Supplemental Materials

New metabolic signature for Chagas disease reveals sex steroid perturbation in mice and humans

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Table S1. Tentative metabolites significantly ($p < 0.01$) differentially expressed (fold-change ≥ ±1.5) in CD patients as compared to CD-negative controls (sorted by fold-change)

#	*m/z* (ppm)[1]	RT [2] (min)	Fold-change [3]	p-value	Ion	Ionization mode	Tentative metabolite [4]
1	210.0760 (0)	9.5	4.0	1.9×10^{-8}	M+H	Positive	Hydroxyphenylacetylglycine
2	333.0593 (0)	10.4	3.6	6.5×10^{-11}	M-H	Negative	Glycerophosphoinositol
3	429.2053 (1)	16.3	3.4	4.7×10^{-8}	M-H	Negative	LysoPA(18:4/0:0)
4	344.1335 (1)	4.1	3.4	2.1×10^{-8}	M+H	Positive	N-(1-Deoxy-1-fructosyl)tyrosine
5	301.1508 (3)	4.5	2.7	1.1×10^{-3}	M-H	Negative	Neuromedin B (1-3)
6	197.0810 (5)	14.2	2.5	1.0×10^{-9}	M-H	Negative	Methylene suberic acid (C10)
7	333.2412 (4)	17.8	2.5	2.6×10^{-10}	M+H	Positive	Hydroxypregnenolone or dihydroxypregnenone or dihydrodeoxycorticosterone
8	267.1339 (0)	4.2	1.9	5.9×10^{-3}	M+H	Positive	Phenylalanyl-threonine (**X**)
9	449.1282 (2)	3.3	1.7	7.9×10^{-9}	M+H	Positive	4-Hydroxy-5-(trihydroxyphenyl)-valeric acid-O-methyl-O-glucuronide
10	305.2122 (4)	18.0	1.5	1.9×10^{-7}	M+H	Positive	Hydroxytestosterone or oxoandrostenediol or dihydroxyandrostenone or hydroxydehydroisoandrosterone or hydroxydehydroepiandrosterone or ketoetiocholanolone
11	126.0220 (3)	3.1	-1.5	3.3×10^{-4}	M+H	Positive	Taurine (**X**)
12	211.0985 (4)	15.1	-2.0	4.9×10^{-6}	M-H	Negative	Methylene azelaic acid (C11)
13	187.0716 (1)	2.8	-2.0	1.1×10^{-3}	M+H	Positive	Pyroglutamyl-glycine (**X**)
14	147.0769 (3)	3.0	-2.1	2.2×10^{-5}	M+H	Positive	Glutamine (**X**)
15	730.4656 (1)	17.0	-2.1	1.3×10^{-5}	M-H	Negative	PS(18:1/14:1)
16	804.5775 (2)	16.7	-2.2	8.2×10^{-3}	M-H	Negative	PS(15:0/22:0)
17	311.2243 (5)	16.6	-2.2	1.3×10^{-6}	M-H	Negative	Hydroperoxylinoleic acid (C18)
18	215.1656 (2)	16.1	-2.2	8.0×10^{-5}	M-H	Negative	Hydroxylauric acid (C12)
19	249.1125 (3)	16.3	-2.4	1.7×10^{-6}	M-H	Negative	Ubiquinone-1
20	289.1047 (2)	17.5	-2.4	5.4×10^{-3}	M-H	Negative	N-Succinyl-diaminopimelate
21	465.2468 (2)	3.1	-2.4	6.5×10^{-3}	M+H	Positive	Asn-Gly-Phe-Lys (**X**)
22	800.5422 (3)	16.3	-2.4	1.1×10^{-5}	M-H	Negative	PS(15:0/22:2)
23	361.1621 (2)	16.8	-2.5	4.1×10^{-5}	M-H	Negative	Thyrotropin releasing hormone

Table S1. Tentative metabolites significantly differentially expressed in CD patients, continued

