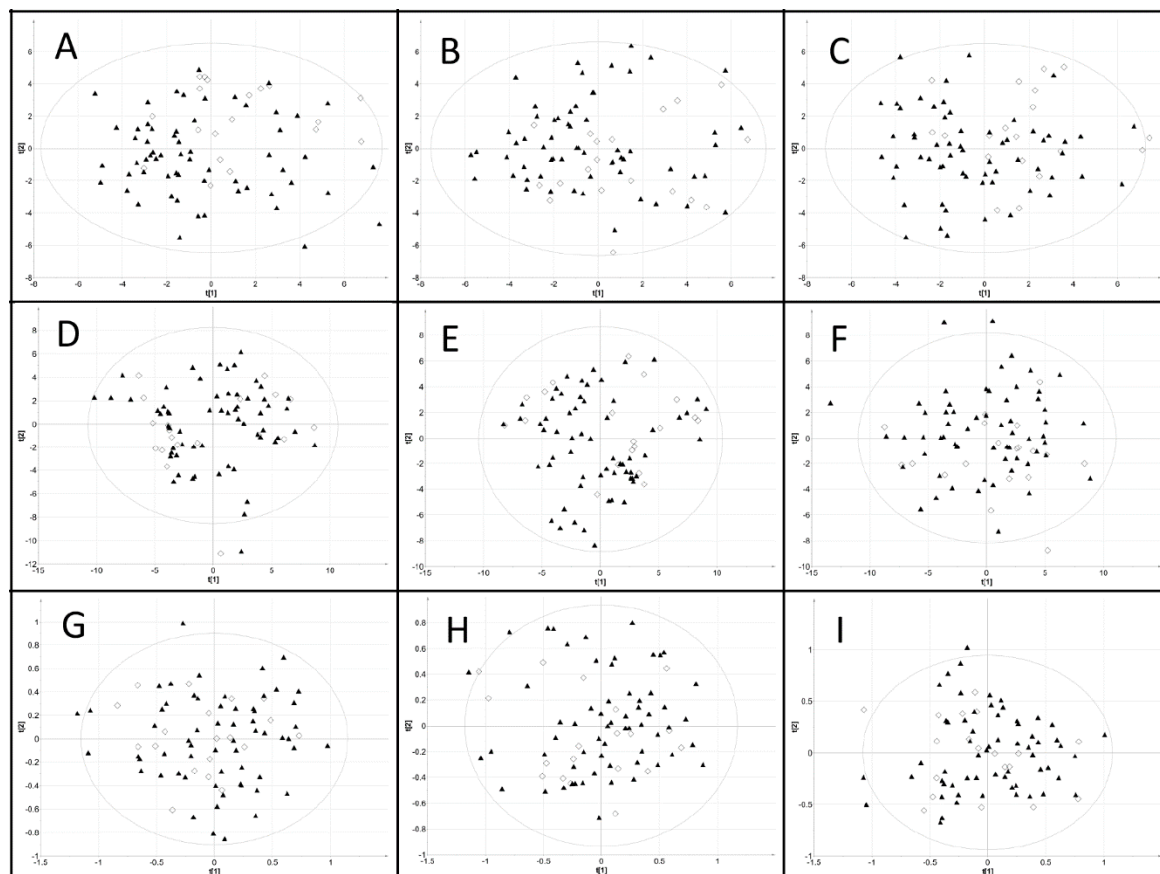# Supplementary Materials: Distinguishing Benign from Malignant Pancreatic and Periampullary Lesions Using Combined Use of 1H-NMR Spectroscopy and Gas Chromatography–Mass Spectrometry

