

Supporting Information

Gut-gonad crosstalk and testicular metabolic impairments
in mice exposed to a “chemical cocktail” and supplemented
with selenium

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Table S1. Parameters and filters used for positive and negative modes used in the UHPLC-QTOF were as follows:

Batch Recursive Feature Extraction	
Extraction	
Peak weight	> 2000 counts
Ion species	
Positive Mode	[M+H] ⁺ , [M+Na] ⁺ , [M+K] ⁺
Negative Mode	[M-H] ⁻ , [M-Cl] ⁻ , [M-HCOO] ⁻
Integration	Agile 2
Alignment Parameters	
RT Tolerance	0.0% ± 0.3 min
Mass Tolerance	20 ppm ± 2.00 mDa
Molecular Feature Extraction Filters	
Score (MFE)	> 95
Tolerance and EIC	
Masses	± 10.00 ppm
RT	0.00% ± 0.3 min
Possible m/z	Symmetric (ppm)
Peak Filter	
Absolute height	> 10000 counts
Chromatogram Format	Centroid
Peak Spectrum	
Average scans	10 % of peak height
Find by ion filters	
Score (Tgt)	> 95
Entities	
Number of entities found	Positive Mode: 2776
	Negative Mode: 1860
Operational Parameters	
m/z range scanned	100-1700 m/z
Drying gas flow rate	13 L min ⁻¹
Temperature	200 °C
Gas nebulizer pressure	35 psi
Sheath gas temperature	350 °C
Flow-rate	11 L min ⁻¹
Fragmentor Voltage	175 V
MS/MS Scan mode	
Scan rate	1 spectrum s ⁻¹
Collision gas	Nitrogen
Voltages Ranges	10-40 V

Figure S1. 3D-Principal component analysis of mice groups studied using their testicular metabolome determined by A) GC-MS, B) UHPLC-(⁺ESI)-QTOF and C) UHPLC-(⁻ESI)-QTOF.

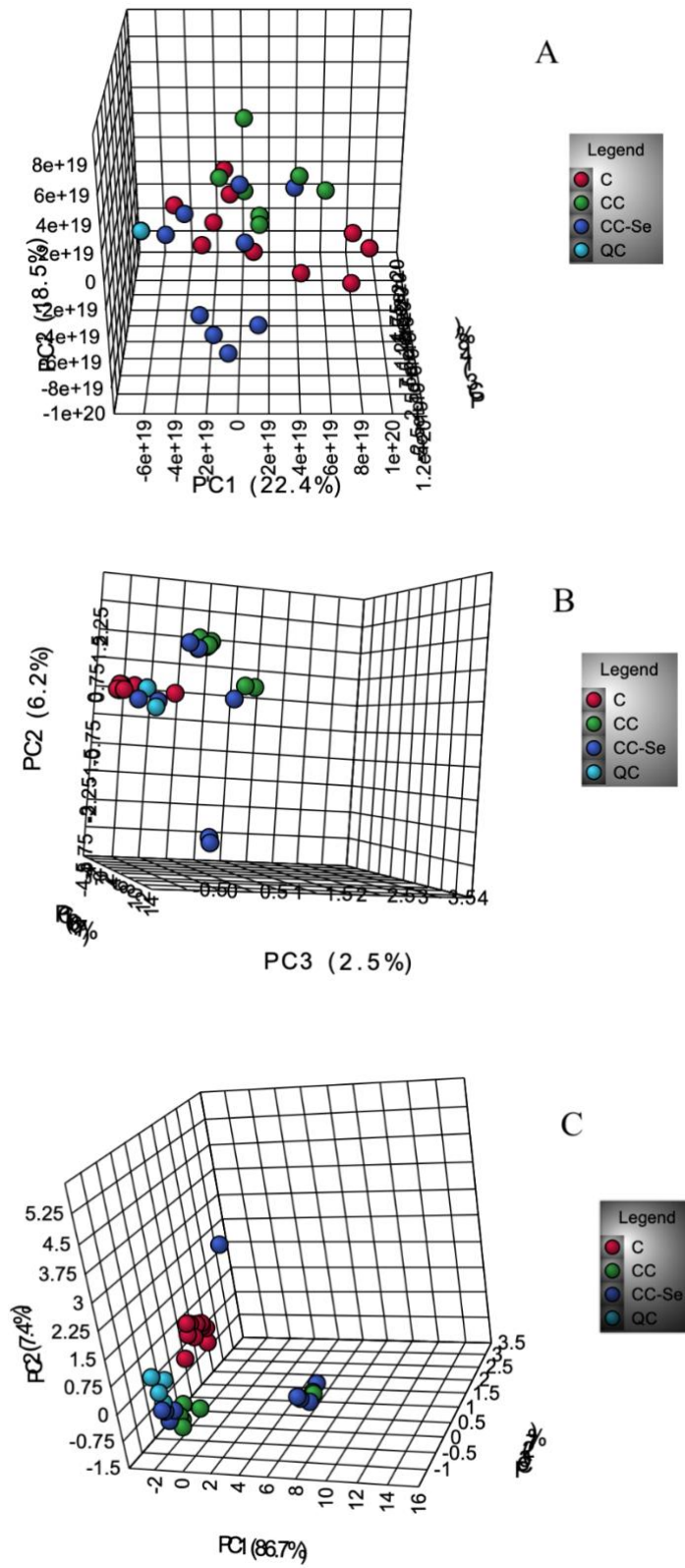
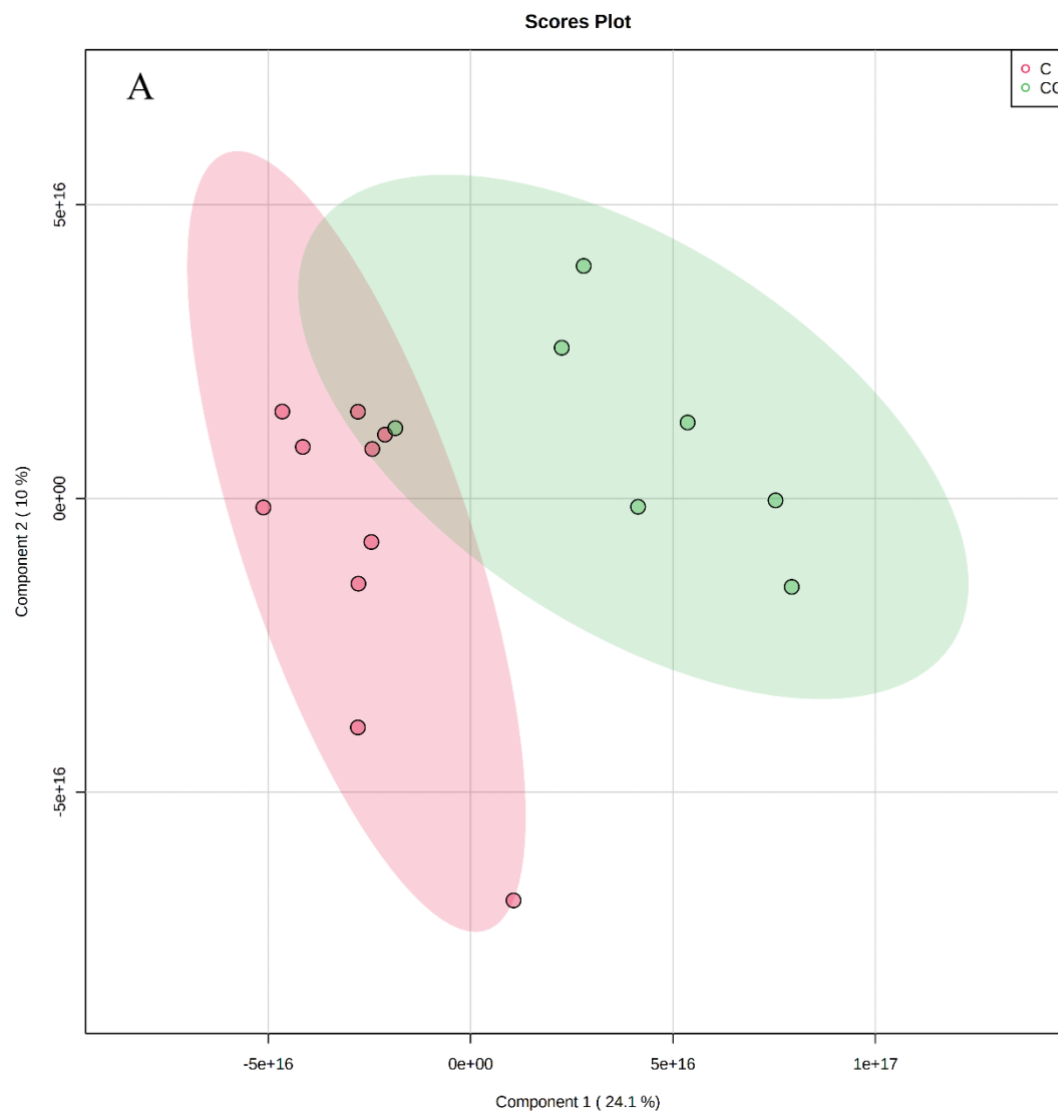