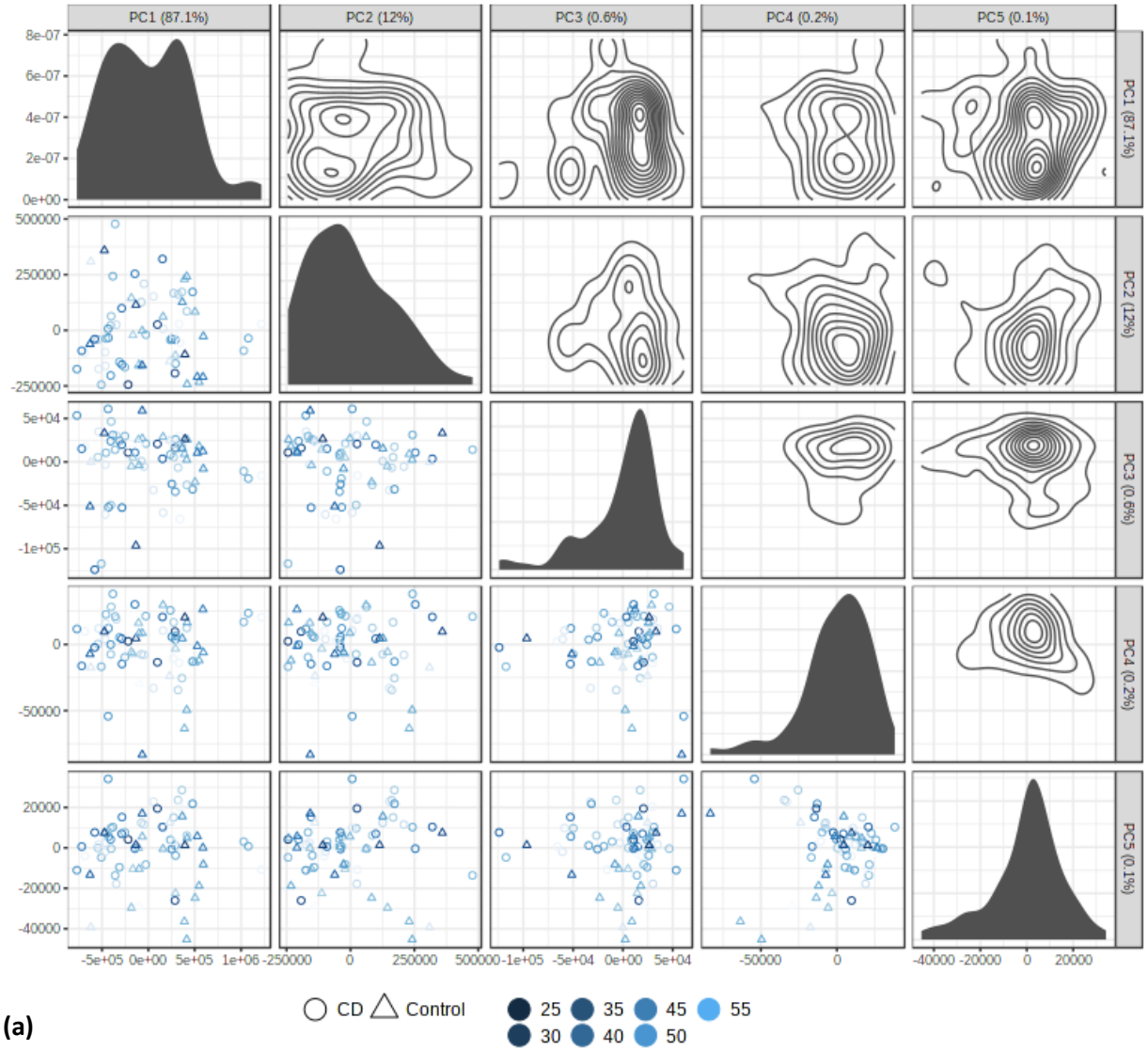
#	*m/z* [1]	RT [2] (min)	Fold-change [3]	p-value	Ion	Ionization mode	Tentative metabolite [4]
24	143.1081 (2)	16.4	-2.5	4.4×10^{-4}	M-H	Negative	Caprylic acid (C8)
25	570.3566 (0)	16.5	-2.6	5.4×10^{-6}	M-H	Negative	LysoPC(22:4)
26	678.4321 (5)	17.2	-2.6	1.8×10^{-6}	M-H	Negative	PS(14:0/14:0)
27	969.7004 (3)	17.4	-2.6	7.2×10^{-3}	M-H	Negative	TG(20:5/22:6/20:5)
28	422.2292 (5)	17.1	-2.8	7.9×10^{-5}	M-H	Negative	LysoPE(14:1/0:0)
29	187.1330 (5)	16.4	-3.0	1.4×10^{-4}	M-H	Negative	Hydroxycapric acid (C10)
30	704.4500 (1)	17.0	-3.0	2.3×10^{-4}	M-H	Negative	PS(14:0/16:1)
31	157.0873 (2)	17.6	-3.1	5.0×10^{-3}	M-H	Negative	Oxocaprylic acid (C8)
32	155.1071 (4)	17.2	-3.1	8.5×10^{-4}	M-H	Negative	Nonanedione (C9)
