

Supplementary Information

Untargeted Metabolomics of Dried Urine Spot Samples from Pompe Disease Patients

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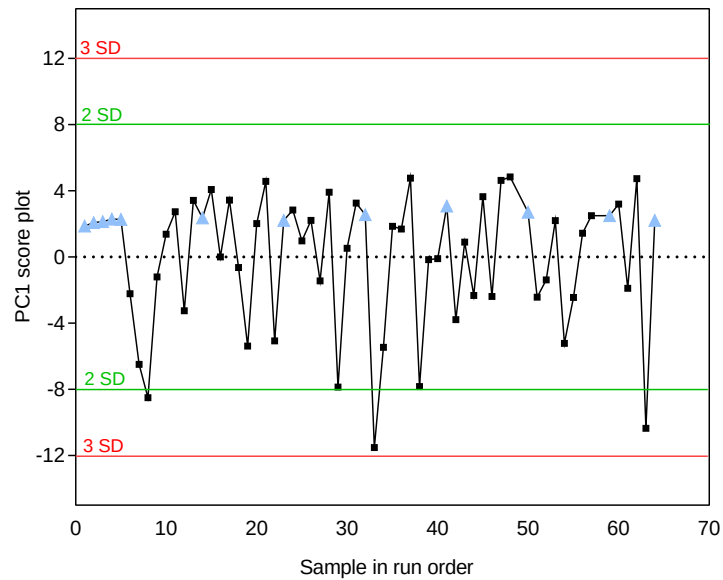


Figure S1. Time series plot of the first principal component *versus* sample run order. Quality control (QC) samples are shown as solid triangles (▲), and samples as solid squares (■). The figure also illustrates the 2- and 3-standard deviation (SD) limits, shown as solid green and red lines, respectively.

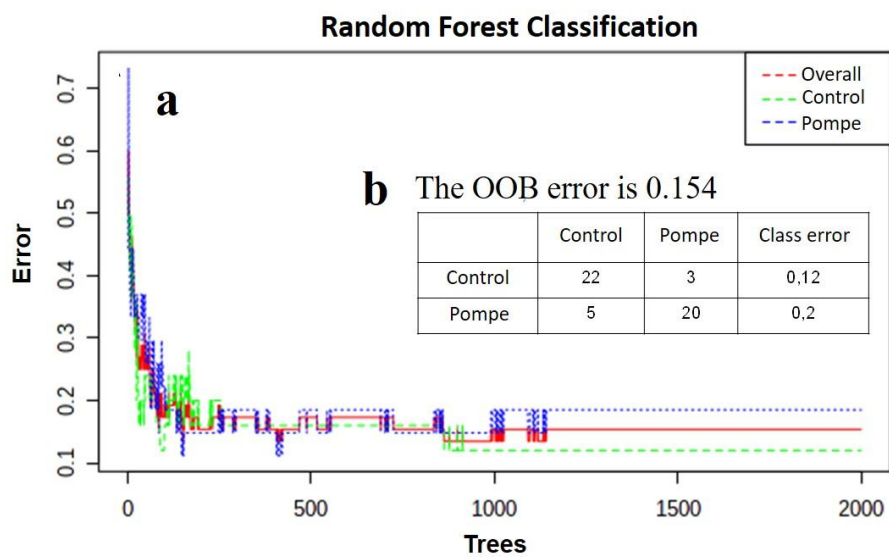


Figure S2. Random forest (RF) classification analysis: (a) the overall error rate is shown as the red line, the control group as the green line, and the Pompe group as the blue line; (b) tabulated data showing the class error for each group; OOB: out-of-bag.

