GC-MS-based endometabolome analysis differentiates prostate cancer from normal prostate cells

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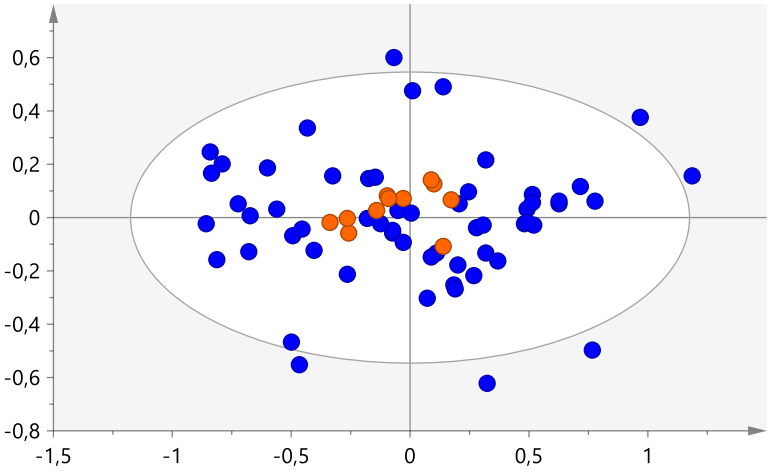
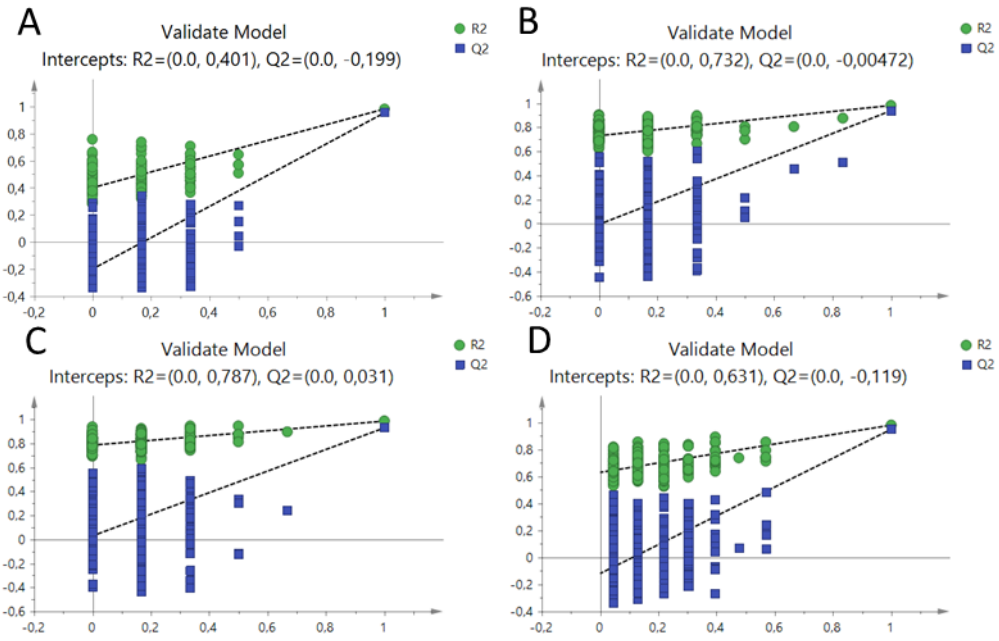


Supplementary Fig. 1: PCA score scatter plot obtained for the GC-MS chromatograms of all samples, namely QCs samples (in orange), and the of all cell lines and blanks (blue) ($R^2X = 0.652$).



Supplementary Fig 2: Statistical validation of the PLS-DA models by permutation testing (200 permutations) (A: 22RV1 vs PNT2; B: PC3 vs PNT2; C: DU145 vs PNT2; D: LNCaP vs PNT2).

Supplementary Table 1: Characteristics of prostate cell lines used in this study.