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Figure S1. Principal Component Analysis (PCA) Scores Scatter plots for three training and validation set allocations of 1H-NMR, GC-MS and combined datasets. All variables are included in the analyses. A, B, and C: three case allocations for 1H-NMR data; D, E and F: three case allocations for GC-MS data; G, H and I: three case allocations for combined 1H-NMR and GC-MS data. Sample sizes were 80 per allocation. Number of variables in each PCA (k) was 83 for 1H-NMR spectroscopy; 146 for GC-MS; and 183 for combined. A= number of components.
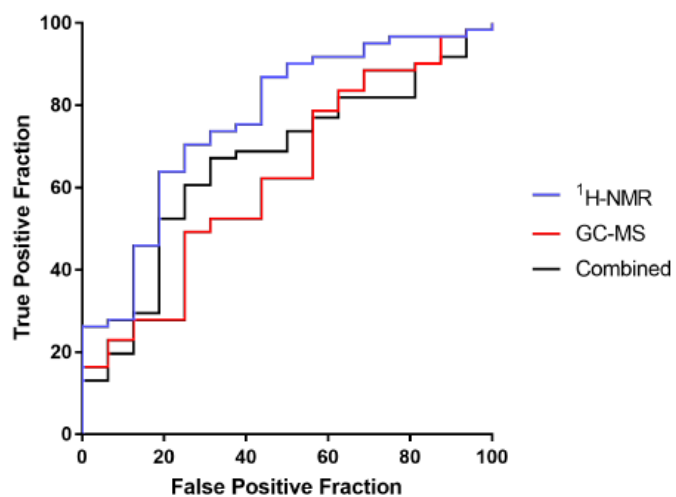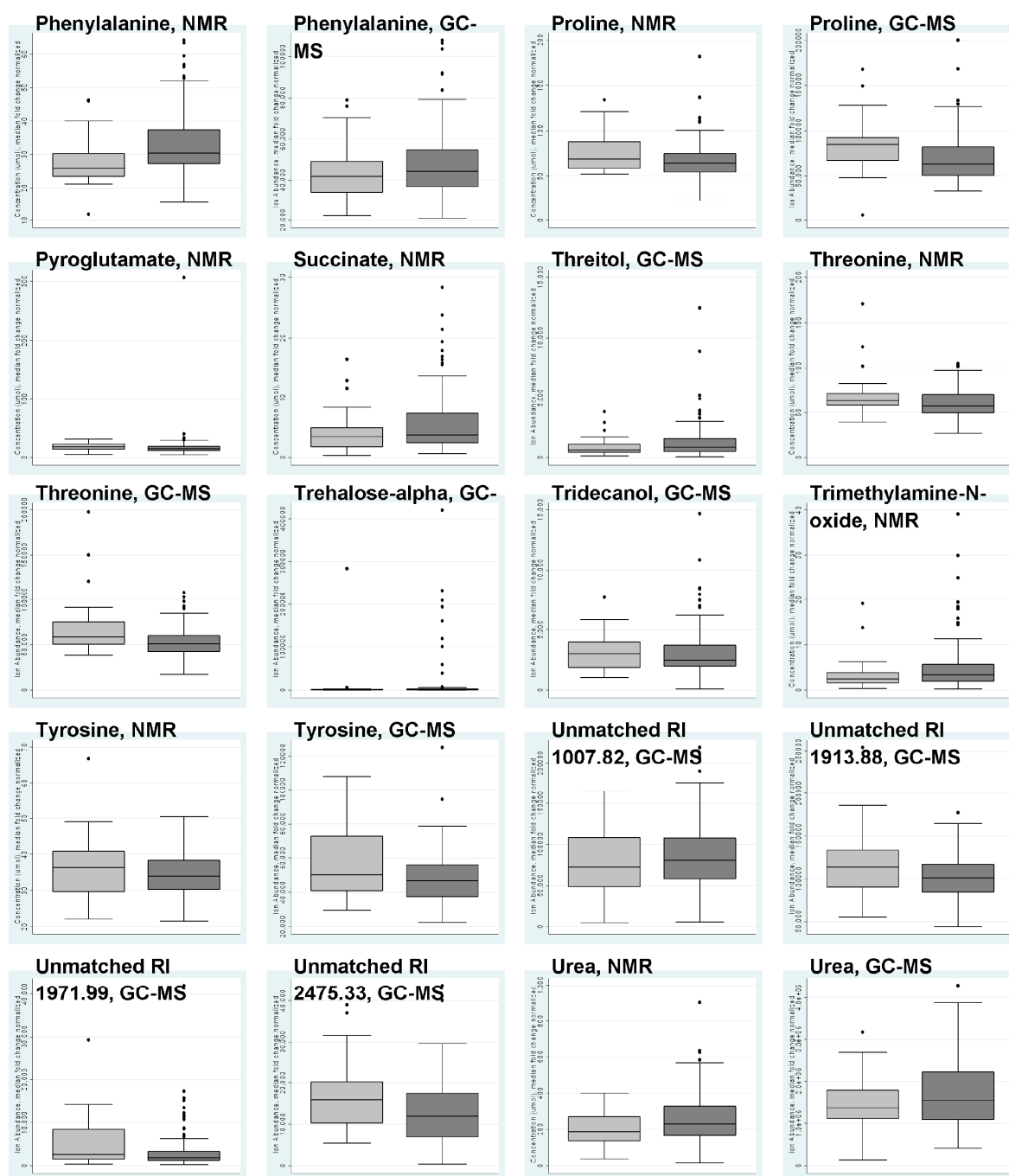
Figure S2. Receiver Operating Characteristic (ROC) curve for detection of benign and malignant periampullary lesions. ^{1}H-NMR: blue, GC-MS: red, Combined model: black.
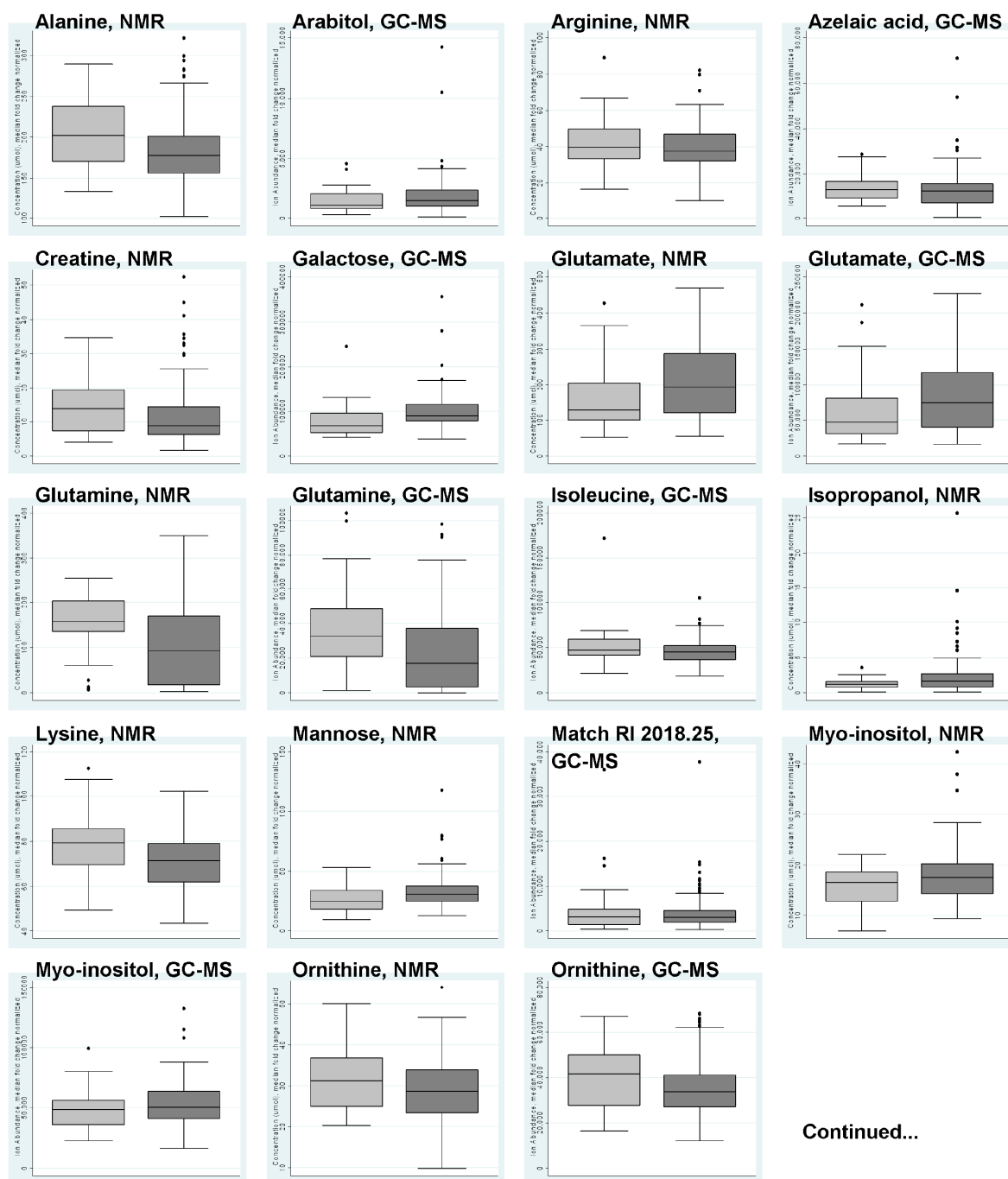
Figure S3. *Cont.*