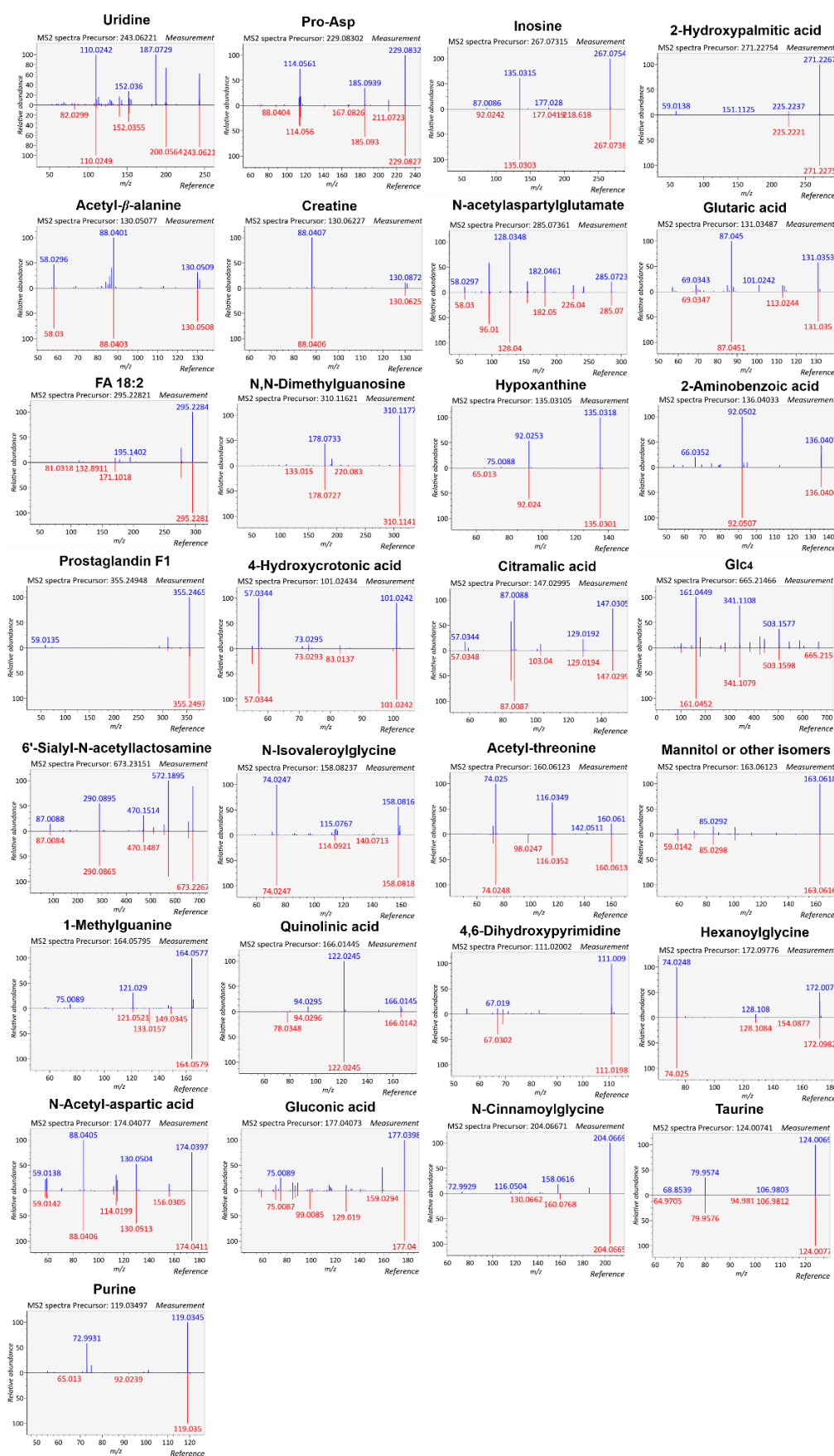


Figure S3. Metabolite annotation using experimental MS/MS spectra compared with the NIST 2020 MS/MS library. Glc4: glucose tetrasaccharide; FA: fatty acid; Pro-Asp: prolyl-aspartic acid.