33	195.1023 (2)	16.7	-3.3	4.9×10^{-6}	M-H	Negative	4-(3-hydroxybutyl)-2-methoxyphenol
34	221.1185 (1)	17.1	-3.5	2.0×10^{-4}	M-H	Negative	2-hydroxy-1-(4-methoxyphenyl)-4-methylpentan-3-one
35	223.1339 (0)	16.4	-3.7	3.6×10^{-7}	M-H	Negative	3-Methyl-5-pentyl-2-furanpropanoic acid
36	776.5440 (1)	17.3	-3.7	2.1×10^{-5}	M-H	Negative	PS(15:0/20:0)
37	732.4824 (0)	17.0	-4.4	5.6×10^{-4}	M-H	Negative	PS(18:1/14:0)
38	367.0181 (1)	17.4	-4.4	1.7×10^{-3}	M-H	Negative	Orotidylic acid
39	718.4651 (2)	16.6	-4.5	7.4×10^{-8}	M-H	Negative	PS(16:1/15:0)
40	829.5770 (2)	17.3	-4.8	7.1×10^{-5}	M-H	Negative	PA(24:1/22:6)
41	165.0914 (4)	16.6	-5.0	2.1×10^{-5}	M-H	Negative	3-(4-methoxyphenyl)propan-1-ol
42	802.5582 (3)	17.0	-6.7	2.1×10^{-4}	M-H	Negative	PS(15:0/22:1)