Figure S3. Whisker plots for consistently contributing metabolites from NMR and GC-MS. Whisker box limits are 1st and 3rd quartiles, whisker bar limits are upper and lower adjacent values. Middle line is median. Y-axis displays normalized concentration/ion abundance data: NMR = μmol, GC-MS = ion abundance. Benign disease: light gray, malignant disease: dark gray.
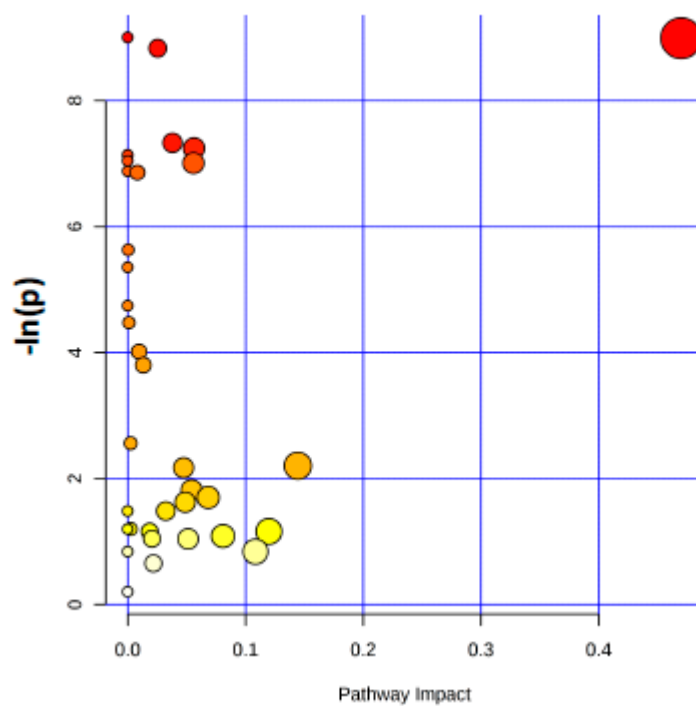
Figure S4. Metaboanalyst 2.0 pathway analysis bubble plot. The plot depicts each pathway's significance (expressed as "–ln(p-value)" on Y-axis) versus its degree of involvement in the biological process (as identified by "impact" on X-axis).