Figure S2. 2D-Least squares principal discriminant analysis of mice groups studied using their testicular metabolome and built from pairwise group comparisons: A) CC vs C and B) CC-Se vs CC.



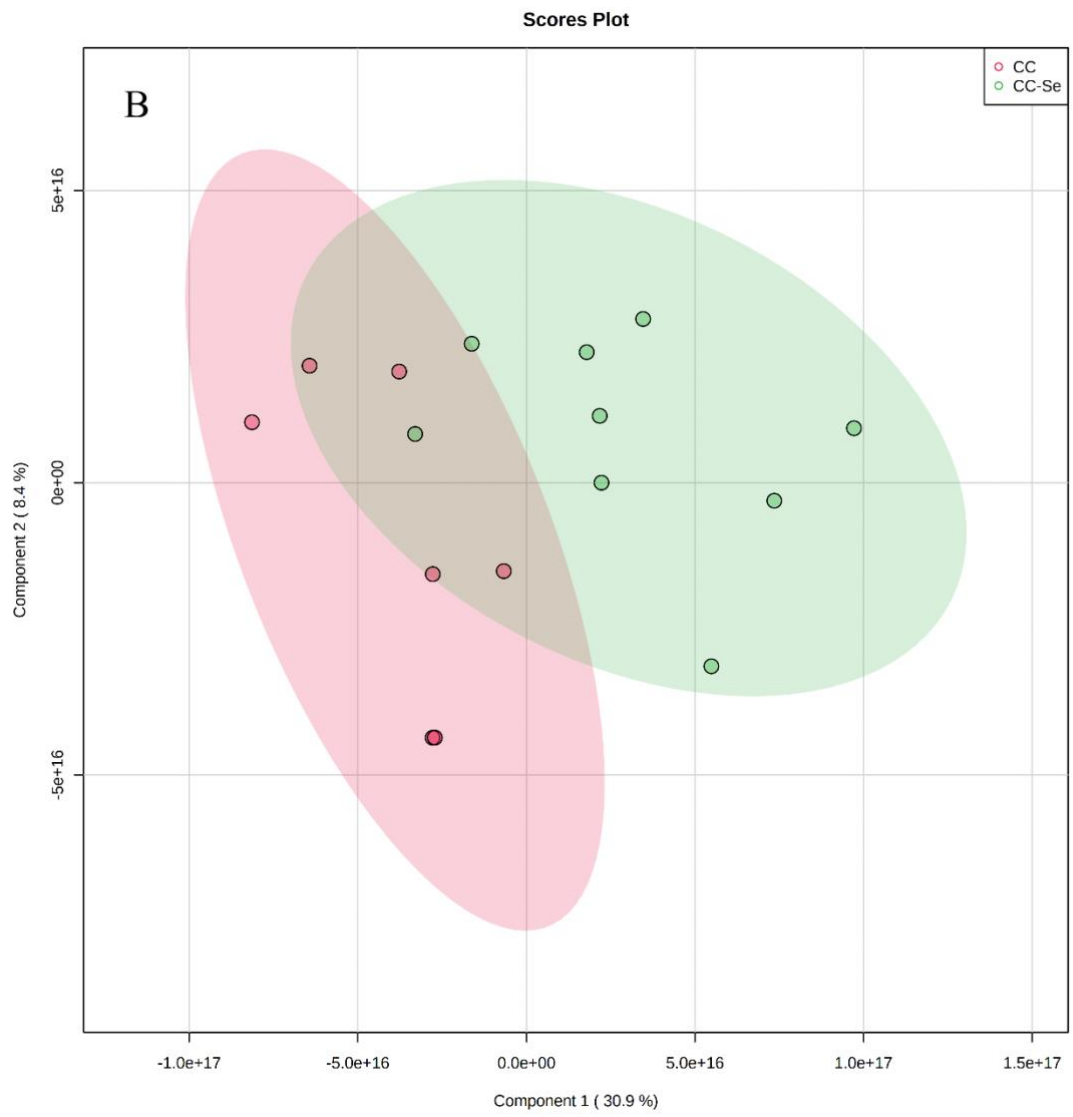


Table S2. Mice testicular metabolites annotated after the analysis by the analytical multiplatform combining GC-MS (c), UHPLC-(+ESI)-QTOF (a) and UHPLC-(-ESI)-QTOF (b). Red color indicated a significant up-regulation of the metabolites when compared with the control group (FC>1.5), while the green color indicated a down-regulation (FC<0.75). RT: retention time, FC: Fold change, N.S.: Not significant, phosphatidylethanolamine (PE); phosphatidylcholine (PC); phosphatidylserine (PS); ceramide (cer); phosphatidylinositol (PI), phosphatidylglycerol (PG); *p*-value obtained from one-way ANOVA followed by Tuckey Test and corrected by Benjamini-Hochberg multiple post-correction. NC: Metabolites detected only in one group for which FC cannot be calculated due to their absence in the group at the denominator of the fraction. NS: Not statistically significant

Experimental Mass (Da)	Theoretical Mass (Da)	RT (min)	Compound	CC vs C		CC-Se vs C		CC-Se vs CC		Mode	Class	RSD in the QC
				p	FC	p	FC	p	FC			
353.3302	353.3294	12.5	Anandamide (20:l. n-9)	<0.001	0.54	NS	0.74	<0.001	1.36	a	Organonitrogen compounds	24.9
261.0798	261.0801	2.3	Flumequine	<0.001	NC	<0.001	NC	NS	2.36	a	Quinolines and derivatives	10.6
497.2761	497.2754	9.7	PS(16:0)	<0.001	4.64	<0.001	3.79	NS	0.82	a	Glycerophospholipids	8.3
310.1784	310.178	7.7	3-Hydroxy-6.8-dimethoxy-7(11)- eremophilin-12.8-olide	<0.001	0.13	<0.001	0.14	NS	1.11	b	Prenol lipids	4.9
408.2872	408.2876	7.8	3a.7a.12b-Trihydroxy-5b-cholanoic acid	NS	NC	<0.001	30.71	<0.001	NC	b	Organooxygen compounds	23.1
785.1567	785.1571	2.2	Flavin adenine dinucleotide (FAD)	0.001	2.73	0.001	2.76	NS	1.01	b	Flavin nucleotides	7.9
420.3111	420.3114	8.3	Stearidonyl carnitine	<0.001	47.05	0.000	42.48	NS	0.90	a	Fatty Acyls	6.1
297.09	297.0896	1.3	5'-Deoxy-5'-(methylthio)adenosine	NS	1.39	0.001	1.59	NS	1.15	a	5'-deoxyribonucleosides	5.0
503.3006	503.3012	9.7	LysoPE(20:3)	NS	1.30	<0.001	1.63	NS	1.25	b	Glycerophospholipids	18.3
339.3505	339.3501	15.0	Docosanamide	<0.001	6.02	<0.001	6.20	NS	1.03	a	Fatty Acyls	59.9
419.3039	419.3036	11.7	Myxalamid S	NS	0.18	<0.001	0.16	NS	0.88	b	Fatty Acyls	3.7
501.2864	501.2855	9.3	LysoPE(20:4)	NS	1.22	0.009	1.52	NS	1.24	a	Glycerophospholipids	12.5
545.2759	545.2754	9.2	PS(20:4)	NS	1.52	0.033	1.59	NS	1.04	a	Glycerophospholipids	33.1

