

Supplementary material

Highly sensitive serum volatolomic biomarkers for pancreatic cancer diagnosis

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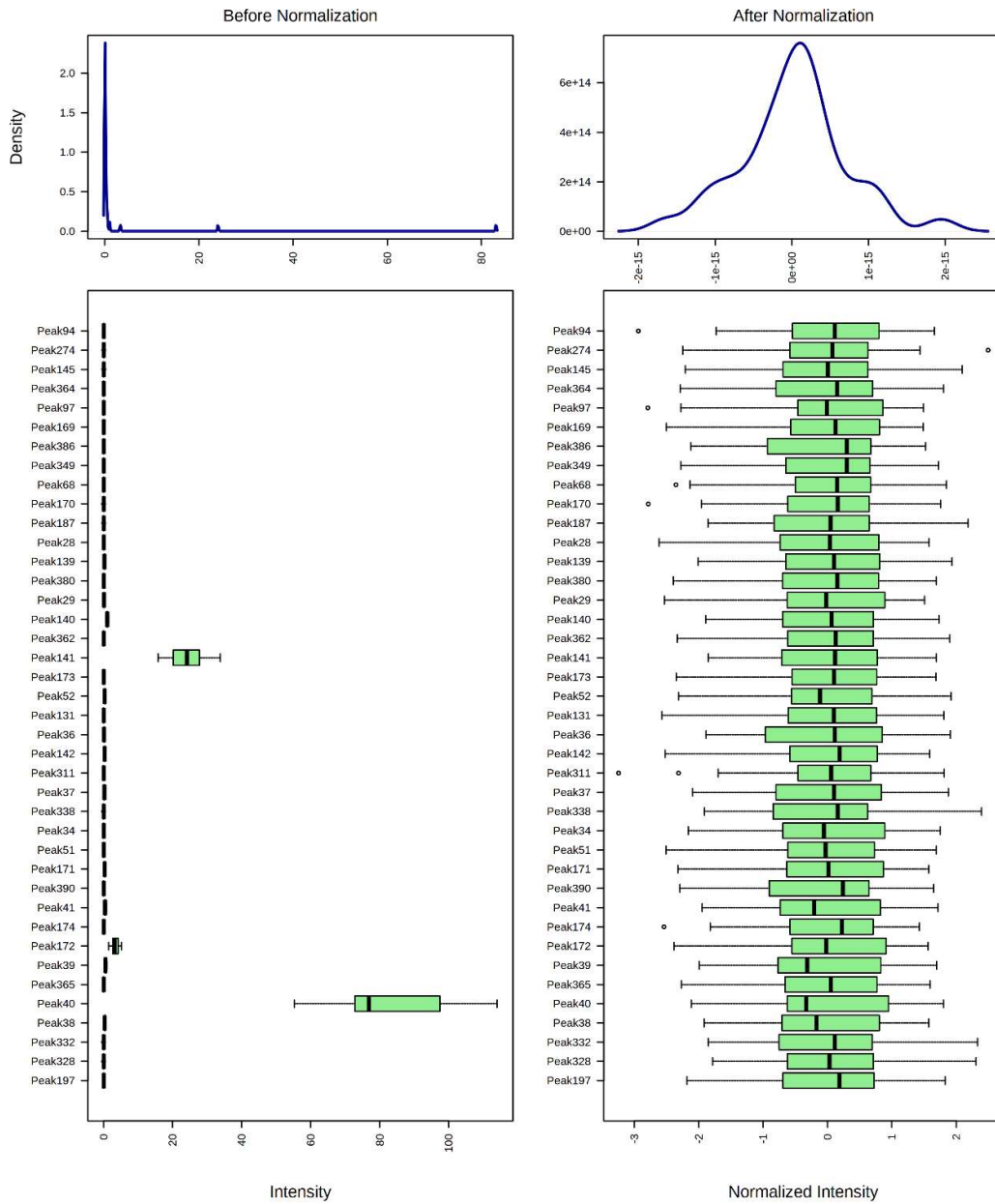
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Table S1. Chromatographic and validation parameters for the selected VOCs. The signal in the procedure blank was normalized by relative area respect to d₄-DCB. Repeatability (% RSD) and intermediate precision (% RSD) were calculated by ANOVA of 3 replicates of a pool of serum from controls (N=5), done three consecutive days.

VOC	RT, min	m/z	Mean relative area	Mean Procedure Blank signal	Repeatability, % RSD	Intermediate precision, % RSD
V197	23.84	71	0.039 ± 0.002	0.0008	8	10
V328	30.17	91	0.017 ± 0.001	0.0006	10	6
V39	5.11	129	1.95 ± 0.10	ND	8	8
V332	30.26	108	0.009 ± 0.001	ND	11	18
V40	5.12	91	244 ± 10	11	6	7
V41	5.13	105	1.56 ± 0.07	0.009	7	5
V172	22.52	57	11.0 ± 0.8	0.65	8	16
V365	31.53	74	0.022 ± 0.001	ND	8	8
V390	32.28	110	0.010 ± 0.001	ND	17	10
V34	4.76	55	0.014 ± 0.001	ND	17	15
V174	22.52	86	0.081 ± 0.005	0.002	10	9
V38	5.11	116	1.13 ± 0.05	0.002	6	7
V338	30.48	108	0.014 ± 0.001	ND	16	16
V311	29.63	86	0.0025 ± 0.0001	ND	6	4
V171	22.49	42	0.91 ± 0.07	0.07	9	18
V173	22.52	63	0.085 ± 0.006	0.005	8	18
V37	5.09	127	0.54 ± 0.03	0.02	7	8
V380	32.00	173	0.081 ± 0.006	0.001	11	10
V36	5.09	176	0.45 ± 0.03	0.004	6	17
V51	8.86	59	0.009 ± 0.001	ND	9	18
V141	20.54	57	52 ± 3	0.35	9	7
V131	19.87	71	0.0066 ± 0.0004	0.0003	6	12
V362	31.41	127	0.0036 ± 0.0004	ND	18	7
V140	20.53	55	2.3 ± 0.1	0.015	9	8
V68	11.89	70	0.023 ± 0.002	0.002	12	17
V142	20.55	113	0.55 ± 0.04	0.002	11	14
V364	31.52	141	0.0071 ± 0.0006	ND	13	9
V29	4.16	55	0.18 ± 0.02	0.03	13	10
V28	4.16	70	0.114 ± 0.009	0.0014	10	15
V139	20.50	63	0.43 ± 0.04	0.002	16	3
V187	23.23	57	0.061 ± 0.003	ND	9	7
V52	8.85	57	1.10 ± 0.05	0.02	8	5
V170	22.49	105	0.039 ± 0.002	ND	4	15
V349	30.97	91	0.0090 ± 0.0005	0.0014	7	12
V386	32.26	205	0.053 ± 0.004	0.011	14	3
V97	16.03	100	0.024 ± 0.001	0.0001	8	10
V169	22.47	89	0.044 ± 0.004	0.0006	16	3
V145	20.86	55	0.069 ± 0.004	0.003	9	11
V274	27.87	91	0.055 ± 0.004	0.0015	10	10
V94	16.01	41	0.097 ± 0.008	0.006	13	14