[1] All listed ions are singly charged (number in parenthesis is mass accuracy in parts per million). [2] LC retention time. [3] Fold-change LC-MS signal intensity (CD/control). [4] PA: phosphatidic acid; PC: phosphorylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PI: phosphatidylinositol; PS: phosphatidylserine; TG: triglyceride. Metabolites marked with **X** were confirmed by LC-MS/MS.

Table S2. Pearson's correlation coefficients (*r*) between CD metabolite biomarkers, sex, age, cardiomyopathy and treatment duration. Taurine and Asn-Gly-Phe-Lys showed moderate correlation ($0.4 < r < 0.8$) with patient's sex. All other correlations were weak.

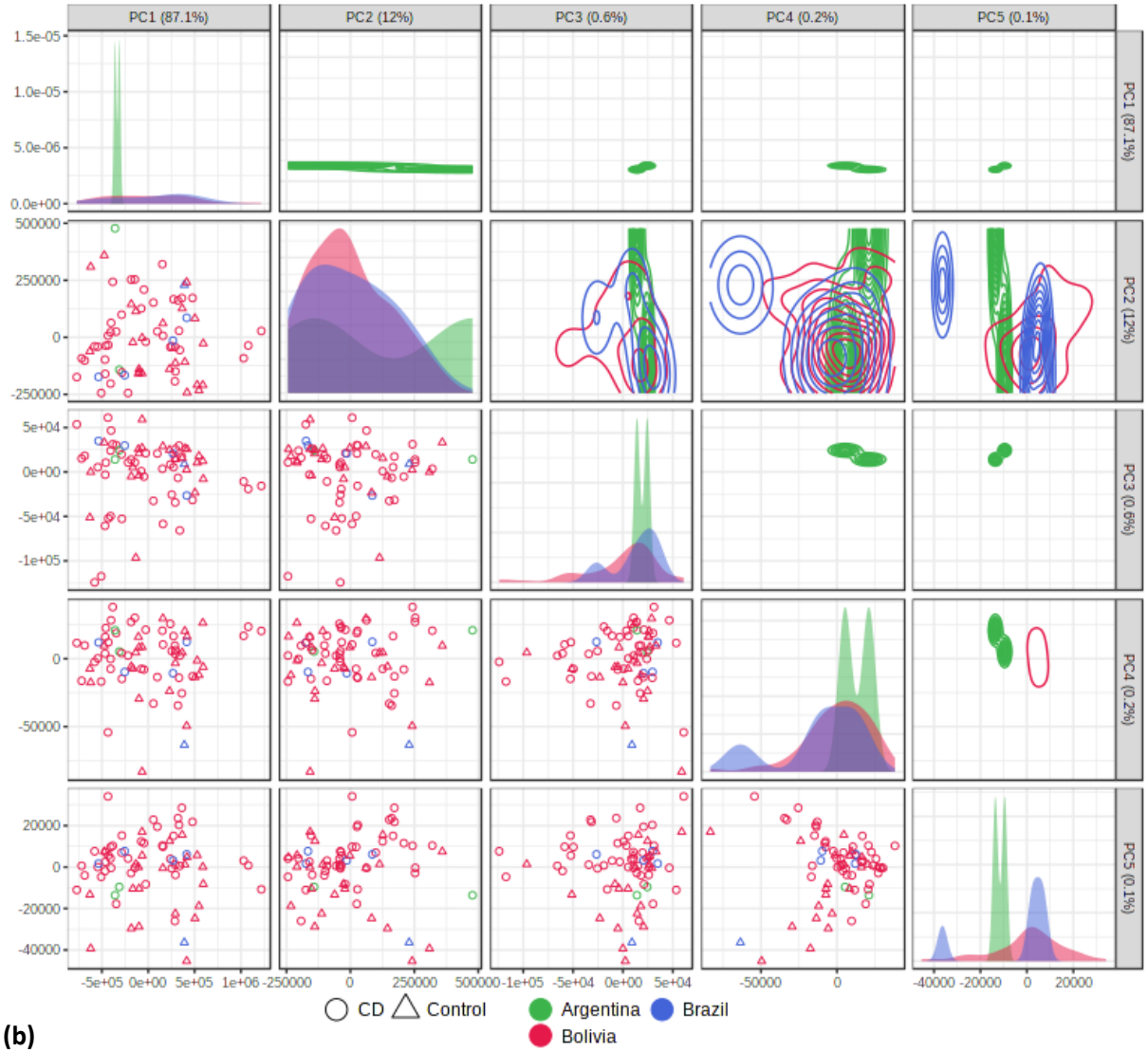
Tentative Metabolite	Sex			Age			Cardiomyopathy		Treatment Duration
	Disease	Follow-up	Control	Disease	Follow-up	Control	Disease	Follow-up	
Glutamine	0.178	0.226	0.181	-0.232	0.123	-0.075	0.022	-0.007	-0.107
Pyroglutamyl-glycine	-0.179	0.121	-0.187	-0.126	0.310	0.369	-0.083	-0.110	0.063
Phenylalanyl-threonine	0.124	-0.098	-0.095	-0.023	0.156	-0.119	0.170	0.238	-0.099
Taurine	-0.112	0.426	-0.117	0.022	0.055	-0.045	0.027	0.041	-0.112
Asn-Gly-Phe-Lys	-0.501	-0.298	0.088	-0.275	0.007	0.070	-0.212	0.189	0.098

Figure S1. Scoring plots from interactive principal component analysis of the identified Chagas disease biomarkers in humans and metadata confounders: age (a), country of origin (b), diagnosis (c), sex (d).