Table S1. Performance measurements in triplicate analysis of ^{1}H-NMR dataset, 50/50 training/test dataset (n = 80/77).

| | OPLS-DA: Filtered | | | | | | OPLS-DA: Minimal[c] | | | | | | | | |
| | Training | | | | Test | | Training | | | | | | | Test | |
Trial	X[a]	R2Y	Q2	p[b]	Core CV[c]	AUROC	SE	X[a]	R2Y	Q2	p[b]	AUROC(i)	Cancer	Non-cancer	AUROC	SE
A	26	0.334	0.174	6.4×10^{-4}	0.487	0.71	0.07	13	0.251	0.122	6.6×10^{-3}		Isopropanol Mannose Phenylalanine Succinate TrimethylamineNoxide TwoHydroxyisobutyrat	Alanine Betaine Glutamine Histidine Lysine Proline Threonine	0.79	0.06
B	28	0.378	0.259	9.8×10^{-6}	0.484	0.73	0.07	14	0.308	0.224	6.1×10^{-5}		Mannose MyoInositol Phenylalanine Urea	Creatine Ethanol Glutamine Histidine Lysine Ornithine Proline Pyroglutamate Threonine Xanthine	0.71	0.05
C	24	0.282	0.140	3.3×10^{-3}	0.491	0.80	0.05	12	0.230	0.128	5.2×10^{-3}	0.756	*Choline Isopropanol Mannose Succinate TrimethylamineN-oxide*	Alanine Creatine Inosine Lysine Ornithine Pyroglutamate Threonine	0.75	0.06
Average	*26*	*0.331*	*0.191*	*1.3×10^{-3}*		*0.75*	*0.06*	*13*	*0.263*	*0.158*	*3.8×10^{-3}*				*0.75*	*0.06*