Fig. S2. Graphical view of the data normalization results. Density graph and boxplot based in all features (a) or samples (b).

a)



b)

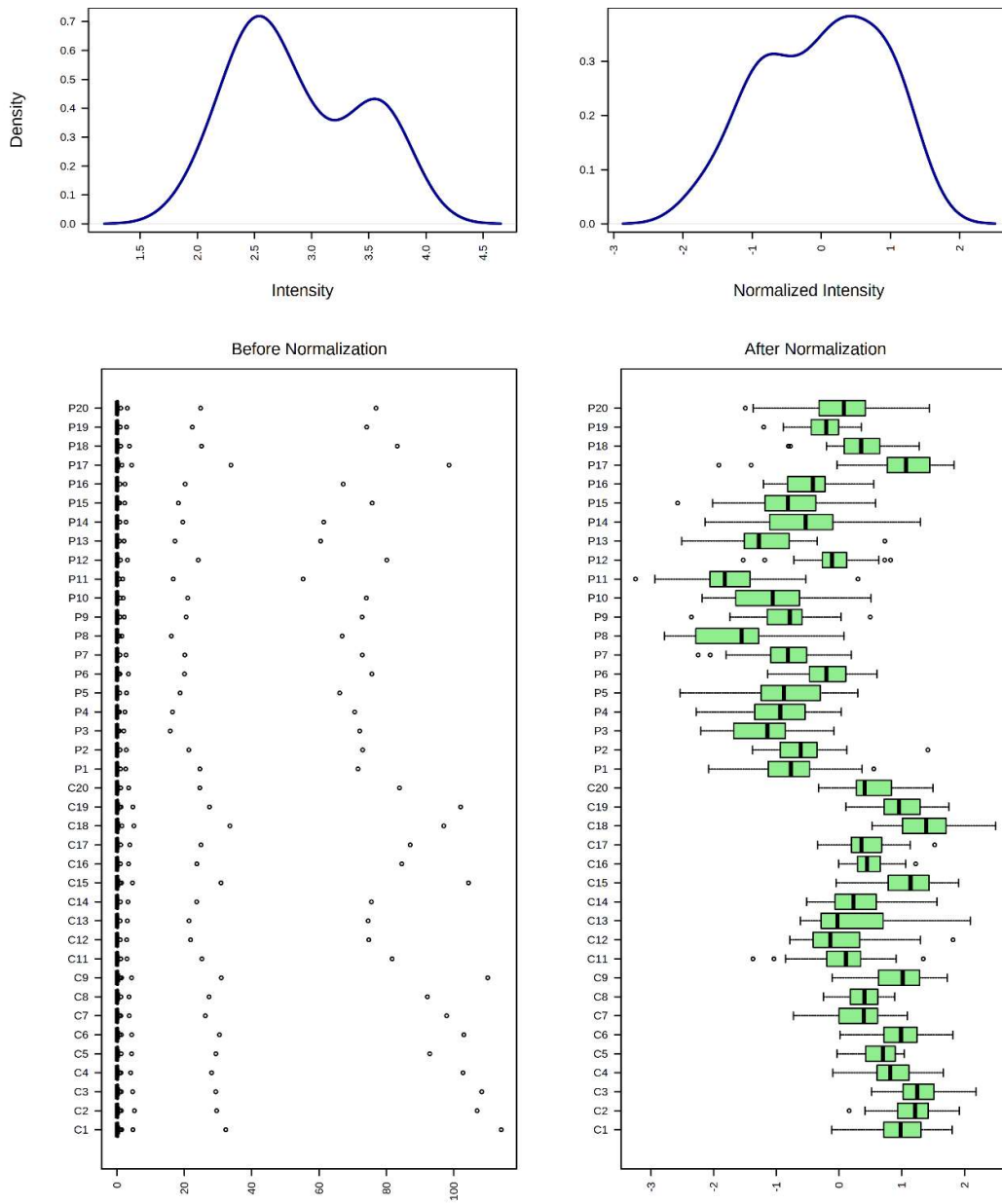


Fig. S3. Scree plot for PCA. Blue line indicates each component explained variance and green line is for accumulated explained variance.