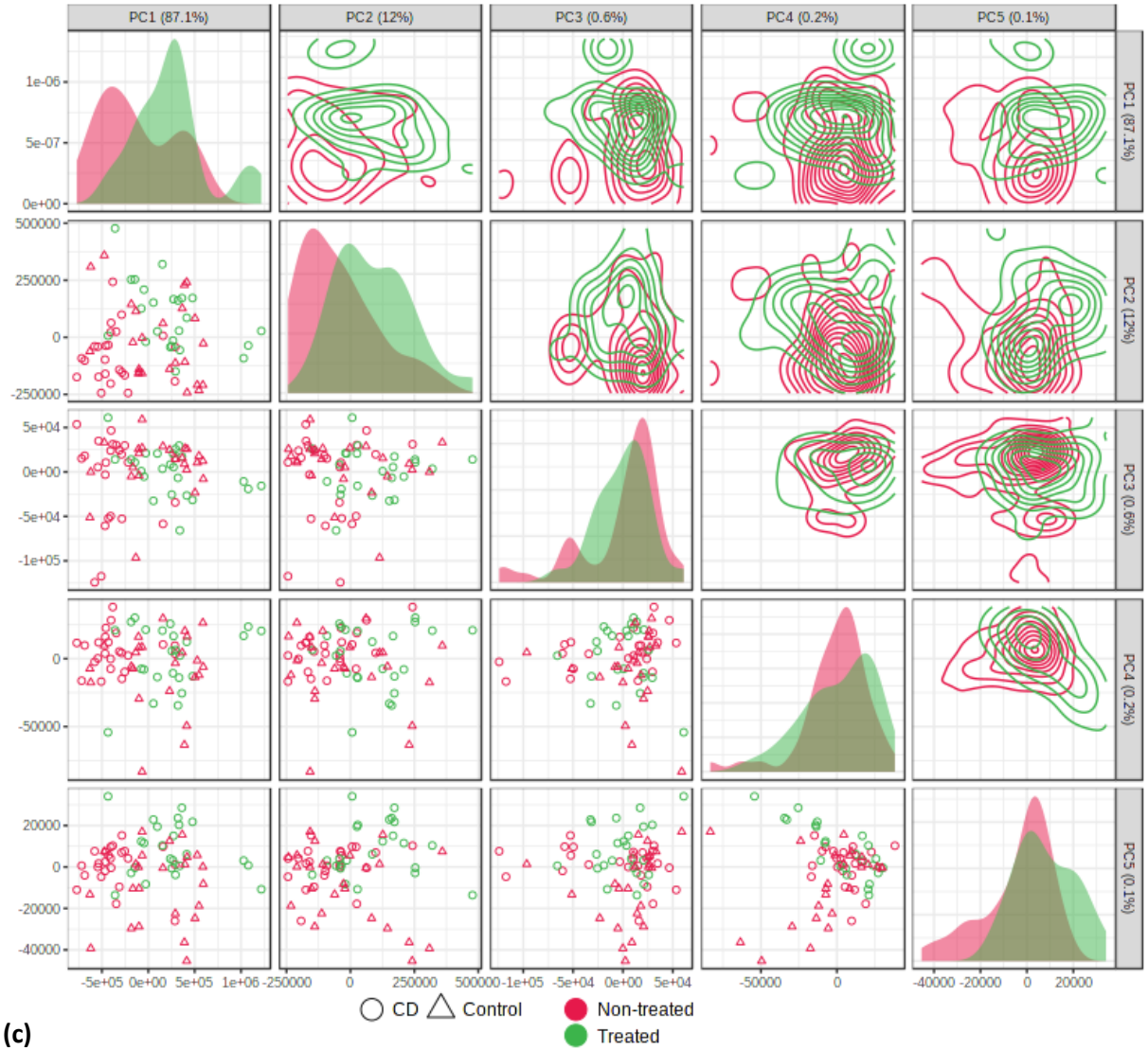
(a)

Figure S1 (cont'd). Scoring plots from interactive principal component analysis of the identified Chagas disease biomarkers in humans and metadata confounders: age (a), country of origin (b), diagnosis (c), sex (d).



(b)

Figure S1 (cont'd). Scoring plots from interactive principal component analysis of the identified Chagas disease biomarkers in humans and metadata confounders: age (a), country of origin (b), diagnosis (c), sex (d).