453.2859	453.2855	8.8	LysoPE(16:0)	<0.001	2.87	<0.001	3.35	NS	1.17	b	Glycerophospholipids	9.6
612.1521	612.152	0.5	Glutathione. oxidized	<0.001	2.81	0.014	2.08	NS	0.74	a	Carboxylic acids and derivatives	141.4
551.3223	551.3223	10.4	PS(20:1)	<0.001	7.25	0.009	7.14	NS	0.99	a	Glycerophospholipids	24.1
326.2087	326.2093	7.8	2.3-Dinor-11b-PGF2a	<0.001	0.12	NS	0.34	<0.001	2.87	b	Fatty Acyls	88.6
326.1923	326.1916	12.3	2-Dodecylbenzenesulfonic acid	NS	0.48	NS	0.59	0.034	1.22	b	Benzene and substituted derivatives	89.9
332.2717	332.2715	13.1	5alpha-Androstan-17beta-ol propionate	<0.001	NC	<0.001	NC	NS	1.04	b	steroid	34.7
447.3356	447.3349	9.6	N-stearoyl tyrosine	NS	1.59	0.008	2.27	NS	1.43	a	Carboxylic acids and derivatives	5.4
335.3192	335.3188	13.5	Pipericine	<0.001	0.27	<0.001	0.26	NS	0.97	a	Fatty Acyls	40.7
210.0927	210.0926	5.9	2-Ethyl-1-hexanol sulfate	<0.001	4.01	<0.001	10.72	NS	2.67	b	Organic sulfuric acids and derivatives	13.3
350.1734	350.1729	11.8	Calonectrin	NS	1.41	<0.001	3.11	0.006	2.21	a	Prenol lipids	47.2
323.3187	323.3188	10.7	N-(14-Methylhexadecanoyl)pyrrolidine	<0.001	NC	NS	1.00	<0.001	0.00	a	Pyrrolidines	32.3
722.5106	722.5098	13.0	PG(16:0/16:0)	0.039	1.57	NS	2.14	0.001	1.36	b	Glycerophospholipids	50.2
294.1839	294.1831	7.9	Nordihydrocapsiate	<0.001	NC	<0.001	NC	NS	1.69	b	Phenols	26.1
328.2615	328.2614	12.1	MG(16:1)	NS	0.13	0.002	0.04	NS	0.34	b	Glycerolipids	3.1
312.1763	312.1759	12.3	N-Undecylbenzenesulfonic acid	<0.001	0.46	NS	0.76	0.006	1.67	b	Benzene and substituted derivatives	7.8
272.2357	272.2351	11.2	16-Hydroxy hexadecanoic acid	NS	0.96	0.021	1.38	0.014	1.43	b	Fatty Acyls	6.3
515.2917	515.2917	5.5	Taurallocholic acid	0.014	0.37	NS	NC	0.001	NC	b	Steroids and steroid derivatives	18.7
60.03236276	60	4.6	Urea	0.015	0.30	0.014	0.36	NS	1.21	c	Organic carbonic acids and derivatives	78.3
92.04734412	92	4.7	Glycerol	0.010	7.80	0.045	5.63	NS	0.72	c	Organooxygen compounds	43.3

94.95507	95	4.8	Phosphate	NS	1.59	NS	2.50	NS	1.57	c	Non-metal oxoanionic compounds	59.7
57.02146	57	5.1	Glycine	NS	4.94	NS	3.87	NS	0.78	c	Carboxylic acids and derivatives	100.4
97.05276	97	7.0	Proline	NS	1.58	0.003	2.69	NS	1.71	c	Carboxylic acids and derivatives	68.8
194.0942943	194	7.1	Ethyl 4-ethoxybenzoate	NS	0.85	NS	0.86	NS	1.01	c	Benzene and substituted derivatives	70.3
140.0238458	140	8.6	Phosphoric acid. monopropyl ester	NS	0.58	NS	0.71	NS	1.23	c	Organic phosphoric acids and derivatives	79.7
256.2402303	256	10.7	Palmitic acid	<0.001	0.21	<0.001	0.27	NS	1.29	c	Fatty Acyls	35.6
180.0633881	180	10.9	Scyllo-Inositol	0.021	0.34	NS	0.476	NS	1.38	c	Organooxygen compounds	70.4
260.0297185	260	12.7	D-myo-inositol phosphate	<0.001	0.02	<0.001	0.02	NS	0.72	c	Organooxygen compounds	63.3
283.0916686	283	14.7	Guanosine	<0.001	0.02	<0.001	0.01	NS	0.42	C	Imidazopyrimidines	66.5

Table S3. Kovat's retention Index (KRI) of the metabolites annotated by GC-MS.

Compound name	Exact Mass (Da)	MW (Da)	Number of TMS groups added	MW-Derivatives (Da)	RT (min)	KRI	Targeted ions (m/z)	Qualifier ions (m/z)
Urea	60.03236276	60	2TMS	204	4.6	625	101	43,73,101,147,173,189,
Glycerol	92.04734412	92	3TMS	308	4.7	1315	232	45,59,73,117,147,205,232,293
Phosphate	94.95507	95	3TMS	314	4.8	839	164	45,59,73,133,164,211,225,283,299,314
Glycine	57.02146	57	3TMS	291	5.1	1160	302	45,59,86,100,133,147,174,177,258,276
Proline	97.05276	97	2TMS	273	7.0	1171	150	43,45,73,147,150,156,214,230,258
6 Azathymine	127.0381764	127	2TMS	271	7.0	1171	272	43,73,100,113,156,228,256,271
Ethyl 4-ethoxybenzoate	194.0942943	194	-	194	7.1	1276	189	63,93,121,138,149,166,189,194
Phosphoric acid propyl ester	140.0238458	140	4TMS	460	8.6	1769	284	73,147,207,211,256,284,299,315,357,445
Palmitic acid	256.2402303	256	1TMS	328	10.7	2098	243	73,75,117,129,201,243,269,285,313
Inositol-scylo	180.0633881	180	6TMS	612	10.9	2711	179	45,73,103,129,147,179,191,204,217,265,291,305,318,393,432,507,612
D-myo-inositol phosphate	260.0297185	260	7TMS	764	12.7	2803	448	73,147,299,315,318,343,387,433,448,470,569,
Guanosine	283.0916686	283	5TMS	643	14.7	2996	630	73,129,147,218,245,280,296,324,352,368,394,410,525,554,628,630,643

MW: Molecular Weight; TMS: Trimethylsilyl derivatives, KIR: Kovat's Retention Index.