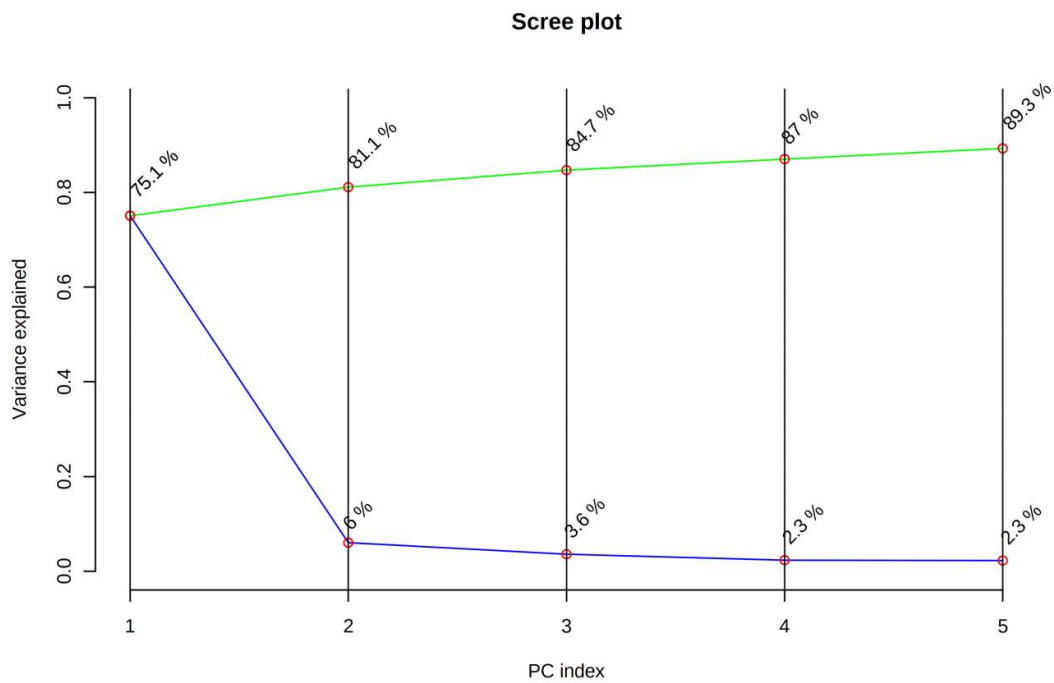
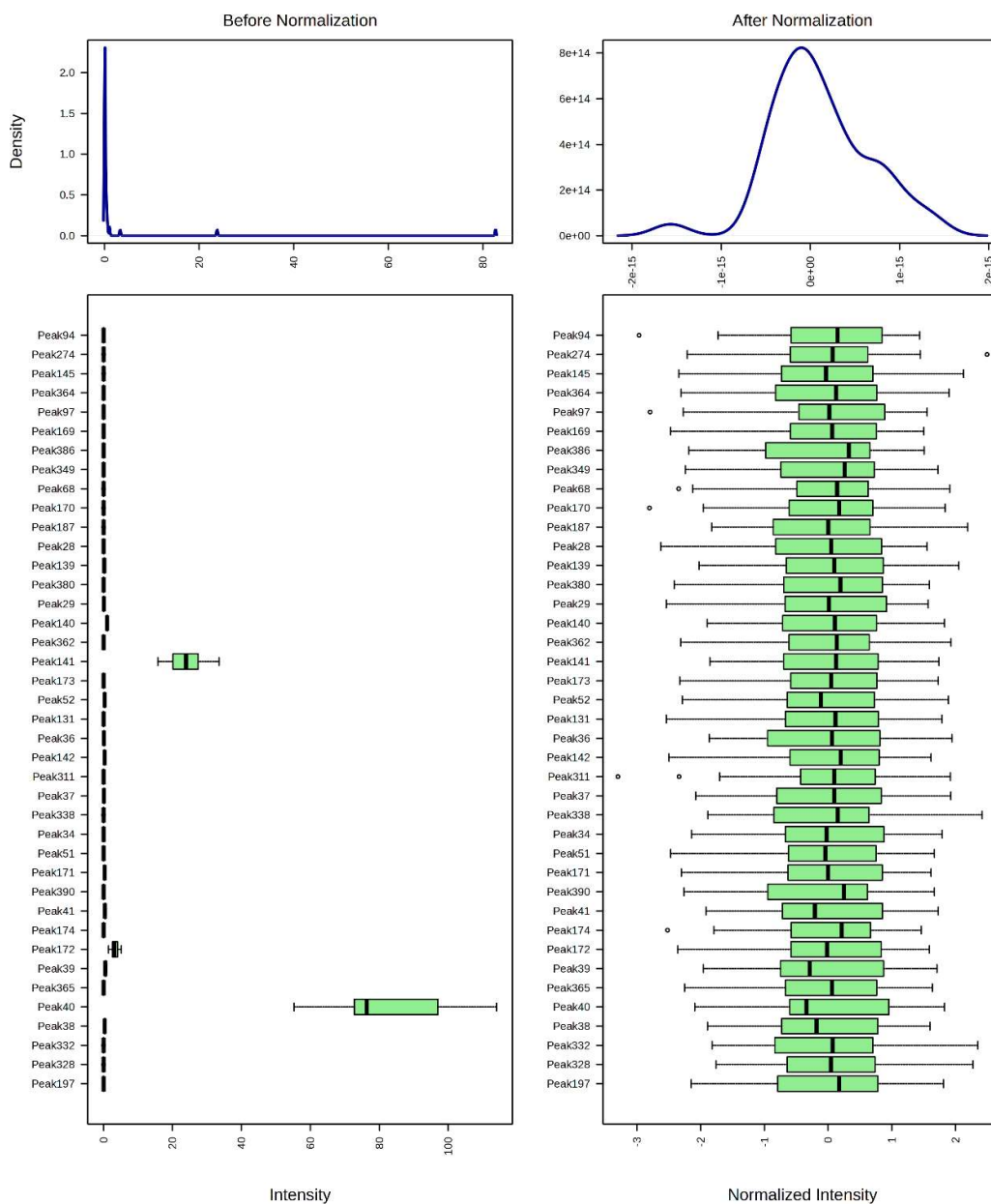
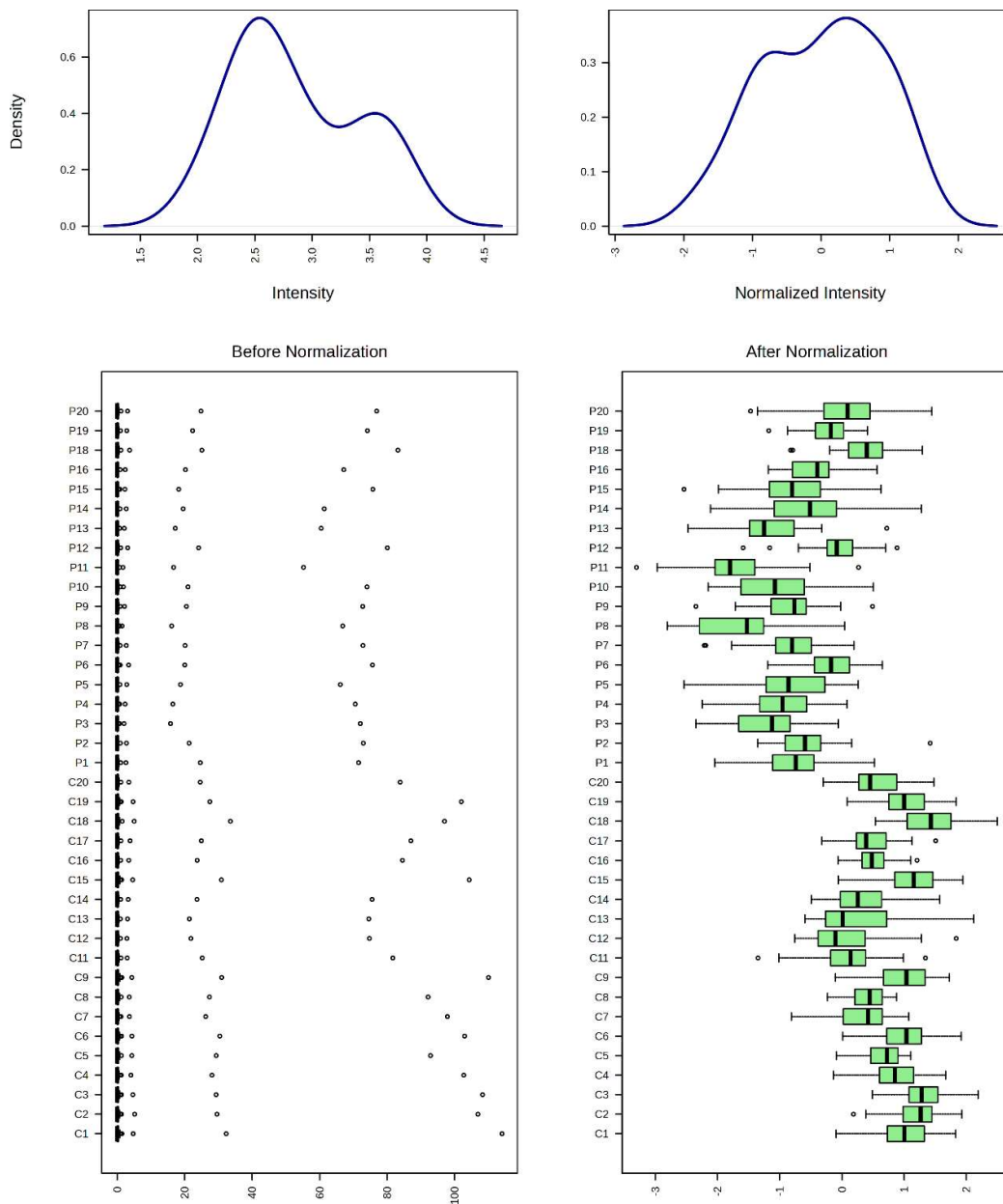