[a] Number of metabolites/features in the filtered (*t*-test *p*-value <0.3) training set

[b] *p*-value for CV-ANOVA testing

[c] Minimal list of metabolites determined by multistep iterative process: selection of metabolites contributing most to class difference by multivariate O2PLS analysis controlling for technical and clinical covariates; then iterative OPLS-DA modeling with sequential exclusion of metabolites based on their contribution to test model parameters- to maximize AUROC.

Table S2. Performance measurements in triplicate analysis of GC-MS dataset, 50/50 training/test dataset (n = 80/77).

| | OPLS-DA: Filtered | | | | | | | OPLS-DA: Minimal[c] | | | | | | | | |
| | Training | | | | | Test | | Training | | | | | | | Test | |
Trial	X[a]	R2Y	Q2	p[b]	Core CV[c]	AUROC	SE	X[a]	R2Y	Q2	p[b]	AUROC(i)	Cancer	Non-cancer	AUROC	SE
A	33	0.440	0.239	4.8×10^{-5}	0.736	0.69	0.07	17	0.336	0.165	1.3×10^{-3}	0.770	Arabitol5TMS Galactose1MEOX MatchRI170922 Threitol4TMS Trehalosealpha UnmatchedRI11763	Isoleucine2TMS MatchRI129451 Ornithine4TMS Proline2TMS Sorbitol6TMS Threonine3TMS Tridecan1oln1TMS UnmatchedRI16313 UnmatchedRI19719 UnmatchedRI24753 UnmatchedRI28215	0.69	0.07
B	43	0.378	0.258	1.3×10^{-5}	0.734	0.52	0.08	21	0.327	0.238	2.9×10^{-5}	0.832	Glutamicacid3TMS Inositolmyo6TMS MatchRI201825 Phenylalanine2TMS UnmatchedRI10078 UnmatchedRI11250 UnmatchedRI11396 UnmatchedRI18425 Urea2TMS	AzelaicAcid2TMS GlutamineDL3TMS Glycerol3TMS Octadecadienoic Ornithine4TMS Tridecan1oln1TMS UnmatchedRI11646 UnmatchedRI13319 UnmatchedRI19138 UnmatchedRI19719 UnmatchedRI22199 UnmatchedRI24753	0.54	0.07
C	33	0.348	0.199	2.6×10^{-4}	0.784	0.70	0.07	17	0.273	0.162	1.2×10^{-3}	*0.755*	*Butanoicacid2am* *Galactose1MEOX* *Inositolmyo6TMS* *Ribitol5TMS* *Threitol4TMS* *UnmatchedRI10078* *Urea2TMS* *UnmatchedRI22127*	AzelaicAcid2TMS GlutamineDL3TMS Isoleucine2TMS MatchRI201825 Ornithine4TMS Tyrosine3TMS UnmatchedRI19138 UnmatchedRI19736 UnmatchedRI24753	0.67	0.08
Average	*36*	*0.389*	*0.232*	*1.1×10^{-4}*	*0.751*	*0.64*	*0.07*	*18*	*0.312*	*0.188*	*8.4×10^{-4}*	*0.786*			*0.67*	*0.07*

[a] Number of metabolites/features in the filtered (*t*-test *p*-value <0.3) training set

[b] *p*-value for CV-ANOVA testing

[c] Minimal list of metabolites determined by multistep iterative process: selection of metabolites contributing most to class difference by multivariate O2PLS analysis controlling for technical and clinical covariates; then iterative OPLS-DA modeling removing metabolites based on their contribution to predictive modeling in test set (YpredPS and T2PS)