Table S4. Pathway analysis details of altered metabolites by pairs comparisons. Match Status: number of altered metabolites of the total metabolites involving in the route; p-value: p-value calculated from the enrichment analysis; Impact: pathway impact value calculated from pathway topology analysis.

CC vs C			
Pathway Name	Match Status	p	Impact
Glutathione metabolism	2/28	0.038169	0.11571
Riboflavin metabolism	1/4	0.044437	0.0
Aminoacyl-tRNA biosynthesis	2/48	0.099858	0.0
Arginine biosynthesis	1/14	0.14754	0.0
Glycerolipid metabolism	1/16	0.16686	0.23676
Galactose metabolism	1/27	0.26597	0.0
Porphyrin and chlorophyll metabolism	1/30	0.29101	0.0
Glyoxylate and dicarboxylate metabolism	1/32	0.30725	0.10582
CC-Se vs C			
Pathway Name	Match Status	p	Impact
Glutathione metabolism	1/28	0.019615	0.11571
Riboflavin metabolism	1/4	0.031525	0.0
Aminoacyl-tRNA biosynthesis	2/48	0.053571	0.0
Glycerolipid metabolism	1/16	0.12071	0.23676
Galactose metabolism	1/27	0.19579	0.0
Porphyrin and chlorophyll metabolism	1/30	0.21522	0.0
Glyoxylate and dicarboxylate metabolism	1/32	0.22793	0.10582
Cysteine and methionine metabolism	1/33	0.23422	0.02089
CC-Se vs CC			
Pathway Name	Match Status	p	Impact
Aminoacyl-tRNA biosynthesis	2/48	0.018865	0.0
Purine metabolism	2/66	0.034453	0.0
Arginine biosynthesis	1/14	0.063409	0.0
Glycerolipid metabolism	1/16	0.07218	0.23676

Table S5. Taxa Abundance at genus level in C, CC, CC-Se groups

Taxa	C	CC	CC-Se
<i>Lachnospiraceae NK4A136 group</i>	35.310	17.438	14.510
<i>Bacteroides</i>	1.310	5.175	4.870
<i>Lactobacillus</i>	1.940	9.288	13.430
<i>Alloprevotella</i>	1.480	4.850	3.810
<i>[Eubacterium] xylanophilum group</i>	3.570	0.825	0.710
<i>Lachnospiraceae_Unclassified</i>	17.550	11.188	13.440
<i>Muribaculaceae_Unclassified</i>	6.760	25.038	18.080
<i>Roseburia</i>	3.440	1.450	1.780
<i>Lachnospiraceae A2</i>	0.730	0.175	0.080
<i>Tannerellaceae_Unclassified</i>	0.040	0.188	0.180
<i>Oscillibacter</i>	1.180	1.338	2.630
<i>Lachnospiraceae UCG-006</i>	2.950	0.950	1.140
<i>Lachnoclostridium</i>	6.690	1.388	2.650
<i>[Clostridium] innocuum group</i>	0.000	0.000	0.070
<i>Enterococcus</i>	0.000	2.988	3.770
<i>Mucispirillum</i>	0.390	0.963	2.160
<i>Muribaculum</i>	0.170	1.375	2.180
<i>Odoribacter</i>	1.630	1.500	1.710
<i>Rikenellaceae RC9 gut group</i>	0.970	0.438	0.020
<i>Escherichia-Shigella</i>	0.020	0.000	0.040
<i>Lachnospiraceae UCG-001</i>	2.310	3.213	0.890
<i>Parabacteroides</i>	0.020	1.850	0.380
<i>Alistipes</i>	1.250	0.175	0.070
<i>Anaerotruncus</i>	0.510	0.038	0.040
<i>Colidextribacter</i>	0.800	0.763	0.890
<i>Intestinimonas</i>	0.430	0.475	1.150
<i>Erysipelatoclostridium</i>	0.000	0.025	0.010

<i>Bilophila</i>	0.250	0.288	0.330
<i>Oscillospiraceae_Unclassified</i>	0.540	1.688	3.050
<i>Streptococcus</i>	0.290	0.000	0.110
<i>Lachnospiraceae GCA-900066575</i>	0.360	0.275	0.250
<i>Anaeroplasma</i>	0.000	0.750	0.320
<i>Lachnospiraceae FCS020 group</i>	0.120	0.163	0.720
<i>Enterorhabdus</i>	1.500	0.075	0.140
<i>Erysipelotrichaceae_Unclassified</i>	0.370	0.063	0.090
<i>[Eubacterium] coprostanoligenes group Unclassified</i>	0.080	0.025	0.020
<i>Peptococcaceae_Unclassified</i>	0.210	0.213	0.230
<i>[Eubacterium] siraeum group</i>	0.420	0.075	0.060
<i>Clostridia UCG-014 Unclassified</i>	0.430	0.063	0.040
<i>Ruminococcaceae_Unclassified</i>	0.600	0.438	0.610
<i>Butyricicoccus</i>	0.480	0.150	0.100
<i>Ruminococcus</i>	0.500	0.250	0.040
<i>Coriobacteriaceae UCG-002</i>	0.000	0.138	0.620
<i>Oscillospiraceae UCG-003</i>	0.330	0.263	0.090
<i>Anaerovoracaceae Family XIII UCG-001</i>	0.190	0.025	0.040
<i>Acetatifactor</i>	0.140	0.325	0.350
<i>Incertae Sedis</i>	0.390	0.413	0.800
<i>Merdibacter</i>	0.010	0.013	0.020
<i>Prevotellaceae UCG-001</i>	0.270	0.025	0.000
<i>Clostridia vadinBB60 group Unclassified</i>	0.020	0.450	0.550
<i>Lachnospiraceae ASF356</i>	0.000	0.163	0.280
<i>[Eubacterium] brachy group</i>	0.130	0.000	0.010
<i>Marvinbryantia</i>	0.020	0.075	0.080
<i>Oscillospira</i>	0.110	0.075	0.010
<i>Parvibacter</i>	0.190	0.013	0.010
<i>Oscillospirales UCG-10 Unclassified</i>	0.070	0.063	0.010