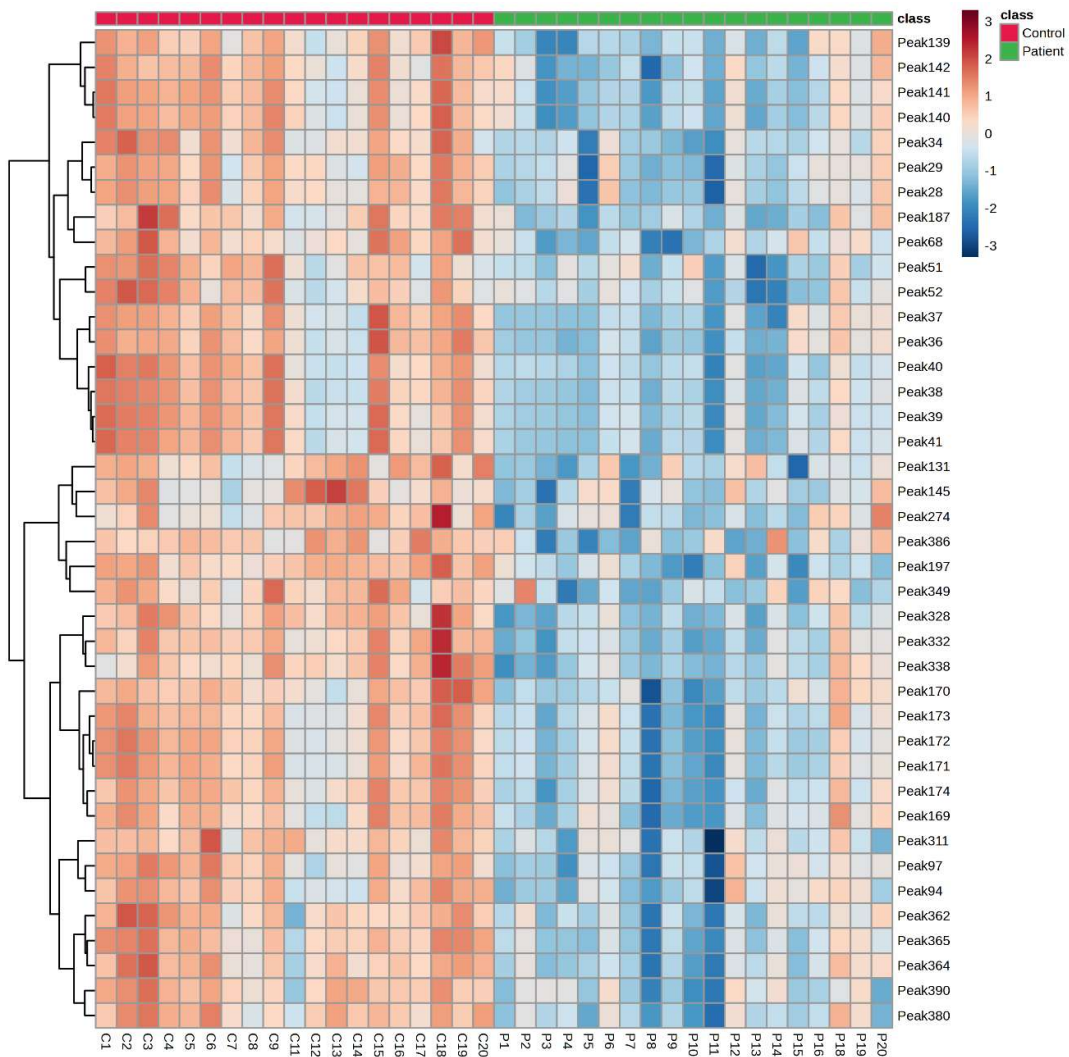
Fig. S4. Results of the control-case study excluding sample P17. A) Normalization results: feature view and sample view; B) heatmap; C) Unsupervised PCA and scree plot; D) Sparse PLS-DA and most significant features. Overall, results do not vary when P17 is excluded from the study.

