Reagents and Chemicals used for the samples preparation and the UHPLC-HRMS Metabolomic Analysis

HPLC grade solvent (acetonitrile, methanol) and formic acid were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water (H_2O) was collected from a Milli-Q system (Millipore, Billerica, MA). Internal Standards (ISs), Carnitine C8:0-d3, Carnitine C16:0-d3, FFA C18:0-d3, CA-d4, CDCA-d5, Phe-d5, and Trp-d5 were purchased from Cambridge Isotope (Tewksbury, MA); FFA C16:0-d3 was purchased from C/D/N Isotopes Inc. (Pointe-Claire, Québec), and LPC 19:0 was supplied by Avanti Polar Lipids (Alabaster, AL).

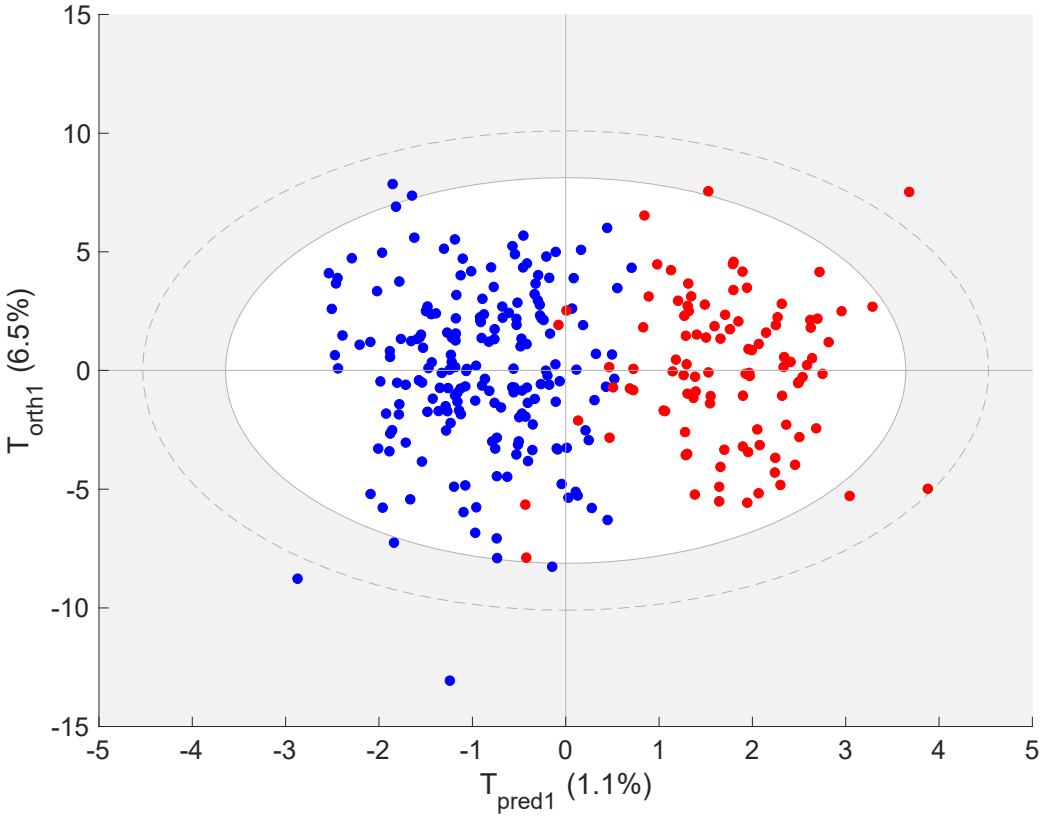
Supplemental Table S1. Internal Standards (ISs) and concentration.

Internal Standards (ISs)	Concentration in extraction solvent (ug/mL)
Carnitine C2:0-d3	0.16
Carnitine C10:0-d3	0.10
Carnitine C16:0-d3	0.15
LPC 19:0	0.75
FFA C16:0-d3	2.50
FFA C18:0-d3	2.50
CA-d4	1.85
CDCA-d4	1.49
Leu-d3	5.00
Val-d8	4.00
Phe-d5	3.61
Trp-d5	4.25

Supplemental Table S2. Important metabolites in the discrimination of participants who developed PCa during the follow-up from controls selected by sPLS-DA.

Metabolites	m/z	Rt	FC	p-value	Adj. p-value	FDR
Eicosadienoic acid	309.2776	6.04	0.18	1.43E-05	3.07E-03	4.38E-04
Ethyl oleate	311.2928	6.97	0.41	5.12E-05	1.10E-02	1.37E-03
Phosphate	98.9843	0.41	0.09	1.02E-05	2.19E-03	4.38E-04
Tiglyl carnitine	244.1539	1.31	-0.10	6.31E-04	1.35E-01	1.35E-02
L-glutamic acid	148.0609	0.38	-0.19	6.75E-11	1.44E-08	7.22E-09
Serotonin	177.1022	0.48	-0.25	1.30E-08	2.78E-06	6.96E-07
2-hydroxyadenine	152.0567	0.39	-0.24	8.77E-10	1.88E-07	6.25E-08
7-Keto cholesterol	401.3407	5.96	-0.10	1.01E-04	2.16E-02	2.40E-03
Sphinganine	302.3046	3.64	-0.33	8.88E-16	1.90E-13	1.90E-13
Sphingosine	300.2902	3.51	-0.21	1.21E-05	2.60E-03	4.33E-04