<i>Tyzzerella</i>	0.010	0.050	0.130
<i>Citrobacter</i>	0.010	0.000	0.000
<i>Oscillospiraceae UCG-005</i>	0.080	0.000	0.000
<i>Oscillospiraceae NK4A214 group</i>	0.010	0.000	0.000
<i>Peptococcus</i>	0.020	0.000	0.000
<i>Dorea</i>	0.010	0.000	0.000
<i>Butyricoccaceae UCG-009</i>	0.020	0.025	0.000
<i>Christensenellaceae Unclassified</i>	0.000	0.000	0.010
<i>Harryflintia</i>	0.000	0.000	0.020
<i>Ruminococcaceae UBA1819</i>	0.080	0.000	0.000

Table S6. Spearman correlation coefficient (rho) of altered gut metabolites and gut microbial genus. phosphatidylethanolamine (PE); phosphatidylcholine (PC); phosphatidylserine (PS); ceramide (cer); phosphatidylinositol (PI).*(p<0.05): **(p<0.01)

Genus	Compound	C		CC		CC-Se	
		rho	p	rho	p	rho	p
<i>Lachnospiraceae NK4A136 group</i>	2-Ethyl-1-hexanol sulfate			.893**	0.007	-.750*	0.020
	Inositol-scylo			.757*	0.049		
	Stearidonyl carnitine					-.667*	0.050
	PG(16:0/16:0)					-.683*	0.042
	2,3-Dinor-11b-PGF2a	-.745*	0.013				
	D-myo-inositol phosphate	-.685*	0.029				
<i>Bacteroides</i>	LysoPE(16:0)			-.786*	0.036		
	5alpha-Androstan-17beta-ol propionate			-.857*	0.014		
	2-Ethyl-1-hexanol sulfate			-.786*	0.036		
	Inositol-scylo			-.802*	0.030		
	5'-Deoxy-5'-(methylthio)adenosine					.717*	0.030
	Pipericine					-.717*	0.030
	2,3-Dinor-11b-PGF2a	.909**	0.000				
	Glycine	.728*	0.017				
	D-myo-inositol phosphate	.768**	0.009				
<i>Lactobacillus</i>	PS(20:1)			-.786*	0.036		
	N-(14-Methylhexadecanoyl)pyrrolidine			-.786*	0.036		
	16-Hydroxy hexadecanoic acid			-.964**	0.000		
	Taurallocholic acid			-.786*	0.036		
	Stearidonyl carnitine					.683*	0.042
	PG(16:0/16:0)					.667*	0.050
	Flavin adenine dinucleotide (FAD)	-.656*	0.039				
	Glutathione, oxidized	-.648*	0.043				
	Inositol-scylo	-.675*	0.032				
<i>Alloprevotella</i>	Flavin adenine dinucleotide (FAD)			.786*	0.036		
	Phosphate			-.906**	0.005		
	Anandamide (20:1, n-9)					-.950**	0.000
	Flumequine			-.964**	0.000	.683*	0.042
	PS(16:0)					.733*	0.025
	Docosanamide					-.733*	0.025
	LysoPE(20:4)					.833**	0.005
	2-Dodecylbenzenesulfonic acid					.728*	0.026
	Pipericine			-.893**	0.007	-.950**	0.000
	PG(16:0/16:0)					.667*	0.050
	N-Undecylbenzenesulfonic acid					.783*	0.013
	Glycine					-.746*	0.021
	D-myo-inositol phosphate	.834**	0.003				