A)

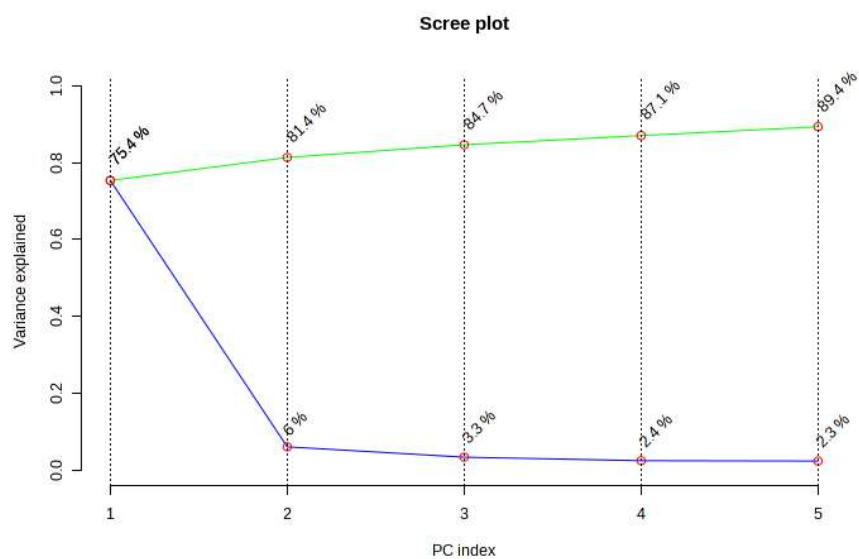
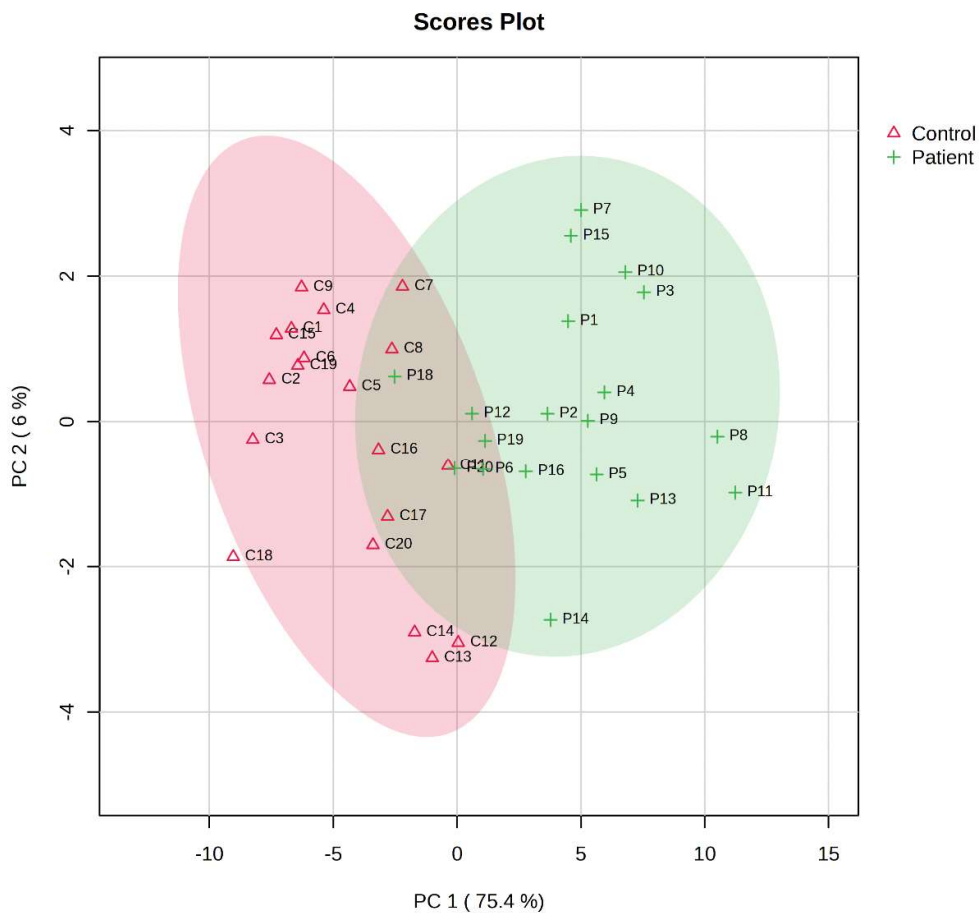




B)



C)



D)

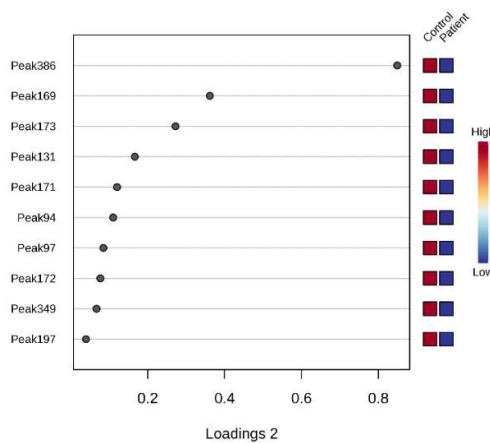
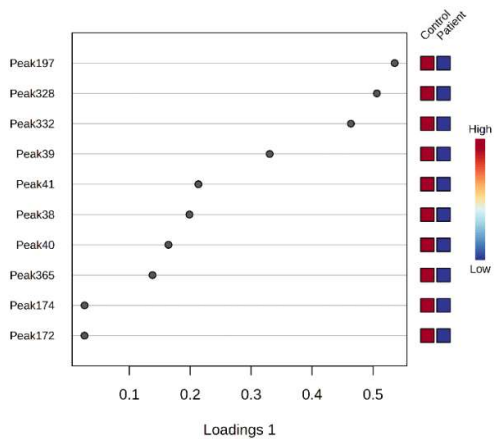
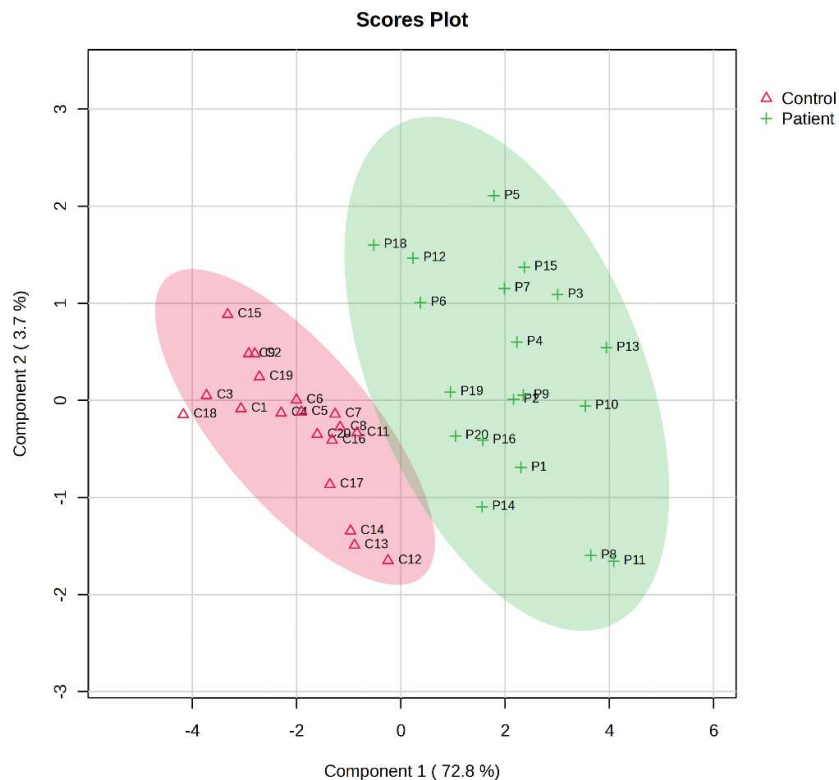


