites using either a PLSDA or Support Vector Machine (SVM) model with three components each is presented. Combining metabolites does not show improvement in AUC.

AUC, Area under the curve. False positive rate = 100 – Specificity. True positive rate = Sensitivity.
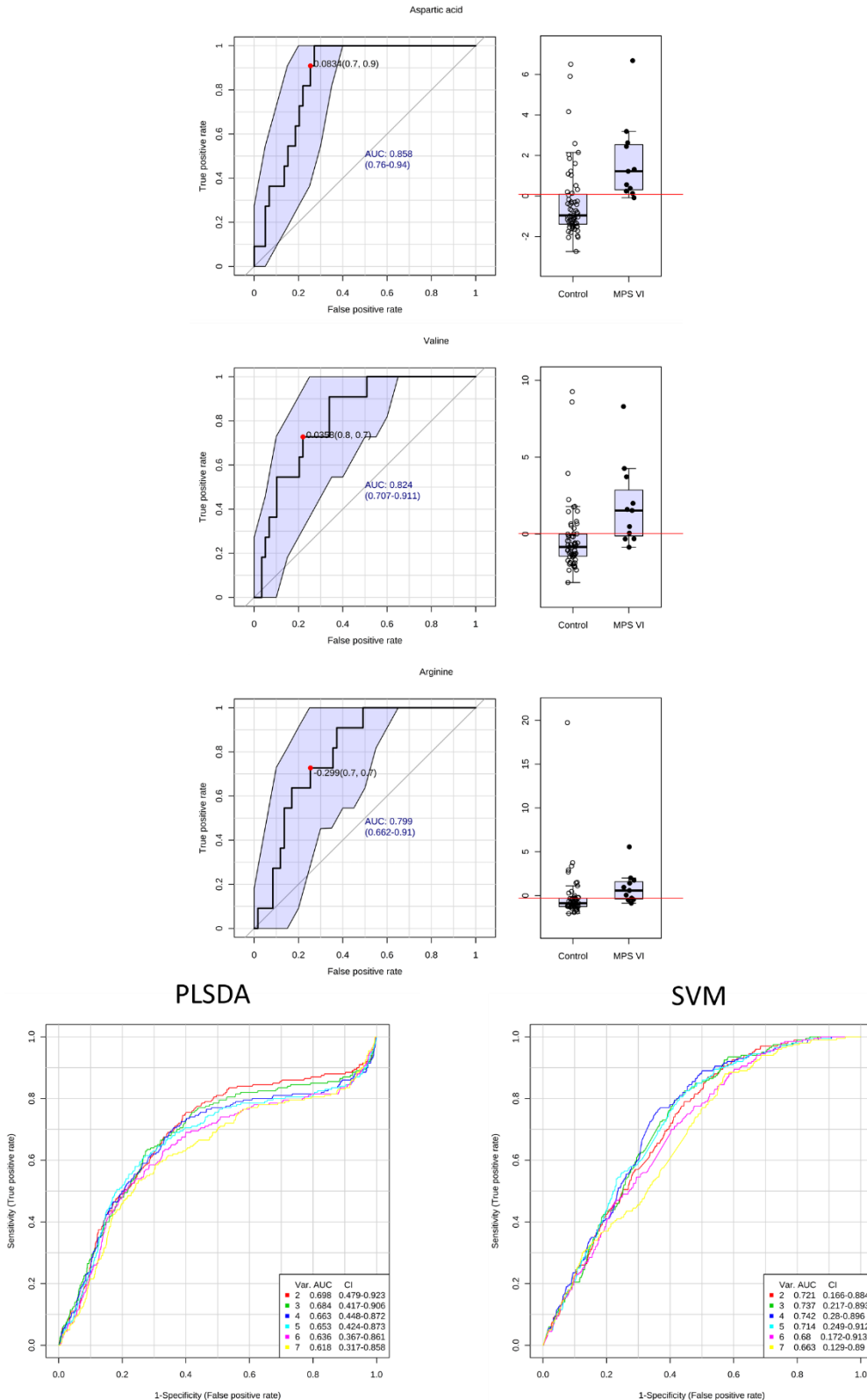
References

1.   Salazar, D.A.; Rodriguez-Lopez, A.; Herreno, A.; Barbosa, H.; Herrera, J.; Ardila, A.; Barreto, G.E.; Gonzalez, J.; Almeciga-Diaz, C.J. Systems biology study of mucopolysaccharidosis using a human metabolic reconstruction network. *Molecular genetics and metabolism* **2016**, *117*, 129-139.