	2,3-Dinor-11b-PGF2a	.873**	0.001				
<i>[Eubacterium] xylanophilum group</i>	Stearidonyl carnitine			-.901*	0.006		
	Proline			.764*	0.046		
	Anandamide (20:1, n-9)					.733*	0.025
	LysoPE(20:3)					-.817**	0.007
	2,3-Dinor-11b-PGF2a					-.667*	0.050
	2-Dodecylbenzenesulfonic acid					-.862**	0.003
	Pipericine					.700*	0.036
	Calonectrin					-.667*	0.050
	PG(16:0/16:0)					-.900**	0.001
	N-Undecylbenzenesulfonic acid					-.917**	0.001
	Glycine					.763*	0.017
<i>Lachnospiraceae_Unclassified</i>	MG(16:1)			-.802*	0.030		
	D-Myo-inositol phosphate			.865*	0.012		
	Stearidonyl carnitine					-.817**	0.007
	Flavin adenine dinucleotide (FAD)	-.779**	0.008				
	Myxalamid S	-.636*	0.048				
	Glutathione, oxidized	-.806**	0.005				
	2-Ethyl-1-hexanol sulfate	.701*	0.024				
	16-Hydroxy hexadecanoic acid	-.709*	0.022				
	Calonectrin	-.745*	0.013				
<i>Muribaculaceae_Unclassified</i>	Flumequine			-.786*	0.036		
	Taurallocholic acid			.786*	0.036		
	Phosphate			-.808*	0.028		
	2,3-Dinor-11b-PGF2a	.782**	0.008			-.767*	0.016
	LysoPE(16:0)					.703*	0.035
	Calonectrin	.661*	0.038			-.700*	0.036
	Glycine	.664*	0.036				
	D-myo-inositol phosphate	.818**	0.004				
<i>Roseburia</i>	Anandamide (20:1, n-9)			-.786*	0.036		
	Calonectrin			.786*	0.036		
	MG(16:1)			-.802*	0.030		
	Flumequine					-.711*	0.032
	PS(16:0)					-.686*	0.041
<i>Lachnospiraceae A2</i>	N-stearoyl tyrosine			.791*	0.034		
	N-(14-Methylhexadecanoyl)pyrrolidine			.791*	0.034		
	D-myo-inositol phosphate			.798*	0.032		
	Guanosine			.791*	0.034		
	3-Hydroxy-6,8-dimethoxy-7(11)-eremophilin-12,8-olide					-.693*	0.039
	Phosphate					.783*	0.013
	LysoPE(16:0)	-.640*	0.046				
	Nordihydrocapsiate	.640*	0.046				
<i>Tannerellaceae_Unclassified</i>	Anandamide (20:1, n-9)			.926**	0.003		

	D-myo-inositol phosphate	.824**	0.003				
<i>Odoribacter</i>	Docosanamide			.865*	0.012		
	2-Dodecylbenzenesulfonic acid			-.775*	0.041		
	D-myo-inositol phosphate	.754*	0.012	.873*	0.010		
	Stearidonyl carnitine					-.756*	0.018
	6 azathymine	.711*	0.021			-.807**	0.009
	2,3-Dinor-11b-PGF2a	.772**	0.009				
	Glycine	.725*	0.018				
<i>Rikenellaceae RC9 gut group</i>	Inositol-scyllo					<0.999**	<0.001
	2,3-Dinor-11b-PGF2a	.763*	0.010				
	Nordihydrocapsiate	-.646*	0.044				
	Glycine	.780**	0.008				
	D-myo-inositol phosphate	.677*	0.032				
	6 azathymine	.745*	0.013				
<i>Escherichia-Shigella</i>	6 azathymine	-.696*	0.025				
<i>Parabacteroides</i>	MG(16:1)			.764*	0.046		
	2,3-Dinor-11b-PGF2a					-.807**	0.009
	LysoPE(16:0)					.671*	0.048
	N-stearoyl tyrosine					-.748*	0.020
	6 azathymine	-.696*	0.025				
<i>Colidextribacter</i>	N-(14-Methylhexadecanoyl)pyrrolidine			-.857*	0.014		
	Taurallocholic acid					-.667*	0.050
	2,3-Dinor-11b-PGF2a	.744*	0.014				
	Phosphate	.738*	0.015				
	6 azathymine	.774**	0.009				
	Glycine	.773**	0.009				
	D-myo-inositol phosphate	.817**	0.004				
<i>Intestinimonas</i>	2-Ethyl-1-hexanol sulfate			.865*	0.012		
	Calonectrin			.757*	0.049		
	Inositol-scyllo			.809*	0.028		
	Glutathione, oxidized					.669*	0.049
	6 azathymine					-.686*	0.041
	3a,7a,12b-Trihydroxy-5b-cholanoic acid	.698*	0.025				
<i>Erysipelatoclostridium</i>	N-stearoyl tyrosine			-.791*	0.034		
	N-(14-Methylhexadecanoyl)pyrrolidine			-.791*	0.034		
	D-myo-inositol phosphate			-.798*	0.032		
	Guanosine			-.791*	0.034		
<i>Bilophila</i>	2-Ethyl-1-hexanol sulfate			.764*	0.046		
	Calonectrin			.764*	0.046		
	Myxalamid S					-.735*	0.024
	LysoPE(16:0)					-.718*	0.029