Table S5. VOCs showing any effect of sex (male/female) in their area. Statistics calculated for all participants (n = 39), the control (n = 19) and the patient (n = 20) groups.

Biomarker		M/F p-val	Male, mean ± 95% CI	Male, C/P pval	Female, mean ± 95% CI	Female, C/P pval
V172	All participants	0.23	3.4 ± 0.4		3.1 ± 0.6	
	Controls	0.93	4.0 ± 0.4	0.0004	4.1 ± 0.6	0.0002
	Patients	0.02	2.9 ± 0.4		2.2 ± 0.4	
V365	All participants	0.44	(6.4 ± 0.5) · 10 ⁻³		(6.2 ± 1.0) · 10 ⁻³	
	Controls	0.35	(7.2 ± 0.5) · 10 ⁻³	0.001	(7.7 ± 0.7) · 10 ⁻³	0.0001
	Patients	0.03	(5.7 ± 0.6) · 10 ⁻³		(4.7 ± 0.7) · 10 ⁻³	
V390	All participants	0.42	(2.5 ± 0.2) · 10 ⁻³		(2.4 ± 0.4) · 10 ⁻³	
	Controls	0.39	(2.8 ± 0.2) · 10 ⁻³	0.001	(3.0 ± 0.3) · 10 ⁻³	0.0003
	Patients	0.04	(2.2 ± 0.2) · 10 ⁻³		(1.8 ± 0.4) · 10 ⁻³	
V34	All participants	0.10	(5.5 ± 0.5) · 10 ⁻³		(4.9 ± 0.9) · 10 ⁻³	
	Controls	0.63	(6.4 ± 0.6) · 10 ⁻³	0.0004	(6.1 ± 1.1) · 10 ⁻³	0.001
	Patients	0.003	(4.8 ± 0.5) · 10 ⁻³		(3.6 ± 0.5) · 10 ⁻³	
V174	All participants	0.17	0.025 ± 0.003		0.022 ± 0.005	
	Controls	0.82	0.031 ± 0.003	6 · 10⁻⁴	0.030 ± 0.004	6 · 10⁻⁵
	Patients	0.03	0.020 ± 0.004		0.013 ± 0.003	
V311	All participants	0.03	(1.44 ± 0.14) · 10 ⁻³		(1.19 ± 0.19) · 10 ⁻³	
	Controls	0.12	(1.63 ± 0.14) · 10 ⁻³	0.006	(1.46 ± 0.12) · 10 ⁻³	0.001
	Patients	0.02	(1.26 ± 0.18) · 10 ⁻³		(0.93 ± 0.22) · 10 ⁻³	
V171	All participants	0.26	0.30 ± 0.03		0.27 ± 0.05	
	Controls	0.94	0.34 ± 0.03	0.0009	0.34 ± 0.04	0.0003
	Patients	0.03	0.26 ± 0.03		0.21 ± 0.03	
V173	All participants	0.16	0.024 ± 0.003		0.021 ± 0.004	
	Controls	0.84	0.028 ± 0.003	0.003	0.028 ± 0.004	0.0002
	Patients	0.02	0.020 ± 0.003		0.015 ± 0.003	
V380	All participants	0.49	0.025 ± 0.002		0.024 ± 0.003	
	Controls	0.13	0.027 ± 0.002	0.002	0.030 ± 0.002	7 · 10⁻⁵
	Patients	0.04	0.023 ± 0.003		0.019 ± 0.003	
V36	All participants	0.18	0.142 ± 0.017		0.124 ± 0.022	
	Controls	0.60	0.170 ± 0.021	0.001	0.159 ± 0.018	7 · 10⁻⁵
	Patients	0.03	0.117 ± 0.017		0.090 ± 0.014	
V362	All participants	0.57	(0.86 ± 0.07) · 10 ⁻³		(0.85 ± 0.17) · 10 ⁻³	
	Controls	0.26	(0.97 ± 0.09) · 10 ⁻³	0.004	(1.09 ± 0.18) · 10 ⁻³	0.0007
	Patients	0.04	(0.76 ± 0.09) · 10 ⁻³		(0.61 ± 0.11) · 10 ⁻³	
V68	All participants	0.10	0.014 ± 0.001		0.013 ± 0.003	
	Controls	0.90	0.016 ± 0.002	0.004	0.016 ± 0.003	0.001
	Patients	0.008	0.012 ± 0.002		0.009 ± 0.002	
V142	All participants	0.16	0.30 ± 0.03		0.27 ± 0.04	
	Controls	0.61	0.34 ± 0.03	0.002	0.33 ± 0.02	3 · 10⁻⁵
	Patients	0.04	0.26 ± 0.03		0.21 ± 0.03	
V364	All participants	0.37	(1.7 ± 0.1) · 10 ⁻³		(1.7 ± 0.3) · 10 ⁻³	
	Controls	0.22	(1.9 ± 0.2) · 10 ⁻³	0.03	(2.1 ± 0.3) · 10 ⁻³	0.0003
	Patients	0.02	(1.6 ± 0.2) · 10 ⁻³		(1.2 ± 0.2) · 10 ⁻³	
V29	All participants	0.10	0.081 ± 0.007		0.071 ± 0.012	
	Controls	0.71	0.091 ± 0.007	0.002	0.089 ± 0.010	5 · 10⁻⁴
	Patients	0.02	0.071 ± 0.009		0.054 ± 0.011	
V28	All participants	0.16	0.053 ± 0.004		0.048 ± 0.007	
	Controls	0.81	0.059 ± 0.004	0.004	0.059 ± 0.006	0.0005
	Patients	0.04	0.047 ± 0.006		0.038 ± 0.007	
V170	All participants	0.13	(8.0 ± 1.3) · 10 ⁻³		(6.7 ± 1.6) · 10 ⁻³	
	Controls	0.92	(9.6 ± 1.7) · 10 ⁻³	0.02	(9.2 ± 1.1) · 10 ⁻³	0.0002
	Patients	0.02	(6.6 ± 1.6) · 10 ⁻³		(4.2 ± 1.5) · 10 ⁻³	
V349	All participants	0.08	(4.2 ± 0.5) · 10 ⁻³		(3.4 ± 0.7) · 10 ⁻³	
	Controls	0.22	(5.0 ± 0.6) · 10 ⁻³	0.003	(4.4 ± 0.7) · 10 ⁻³	0.0004
	Patients	0.04	(3.4 ± 0.7) · 10 ⁻³		(2.4 ± 0.5) · 10 ⁻³	
V97	All participants	0.20	(9.0 ± 2.2) · 10 ⁻³		(10.0 ± 1.2) · 10 ⁻³	
	Controls	0.83	(11.7 ± 2.7) · 10 ⁻³	0.01	(12.1 ± 2.5) · 10 ⁻³	0.002
	Patients	0.03	(8.5 ± 1.6) · 10 ⁻³		(5.9 ± 1.7) · 10 ⁻³	
V169	All participants	0.17	(11.0 ± 1.8) · 10 ⁻³		(9.3 ± 2.7) · 10 ⁻³	
	Controls	0.84	(13.5 ± 2.2) · 10 ⁻³	0.006	(13.1 ± 3.0) · 10 ⁻³	0.001
	Patients	0.04	(8.6 ± 2.1) · 10 ⁻³		(5.5 ± 2.1) · 10 ⁻³	