	PNT2	22RV1	PC3	DU145	LNCaP
Organism	*Homo sapiens*	*Homo sapiens*	*Homo sapiens*	*Homo sapiens*	*Homo sapiens*
Age	33 years	NA	62 years	69 years	50 years
Ethnicity	NA	NA	Caucasian	Caucasian	Caucasian
Tissue	Prostate	Prostate	Prostate; derived from metastatic site: bone	Prostate; derived from metastatic site: brain	Prostate; derived from metastatic site: left supraclavicular lymph node
Morphology	Epithelial	Epithelial	Epithelial	Epithelial	Epithelial
Culture Properties	Adherent	Adherent	Adherent	Adherent	Adherent
Disease	Healthy	Carcinoma	Grade IV, adenocarcinoma	Carcinoma	Carcinoma
Tumorigenic	No	Yes	Yes	Yes	Yes
AR expression	Yes	Yes	No	No	Yes
Metastatic potential	—	NA	High	Moderate	Low

NA: not available

Supplementary Table 2: List of metabolites selected from PLS-DA of 22RV1 vs PNT2, PC3 vs PNT2, DU145 vs PNT2 and LNCaP vs PNT2 (VIP>1) as potentially important for discrimination between PCa and normal cell lines. The identification of the metabolites is based on the NIST (2014) and standards. They are characterized by their IUPAC name, RT, characteristic ions, Kovat indices from literature, experimental Kovat indices, NIST R-match, Cas registry number and HMDB code (when available).

Name	RT	Characteristic ions	KI from literature	Experimental KI or standards	MS-R match	Cas number	HMDB
Unknown 1	**4.56**	**69/140**	**NA**	**983**	**NA**	**NA**	**NA**
Ethanolamine, 2TMS derivative	**5.08**	**102/147**	**1021**	**1027**	**890**	**17165-52-5**	**HMDB00149**
Lactic Acid, 2TMS derivative*	**5.43**	**73/147**	**1066**	**1057**	**927**	**17596-96-2**	**HMDB00190**
L-Alanine, 2TMS derivative	**5.94**	**116/147/190**	**—**	**S**	**935**	**27844-07-1**	**HMDB00161**
Glycine, 2TMS derivative	**6.14**	**102/147/204**	**—**	**S**	**950**	**7364-42-3**	**HMDB00123**
Sarcosine, 2TMS derivative	**6.32**	**73**	**1161**	**S**	**845**	**7364-43-4**	**HMDB00271**
3-Hydroxypropionic acid 2TMS derivative	**6.41**	**127**	**1151**	**1140**	**842**	**55162-32-8**	**HMDB00700**
ß-Alanine, 2TMS derivative	**6.90**	**102/176**	**1190**	**1186**	**899**	**17891-86-0**	**HMDB00056**
2-Butenoic acid, 2-[(trimethylsilyl)oxy]-trimethylsilyl ester	**6.96**	**73/147**	**1186**	**1192**	**793**	**55590-70-0**	**HMDB10720**
3-Hydroxyisovaleric acid, 2TMS derivative	**7.10**	**73/131**	**1216**	**1202**	**937**	**55124-90-8**	**HMDB00754**
L-Valine, 2TMS derivative	**7.17**	**144/218**	**—**	**S**	**920**	**7364-44-5**	**HMDB00883**
Urea, 2TMS derivative	**7.43**	**73/147**	**1249**	**1219**	**921**	**18297-63-7**	**HMDB00294**
L-Leucine, 2TMS derivative	**7.94**	**102/158**	**—**	**S**	**931**	**7364-46-7**	**HMDB00687**
L-Proline, 2TMS derivative	**8.01**	**73/142/216**	**—**	**S**	**909**	**7364-47-8**	**HMDB00162**
Unknown 2	**8.54**	**147/204**	**NA**	**1277**	**NA**	**NA**	**NA**
Toluic acid, TMS derivative	**8.64**	**65/119/193**	**1282**	**1282**	**818**	**NA**	**HMDB62810**
L-Threonine, 2TMS derivative	**8.77**	**73/117/130**	**—**	**S**	**737**	**7536-82-5**	**HMDB00167**
Glycerol, 3TMS derivative	**8.92**	**103/147/205**	**1289**	**1297**	**789**	**6787-10-6**	**HMDB00131**
L-Aspartic acid, 3TMS derivative	**9.93**	**100**	**—**	**S**	**890**	**55268-53-6**	**HMDB00191**
Creatinine, N,N,O-tris(trimethylsilyl)	**10.26**	**75**	**1445**	**1447**	**758**	**NA**	**HMDB00562**
L- Glutamine, 3TMS derivative	**10.73**	**73/75/246**	**—**	**S**	**570**	**70591-28-5**	**HMDB00641**
Phenylalanine, 2TMS derivative	**10.84**	**73/147/218**	**—**	**S**	**876**	**2899-52-7**	**HMDB00159**
Unknown 3	**11.77**	**73**	**NA**	**1646**	**NA**	**NA**	**NA**
Tridecanoic acid, TMS derivative	**12.43**	**117**	**1705**	**1739**	**688**	**169597-14-2**	**HMDB00910**
Sorbose, 5TMS derivative	**13.02**	**103/217**	**1867**	**1826**	**809**	**NA**	**HMDB01266**
L-Tyrosine, 3TMS derivative	**13.06**	**73/100/218/280**	**—**	**S**	**923**	**51220-73-6**	**HMDB00158**
Unknown 4	**13.61**	**73/117/129**	**NA**	**1904**	**NA**	**NA**	**NA**
Palmitic Acid, TMS derivative	**13.77**	**73/132/129/313**	**—**	**S**	**935**	**55520-89-3**	**HMDB00220**
Galacturonic acid, 5TMS derivative	**14.04**	**75**	**1943**	**1924**	**729**	**NA**	**HMDB02545**
Unknown 5	**14.21**	**131**	**NA**	**1932**	**NA**	**NA**	**NA**
Unknown 6	**14.25**	**73/145/311**	**NA**	**1934**	**NA**	**NA**	**NA**
Unknown 7	**14.42**	**117**	**NA**	**1942**	**NA**	**NA**	**NA**
Unknown 8	**14.47**	**117/327**	**NA**	**1944**	**NA**	**NA**	**NA**