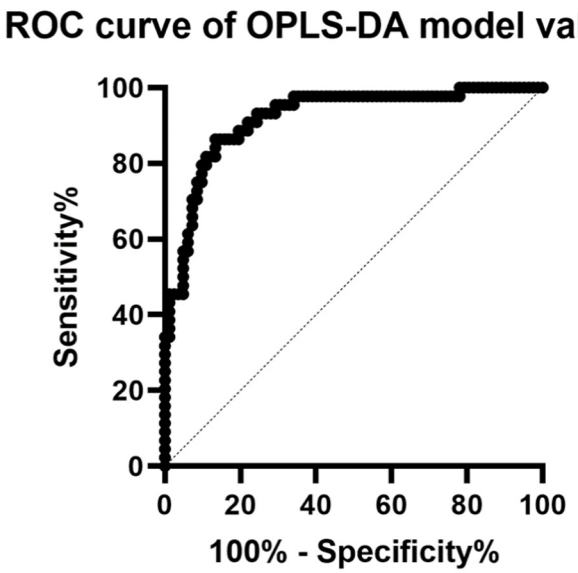
m/z: detected mass; Rt, retention time (in minutes); FC, fold change; FDR, false discovery rate; p-value were computed by using Multiple Experiment Viewer (MeV_4_9_0_r2731_win); Adj. p-value, p-value with Bonferroni adjustment.



Supplemental Figure S1. Score plot of OPLS-DA model for the Discovery cohort.

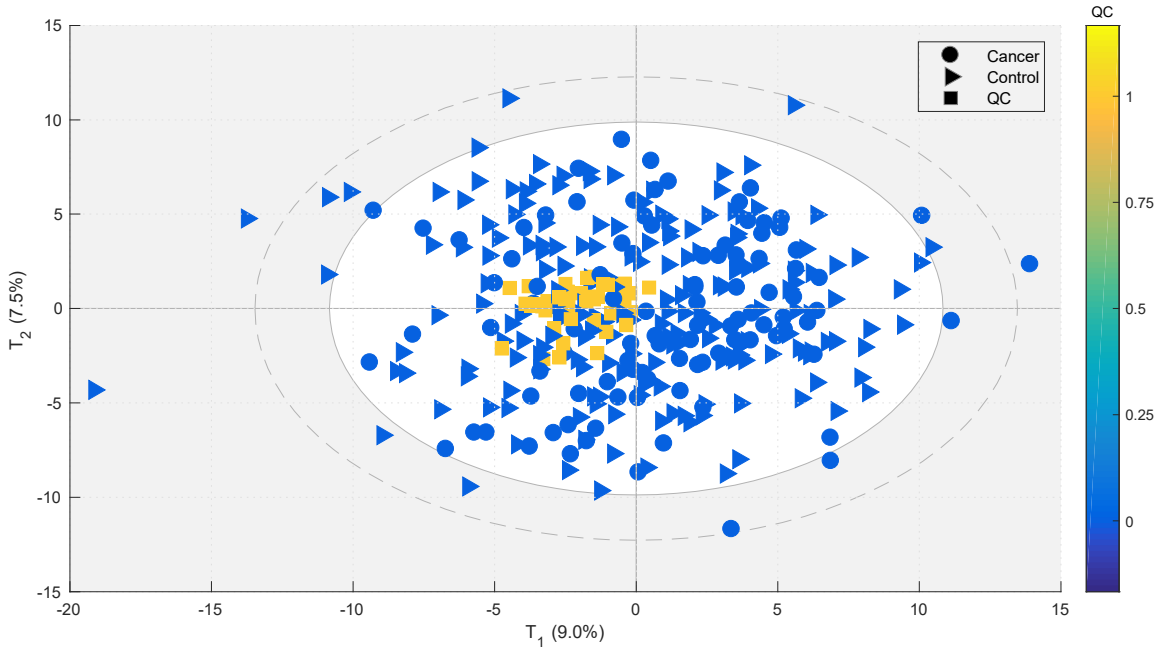
Prostate cancer cases (n=102, red circle), matched controls (n=190, blue circle).

Components: 1(predictive) + 4(orthogonal); R2: 0.73, Q2cum: 0.48.



Supplemental Figure S2. AU-ROC for the OPLS-DA model validation.

The OPLS-DA model from the discovery cohort was validated by prediction of samples in the validation cohort (remaining 30% of all samples, PCa cases: n= 44 / Controls: n= 82). AU-ROC: 0.92 (Sensitivity: 86.36%; Specificity: 86.59%), 95% confidence interval (0.87, 0.97), P value: < 0.0001.



Supplemental Figure S3. Score plot of PCA with QC samples (highlighted).