	2-Dodecylbenzenesulfonic acid			-.802*	0.030		
	Nordihydrocapsiate			.802*	0.030		
	3-Hydroxy-6,8-dimethoxy-7(11)- eremophilin-12,8-olide					-.708*	0.033
<i>Clostridia UCG-014 Unclassified</i>	PS(16:0)			-.777*	0.040		
	Docosanamide			.896**	0.006		
	2-Dodecylbenzenesulfonic acid			-.837*	0.019		
	D-myo-inositol phosphate	.839**	0.002	.784*	0.037	.680*	0.044
	2,3-Dinor-11b-PGF2a	.777**	0.008				
	Glycine	.818**	0.004				
<i>Ruminococcaceae Unclassified</i>	PS(20:1)			.879**	0.009		
	N-(14-Methylhexadecanoyl)pyrrolidine			.898**	0.006		
	16-Hydroxy hexadecanoic acid			.954**	0.001		
	Taurallocholic acid			.842*	0.017		
	Flavin adenine dinucleotide (FAD)					.672*	0.047
	Stearidonyl carnitine					-.834**	0.005
	Phosphoric acid propyl ester	-.745*	0.013				
<i>Butyricoccus</i>	Anandamide (20:1, n-9)			-.926**	0.003		
	Stearidonyl carnitine					-.695*	0.038
	Nordihydrocapsiate	-.767**	0.010				
<i>Coriobacteriaceae UCG-002</i>	Anandamide (20:1, n-9)			.767*	0.044		
	Stearidonyl carnitine					.757*	0.018
<i>Oscillospiraceae UCG-003</i>	2,3-Dinor-11b-PGF2a	.841**	0.002				
	D-myo-inositol phosphate	.772**	0.009				
<i>Anaerovoracaceae Family XIII UCG-001</i>	3-Hydroxy-6,8-dimethoxy-7(11)- eremophilin-12,8-olide	-.809**	0.005				
	Stearidonyl carnitine	.645*	0.044				
	Palmitic acid	.689*	0.027				
<i>Peptococcus</i>	Glycine	.750*	0.013				
	6 azathymine	.696*	0.025				
<i>Acetatifactor</i>	Anandamide (20:1, n-9)			-.867*	0.012		
	Guanosine					.708*	0.033
	Flavin adenine dinucleotide (FAD)	.695*	0.026				
	N-stearoyl tyrosine	.655*	0.040				
	Calonectrin	.636*	0.048				
<i>Lachnospiraceae UCG-001</i>	2-Ethyl-1-hexanol sulfate			.937**	0.002		
	Calonectrin			.847*	0.016		
	LysoPE(16:0)	-.659*	0.038				
	2,3-Dinor-11b-PGF2a	.695*	0.026				
<i>Alistipes</i>	2,3-Dinor-11b-PGF2a	.853**	0.002	-.867*	0.011		
	Urea			.857*	0.014		
	6 Azathymine	.632*	0.050	.767*	0.044		
	Myxalamid S					-.825**	0.006
	LysoPE(16:0)					-.680*	0.044

<i>Incertae Sedis</i>	2,3-Dinor-11b-PGF2a			-.805*	0.029		
	MG(16:1)			-.809*	0.028		
	16-Hydroxy hexadecanoic acid			.775*	0.041		
	Stearidonyl carnitine					-.683*	0.042
	Ethyl 4-ethoxybenzoate	-.636*	0.048				
<i>Merdibacter</i>	MG(16:1)					>.999**	< 0.001
<i>Prevotellaceae UCG-001</i>	2,3-Dinor-11b-PGF2a	.924**	0.000				
	Glycine	.815**	0.004				
	D-Myo-inositol phosphate	.846**	0.002				
<i>Clostridia vadinBB60 group Unclassified</i>	Docosanamide			.757*	0.049		
	D-Myo-inositol phosphate			.836*	0.019		
	PG(16:0/16:0)					-.732*	0.025
	2,3-Dinor-11b-PGF2a	.696*	0.025				
	N-Stearoyl tyrosine	.696*	0.025				
	Glycerol	.667*	0.035				
	Glycine	.857**	0.002				
	Phosphate	.696*	0.025				
<i>Lachnospiraceae ASF356</i>	Ethyl 4-ethoxybenzoate			-.759*	0.048		
	Stearidonyl carnitine					-.923**	0.000
	6-zathymine					-.675*	0.046
<i>Marvinbryantia</i>	Stearidonyl carnitine			-.869*	0.011		
	Docosanamide			.756*	0.049		
	D-myo-inositol phosphate			.782*	0.038		
	Myxalamid S					.840**	0.005
	LysoPE(16:0)					.760*	0.017
	5 alpha-Androstan-17beta-ol propionate					.807**	0.009
	PG(16:0/16:0)	-.696*	0.025				
	MG(16:1)	.705*	0.023				
	Urea	-.696*	0.025				
<i>Oscillospira</i>	Guanosine			-.772*	0.042		
	Inositol-scylo					1.000**	< 0.001
	PS(16:0)	-.754*	0.012				
	Myxalamid S	-.699*	0.024				
	Glutathione, oxidized	-.884**	0.001				
	PS(20:1)	.641*	0.046				
<i>Parvibacter</i>	LysoPE(16:0)	-.648*	0.043				
<i>Oscillospirales UCG-10 Unclassified</i>	PS(20:1)			.926**	0.003		
	Taurallocholic acid			.849*	0.016		
	Urea			.830*	0.021		
	Glycerol					1.000**	< 0.001
	2,3-Dinor-11b-PGF2a	.888**	0.001				
	D-Myo-inositol phosphate	.868**	0.001				
<i>Tuzzerella</i>	Calonectrin			.866*	0.012		
	Flavin adenine dinucleotide (FAD)					.791*	0.011

<i>Oscillospiraceae</i> UCG-005	LysoPE(16:0)	-.690*	0.027			
	Pipericine	-.905**	0.000			
<i>Butyricocccaceae</i> UCG-009	PS(20:1)			.791*	0.034	
	16-Hydroxy hexadecanoic acid			.791*	0.034	
	3a,7a,12b-Trihydroxy-5b-cholanoic acid	.994**	0.000			
	Ethyl 4-ethoxybenzoate	.696*	0.025			
<i>Ruminococcaceae</i> UBA1819	Stearidonyl carnitine	1.000**	<0.001			
	Docosanamide	.745*	0.013			

Table S7. Cross validation from 3D-PLS-DA of testicular samples corresponding to A) GC-MS analysis, B) UHPLC-ESI⁺-QTOF-MS and C) UHPLC-ESI-QTOF-MS.

A					
Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.74	0.91	0.91	0.96	0.96
R2	0.84	0.96	0.99	1.00	1.00
Q2	0.60	0.63	0.62	0.61	0.60
B					
Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.73	0.85	0.81	0.73	0.77
R2	0.80	0.93	0.96	0.98	0.99
Q2	0.69	0.77	0.76	0.68	0.66
C					
Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.58	0.76	0.57	0.57	0.64
R2	0.63	0.68	0.69	0.69	0.69
Q2	0.43	0.35	0.26	0.09	-0.07