Table S3. Performance measurements in triplicate analysis of Combined dataset, 50/50 training/test dataset (*n* = 80/77).

| Trial | OPLS-DA: Filtered | | | | | | | OPLS-DA: Minimal[c] | | | | | | | | |
| | Training | | | | | Test | | Training | | | | | | | | Test | |
	X[a]	R2Y	Q2	*p*[b]	Core CV[c]	AUROC	SE	X[a]	R2Y	Q2	*p*[b]	AUROC(i)	Cancer	Non-cancer	AUROC	SE
A	50	0.451	0.238	4.1×10^{-5}	-	0.67	0.08	24	0.341	0.177	7.8×10^{-4}	0.802	Arabitol5TMS-G Isopropanol-N Mannose-N MatchRI170922-G MyoInositol-N Phenylalanine-C Ribitol5TMS-G Succinate-N Threitol4TMS-G Trehalosealpha-G TwoHydroxyisobutyrat Urea-C	Histidine-C Lysine-N MatchRI129451-G Methionine-C Ornithine-C Proline-C Sorbitol6TMS-G Threonine-C UnmatchedRI16313-G UnmatchedRI22199-G UnmatchedRI24753-G UnmatchedRI28215-G	0.67	0.07
B	62	0.402	0.239	3.7×10^{-7}	-	0.63	0.08	26	0.367	0.231	5.2×10^{-5}	0.839	Glucuronicacid1M-G Mannose-N MyoInositol-N Phenylalanine-C UnmatchedRI10078-G UnmatchedRI10325-G UnmatchedRI11396-G UnmatchedRI18377-G UnmatchedRI18425-G Urea-C	Arginine-N AzelaicAcid2TMS-G Creatine-N Glutamine-C Glycerol-C Histidine-C Lysine-N Methanol-N Ornithine-C Proline-C UnmatchedRI11646-G UnmatchedRI13319-G UnmatchedRI19736-G UnmatchedRI22199-G UnmatchedRI24753-G Xanthine-N	0.66	0.07

[a] Number of metabolites/features in the filtered (*t*-test *p*-value <0.3) training set

[b] *p*-value for CV-ANOVA testing

[c] Minimal list of metabolites determined by multistep iterative process: selection of metabolites contributing most to class difference by multivariate O2PLS analysis controlling for technical and clinical covariates; then iterative OPLS-DA modeling removing metabolites based on their contribution to predictive modeling in test set (YpredPS and T2PS)

Table S3. *Cont.*

| | OPLS-DA: Filtered | | | | | | | OPLS-DA: Minimal[c] | | | | | | | | |
| | Training | | | | Test | | Training | | | | | | | | Test | |
Trial	X[a]	R2Y	Q2	*p*[b]	Core CV[c]	AUROC	SE	X[a]	R2Y	Q2	*p*[b]	AUROC(i)	Cancer	Non-cancer	AUROC	SE
C	47	0.369	0.219	9.3×10^{-5}	-	0.73	0.06	19	0.311	0.204	1.7×10^{-4}	0.795	Choline-N Erythritol4TMS-G Galactose1MEOX-G Isopropanol-N Mannose-N MyoInositol-N Phenylalanine-C Ribitol5TMS-G Threitol4TMS-G TrimethylamineNoxide UnmatchedRI22127-G	Arginine-N Creatine-N Inosine-N Lysine-N MatchRI201825-G Threonine-C UnmatchedRI19138-G UnmatchedRI19736-G	0.68	0.07
Average	*53*	*0.407*	*0.232*	*4.5×10^{-5}*		*0.68*	*0.07*	*23*	*0.34*	*0.204*	*3.3×10^{-4}*	*0.812*			*0.67*	*0.07*

[a] Number of metabolites/features in the filtered (*t*-test *p*-value <0.3) training set

[b] *p*-value for CV-ANOVA testing

[c] Minimal list of metabolites determined by multistep iterative process: selection of metabolites contributing most to class difference by multivariate O2PLS analysis controlling for technical and clinical covariates; then iterative OPLS-DA modeling removing metabolites based on their contribution to predictive modeling in test set (YpredPS and T2PS)