Table S6. List of VOCs that showed a significant response (t-test, equal variances, $\alpha = 0.05$) when patients that survived more than 1 year after diagnosis were compared with those that did not survive. Mean normalized values for both groups, fold change and p-values obtained from the t-test are shown. The p-values and fold change from the patient/control comparison are included.

VOC	RT (min)	Quant Ion m/z	Controls Mean \pm 95% CI	FC (P/C)	Raw P-val C/P	Patient Survival < 1 year Mean \pm 95% CI	Patient Survival > 1 year Mean \pm 95% CI	FC (N/Y)	Raw P-value N/Y
V109	18.2	55	0.012 \pm 0.005	0.8	0.8	0.012 \pm 0.004	0.005 \pm 0.002	2.5	0.005
V345	30.8	119	(0.76 \pm 0.11) $\cdot 10^{-3}$	0.8	0.01	(0.71 \pm 0.12) $\cdot 10^{-3}$	(0.49 \pm 0.08) $\cdot 10^{-3}$	1.5	0.006
V107	17.8	55	0.018 \pm 0.011	0.7	0.03	0.019 \pm 0.010	0.005 \pm 0.002	4.0	0.010
V82	13.5	57	0.027 \pm 0.018	1.0	0.5	0.036 \pm 0.017	0.013 \pm 0.005	2.8	0.018
V386	32.3	205	0.0213 \pm 0.0019	0.6	3 $\cdot 10^{-5}$ *	0.015 \pm 0.004	0.009 \pm 0.002	1.6	0.020
V412	33.4	215	(1.3 \pm 0.3) $\cdot 10^{-3}$	1.1	0.1	(1.7 \pm 0.2) $\cdot 10^{-3}$	(1.3 \pm 0.3) $\cdot 10^{-3}$	1.3	0.024
V317	29.8	120	(1.4 \pm 0.9) $\cdot 10^{-3}$	0.7	0.6	(1.2 \pm 0.3) $\cdot 10^{-3}$	(0.7 \pm 0.1) $\cdot 10^{-3}$	1.6	0.025
V108	18.2	43	0.037 \pm 0.009	0.8	0.1	0.031 \pm 0.010	0.017 \pm 0.005	1.8	0.030
V85	14.2	55	0.012 \pm 0.005	0.9	0.8	0.018 \pm 0.009	0.007 \pm 0.003	2.7	0.033
V13	2.4	43	0.138 \pm 0.011	1.0	0.6	0.128 \pm 0.015	0.154 \pm 0.017	0.8	0.036
V72	12.6	57	0.020 \pm 0.007	0.8	0.4	0.019 \pm 0.010	0.008 \pm 0.003	2.2	0.046
V144	20.8	71	0.021 \pm 0.007	0.9	0.4	0.019 \pm 0.007	0.010 \pm 0.004	1.8	0.046
V102	16.8	57	0.40 \pm 0.12	0.7	0.1	0.33 \pm 0.14	0.16 \pm 0.06	2.0	0.047
V104	16.9	71	0.16 \pm 0.05	0.7	0.1	0.13 \pm 0.06	0.06 \pm 0.02	2.0	0.047
V219	24.9	57	0.007 \pm 0.002	0.7	0.009	0.004 \pm 0.002	0.007 \pm 0.002	0.6	0.049
V323	30.0	87	0.014 \pm 0.003	0.6	0.001	0.010 \pm 0.004	0.005 \pm 0.002	1.8	0.049

Table S7. ROC analysis of the VOCs showing significant differences between the survival-based groups: AUC, cut off point, sensitivity, and specificity.