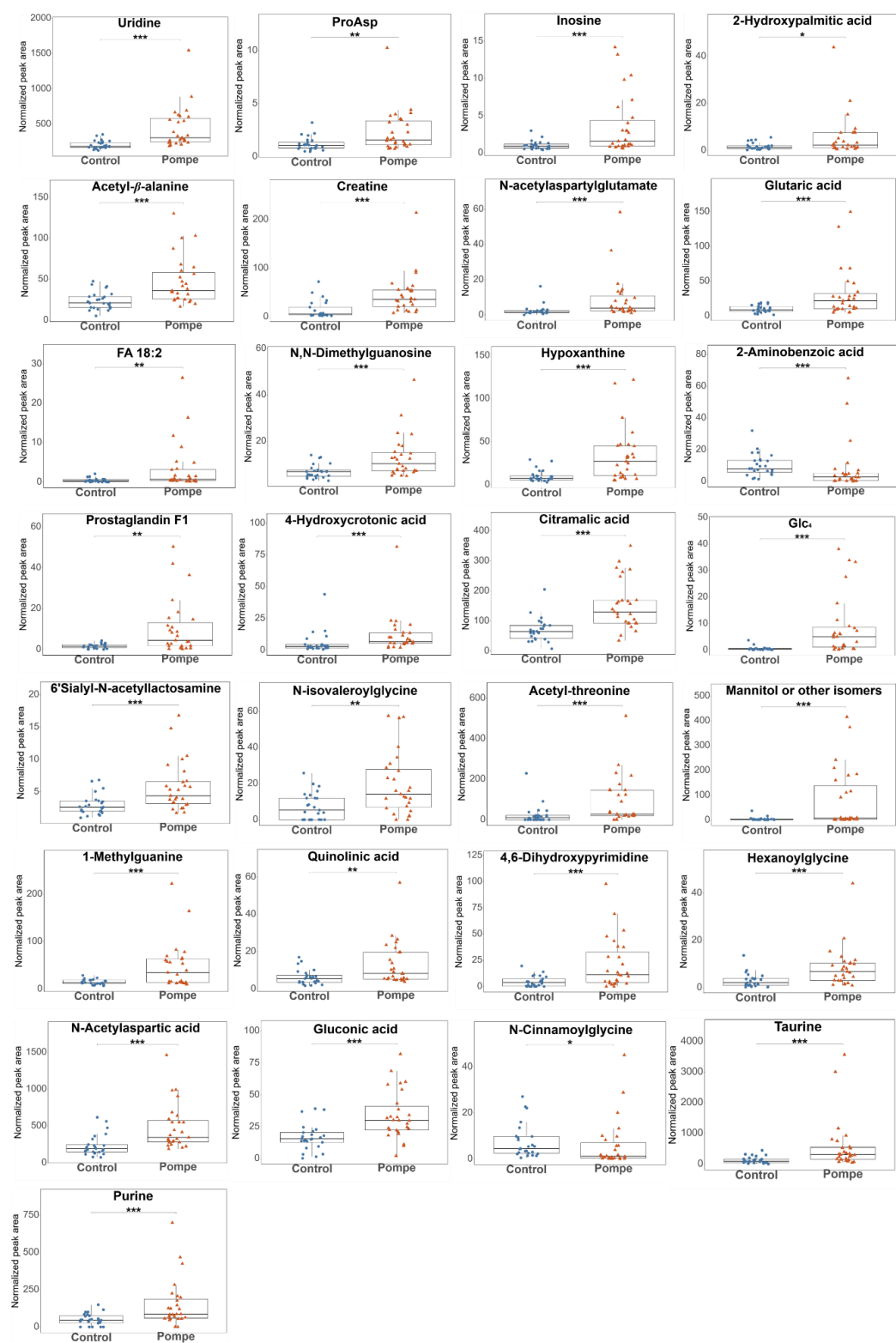


Figure S4. Boxplots showing significantly altered urinary levels of metabolites in Pompe disease patients compared to controls. *P*-values are from the Mann-Whitney test. Statistically significant are showed: *** < 0.001, ** < 0.01 and * < 0.05; Glc₄: glucose tetrasaccharide; FA: fatty acid; Pro-Asp: prolyl-aspartic acid.



Figure S5. Sunburst chart showing the 29 annotated compounds in DUS from Pompe patients, along with their respective chemical classes; Glc4: glucose tetrasaccharide; FA: fatty acid; Pro-Asp: prolyl-aspartic acid; NAAG: *N*-acetylaspartylglutamate.

Table S1. Concentration of glucose tetrasaccharide (Glc₄) in dried urine spots (DUS) from Pompe patients and controls using LC-HRMS¹

Pompe					Control		
Sample ID	ERT	Phenotype	Glc ₄ / (mmol mol ⁻¹ creatinine)	Sex	Sample ID	Glc ₄ / (mmol mol ⁻¹ creatinine)	Sex
P03	yes	IOPD	20.6	M	C1	0.685	M
P04	yes	IOPD	8.62	M	C2	0.197	M
P05	yes	IOPD	5.04	M	C3	0.43	F
P06	yes	IOPD	166	M	C4	< LOQ	F
P09	yes	IOPD	43.4	M	C5	0.171	M
P10	yes	IOPD	38.9	M	C6	1.28	F
P11	yes	IOPD	33.1	M	C7	0.325	M
P17	no	IOPD	74.3	F	C8	< LOQ	M
P18	yes	IOPD	87.7	F	C9	0.602	F
P23	no	LOPD	1.59	F	C10	< LOQ	F
P24	yes	LOPD	0.705	F	C11	0.475	M
P32	yes	LOPD	3.81	M	C12	2.53	M
P33	yes	LOPD	1.54	M	C13	0.267	M
P35	yes	LOPD	11.5	M	C14	0.912	F
P36	yes	LOPD	12.9	M	C15	0.403	F
P37	yes	LOPD	7.67	M	C16	0.543	F
P38	yes	LOPD	3.35	M	C17	0.811	F
P39	yes	LOPD	12.2	M	C18	0.617	F
P52	yes	LOPD	1.95	F	C19	0.227	M
P53	yes	LOPD	3.57	F	C20	< LOQ	F
P61	no	IOPD	202	M	C21	< LOQ	M
P73	yes	LOPD	0.462	F	C22	1.05	M
P74	yes	LOPD	1.23	M	C23	< LOQ	F
P75	yes	IOPD	144	M	C24	0.023	M
P78	no	IOPD	14.8	F	C25	0.26	M