Name	RT	Characteristic ions	KI from literature	Experimental KI or standards	MS-R match	Cas number	HMDB
Unknown 9	**14.94**	**117/129/339**	**NA**	**1966**	**NA**	**NA**	**NA**
Unknown 10	**15.14**	**117/129/145/341**	**NA**	**1975**	**NA**	**NA**	**NA**
Unknown 11	**15.62**	**57/69/84/**	**NA**	**1997**	**NA**	**NA**	**NA**
9-Hexadecenoic acid, TMS derivative **(palmitoleic acid)**	**15.80**	**55/75/145**	**2027**	**2006**	**701**	**NA**	**HMDB03229**
Unknown 12	**16.28**	**117**	**NA**	**2028**	**NA**	**NA**	**NA**
Unknown 13	**16.78**	**75/129**	**NA**	**2051**	**NA**	**NA**	**NA**
Unknown 14	**17.30**	**55**	**NA**	**2075**	**NA**	**NA**	**NA**
Methyl 2-acetamido-2-deoxy-3-O-methyl-a-D-galactopyranoside, 2TMS	**17.94**	**73/117**	**2134**	**2105**	**660**	**56196-89-5**	**NA**
Unknown 15	**19.00**	**73/117**	**NA**	**2154**	**NA**	**NA**	**NA**
13-Octadecenoic acid, TMS derivative	**19.72**	**69/75/117**	**2228**	**2187**	**634**	**NA**	**HMDB41480**
Cholesterol, TMS derivative	**23.06**	**129/329/368**	**—**	**S**	**912**	**1856-05-9**	**HMDB00067**

KI: Kovat indices; HMDB: Human Metabolome Database; NA: not available; S: identified with standards;