VOC	AUC	AUC 95% IC	Cutoff	Sensitivity, %	Specificity, %
V109	0.928	0.767-1.000	0.00796	100	89
V345	0.900	0.711-1.000	0.00057	80	89
V107	0.867	0.667-0.978	0.00750	80	79
V82	0.800	0.533-1.000	0.0278	100	67
V386	0.778	0.544-0.950	0.0118	90	67
V412	0.833	0.589-1.000	0.00151	90	89
V317	0.800	0.516-0.972	0.000959	90	67
V108	0.789	0.528-0.989	0.0278	100	67
V85	0.744	0.467-0.939	0.00664	70	78
V13	0.767	0.528-0.967	0.141	70	78
V72	0.756	0.478-0.961	0.0136	90	67
V144	0.767	0.528-0.950	0.0191	100	67
V102	0.767	0.511-0.956	0.296	100	67
V104	0.767	0.500-1.000	0.119	100	67
V219	0.767	0.500-0.956	0.00585	70	78
V323	0.744	0.472-0.933	0.00896	100	55

Table S8. Chemicals with high coincidence score of the mass spectra found using NIST library were purchased and tested. VOCs RT may differ from initially assigned due to system maintenance operations.

VOC	VOC RT, min	NIST Proposed compound	NIST Match score, %	CAS	StandardRT, min
V197	23.5	3,5-dimethylcyclohexanol	58	5441-52-1	20.5+21.0
		2,5-dimethylcyclohexanol	58	3809-32-3	22.2+22.8
		3,4-dimethylcyclohexanol	58	5715-23-1	24.0
		2,3-dimethylcyclohexanol	58	1502-24-5	23.7+24.2
		2,6-dimethylcyclohexanol	58	5337-72-4	19.8+19.9+22.4
		2,4-dimethylcyclohexanol	58	69542-91-2	20.4+20.6
		2,6-octadiene-4,5-diol	57	4486-59-3	28.93
V328	30.0	butoxymethylbenzene	86	588-67-0	30.0
		2,5-dibenzoyloxynitrobenzene	78	51792-85-9	2.7
		4-benzoyloxy-3-methoxy-2-nitrobenzoic acid	78	2450-27-3	2.7
		1,3,5-cycloheptatriene	76	544-25-2	5.7
V332	30.1	propionic acid 2-phenylhydrazide	83	20730-02-3	Nd
		metolcarb	78	1129-41-5	26.2
		1-phenyl-1-pentanol	77	583-03-9	30.9
		1,2-diphenyl-1,2-ethanediol	75	52340-78-0	32.4
		4-pentyl-phenol	75	14938-35-3	32.0
		meso-hydrobenzoin	73	579-43-1	28.7
		n-formyl-L-tyrosine	70	13200-86-7	32.0
chloroacetyl-L-tyrosine	70	1145-56-8	28.8		
V40	4.8	toluene	85	108-88-3	4.8
		1,3,5-cycloheptatriene	78	544-25-2	5.7
		1-phenyl-2-propanol	75	698-87-3	28.5
V172	22.2	2-propyl-1-pentanol	86	58175-57-8	21.7
		3,5,5-trimethyl-1-hexene	86	4316-65-8	26.5
		2-ethyl-1-hexanol	86	104-76-7	22.2
		1,1'-oxybis-octane	85	629-82-3	32.4
V365	31.4	2-Amino-2-methyl-1,3-propanediol	67	115-69-5	29.2
		diethanolamine	54	111-42-2	31.9
V390	32.2	1H-imidazole, 2-ethyl-4-methyl-	53	931-36-2	29.8
		2-hexanoylfuran	71	14360-50-0	30.8
V174	22.1	4-chloro-N-(2-(diethylamino)ethyl)benzamide	66	2852-23-5	Nd
		amiodarone	60	1951-25-3	Nd
V338	30.4	4-pentyl-phenol	77	14938-35-3	32.0
		metolcarb	79	1129-41-5	26.2
		propionic acid 2-phenylhydrazide	81	20730-02-3	nd
		chloroacetyl-L-tyrosine	65	1145-56-8	28.8
		5-methyl-2-pyridinamine	64	1603-41-4	28.7
		N-formyl-L-tyrosine	69	13200-86-7	32.0
		1-phenyl-1-pentanol	77	583-03-9	30.9
1,2-diphenyl-1,2-ethanediol	76	52340-78-0	32.4		
meso-hydrobenzoin	72	579-43-1	28.7		
V187	22.9	vinyl pivalate	84	3377-92-2	28.8
		trimethylacetic anhydride	83	1538-75-6	3.4
		hexamethylacetone	87	815-24-7	12.1
V274	27.5	pentylbenzene	84	538-68-1	27.5
V94	15.5	heptane	87	142-82-5	1.7
		2-methyl-3-pentanone	85	565-69-5	4.4
		3-hexanone	89	589-38-8	5.7
		2,2-dimethyl-3-hexanone	77	18641-70-8	10.2
		2,2-dimethylbutane	77	75-83-2	2.7

nd: not detected

Table S9. ROC results including AUC with 95%CI, cut-off point, sensitivity, specificity, and p-value for the combinations of four identified VOCs: butoxymethylbenzene (V328), toluene (V40), 2-ethyl-1-hexanol (V172) and pentylbenzene (V274). Validation results indicated by false negatives (FN) and false positives (FP) out of 5 controls and 5 patients.

Combination	AUC	95% CI	Cut-off	Sensitivity	Specificity	p-value	FN	FP
V328+V40	0.968	(0.897-1.000)	0.53	90	100	0.0006	1	1
V328+V40+V274	0.966	(0.883-1.000)	1.88	90	95	0.0006	0	1
V328+V172+V40	0.961	(0.875-1.000)	1.14	90	95	0.0007	0	1
V328+V172+V40+V274	0.958	(0.880-1.000)	2.19	90	95	0.0011	0	1
V328+V172	0.957	(0.866-1.000)	1.05	90	95	0.0006	1	0
V328+V172+V274	0.953	(0.863-0.997)	1.16	85	95	0.0009	1	0
V328+V274	0.947	(0.854-0.995)	0.69	85	95	0.0013	1	0
V172+V40+V274	0.947	(0.839-0.996)	0.87	85	95	0.0010	0	1
V40+V274	0.945	(0.841-1.000)	1.12	90	90	0.0009	0	2
V172+V40	0.939	(0.851-0.989)	0.57	85	80	0.009	0	2
V172+V274	0.908	(0.782-0.995)	0.68	85	90	0.008	0	0