LC-HRMS: liquid chromatography-high-resolution mass spectrometry; ID: sample identification number; IOPD: infantile-onset Pompe disease; LOPD: late-onset Pompe disease; ERT: enzyme replacement therapy; M: male, F: female; LOQ: limit of quantification.

Table S2. Receiver operating characteristic (ROC) curve analysis of metabolites in dried urine spots (DUS) from Pompe patients

Compound	AUC	Specificity	Sensitivity	Confidence interval (95%)	<i>p</i> -valor
Uridine	0.878	0.9	0.7	0.771-0.954	1.32×10^{-3}
Glc ₄	0.873	0.9	0.8	0.762-0.963	1.24×10^{-6}
Citramalic acid	0.862	0.8	0.9	0.749-0.959	1.48×10^{-3}
Creatine	0.857	0.7	0.9	0.749-0.947	3.60×10^{-5}
2-Aminobenzoic acid	0.830	0.8	0.8	0.699-0.933	6.83×10^{-4}
Hypoxanthine	0.827	0.8	0.8	0.697-0.926	3.54×10^{-3}
<i>N</i> -Acetyl- <i>L</i> -aspartic acid	0.824	0.8	0.8	0.686-0.922	1.15×10^{-3}
Taurine	0.816	0.8	0.8	0.682-0.914	1.45×10^{-3}
Gluconic acid	0.808	0.8	0.8	0.673-0.927	2.68×10^{-2}
Purine	0.801	0.7	0.8	0.664-0.912	1.35×10^{-2}
Acetyl-beta-alanine	0.796	0.8	0.6	0.668-0.898	9.23×10^{-3}
Mannitol or other isomers	0.795	0.8	0.6	0.673-0.907	1.64×10^{-3}
<i>N</i> -Acetylaspartylglutamate	0.793	0.7	0.8	0.662-0.913	7.33×10^{-4}
Hexanoylglycine	0.792	0.7	0.8	0.657-0.903	5.14×10^{-3}
<i>N,N</i> -Dimethylguanosine	0.782	0.7	0.8	0.658-0.894	3.66×10^{-2}
6'-Sialyl- <i>N</i> -acetyllactosamine	0.782	0.8	0.7	0.654-0.902	3.01×10^{-2}
4-Hydroxycrotonic acid	0.780	0.8	0.8	0.658-0.940	3.24×10^{-2}
Acetyl- <i>L</i> -threonine	0.776	0.8	0.7	0.642-0.903	8.84×10^{-2}
1-Methylguanine	0.769	0.9	0.6	0.630-0.885	5.48×10^{-3}
Inosine	0.763	0.7	0.7	0.627-0.878	3.77×10^{-3}
Glutaric acid	0.763	0.9	0.6	0.615-0.886	1.58×10^{-2}
FA 18:2	0.761	0.7	0.7	0.625-0.882	1.39×10^{-2}
4,6-Dihydroxypyrimidine	0.760	0.8	0.6	0.611-0.882	6.52×10^{-3}
Quinolinic acid	0.744	0.7	0.6	0.589-0.865	3.03×10^{-2}
Prostaglandin F1	0.739	0.8	0.6	0.585-0.875	1.22×10^{-4}
<i>N</i> -Isovaleroylglycine	0.739	0.6	0.7	0.577-0.863	1.97×10^{-2}
Pro-Asp	0.726	0.7	0.7	0.579-0.856	4.64×10^{-2}
2-Hydroxypalmitic acid	0.710	0.8	0.6	0.521-0.830	4.72×10^{-2}
<i>N</i> -Cinnamoylglycine	0.705	0.8	0.6	0.510-0.828	3.64×10^{-4}

AUC: area under the curve, Glc₄: glucose tetrasaccharide; FA: fatty acid; Pro-Asp: prolyl-aspartic acid.

References

1. De Souza, H. M. R.; Scalco, F. B.; Garrett, R.; Ferreira, F.; Marques, C.; *Anal. Methods* **2023**, *15*, 3932. [Crossref]