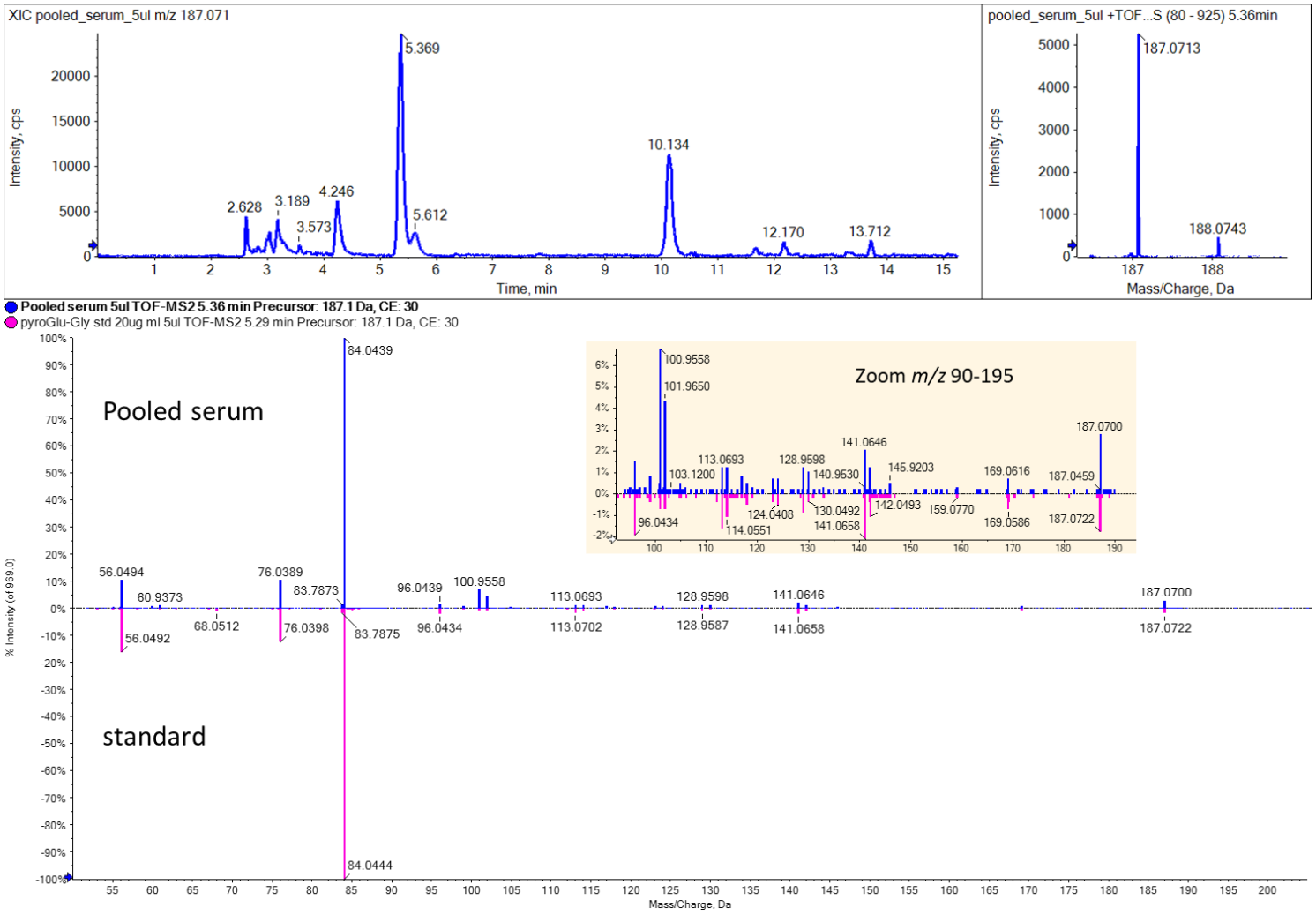
(c)

Figure S1 (cont'd). Scoring plots from interactive principal component analysis of the identified Chagas disease biomarkers in humans and metadata confounders: age (a), country of origin (b), diagnosis (c), sex (d).

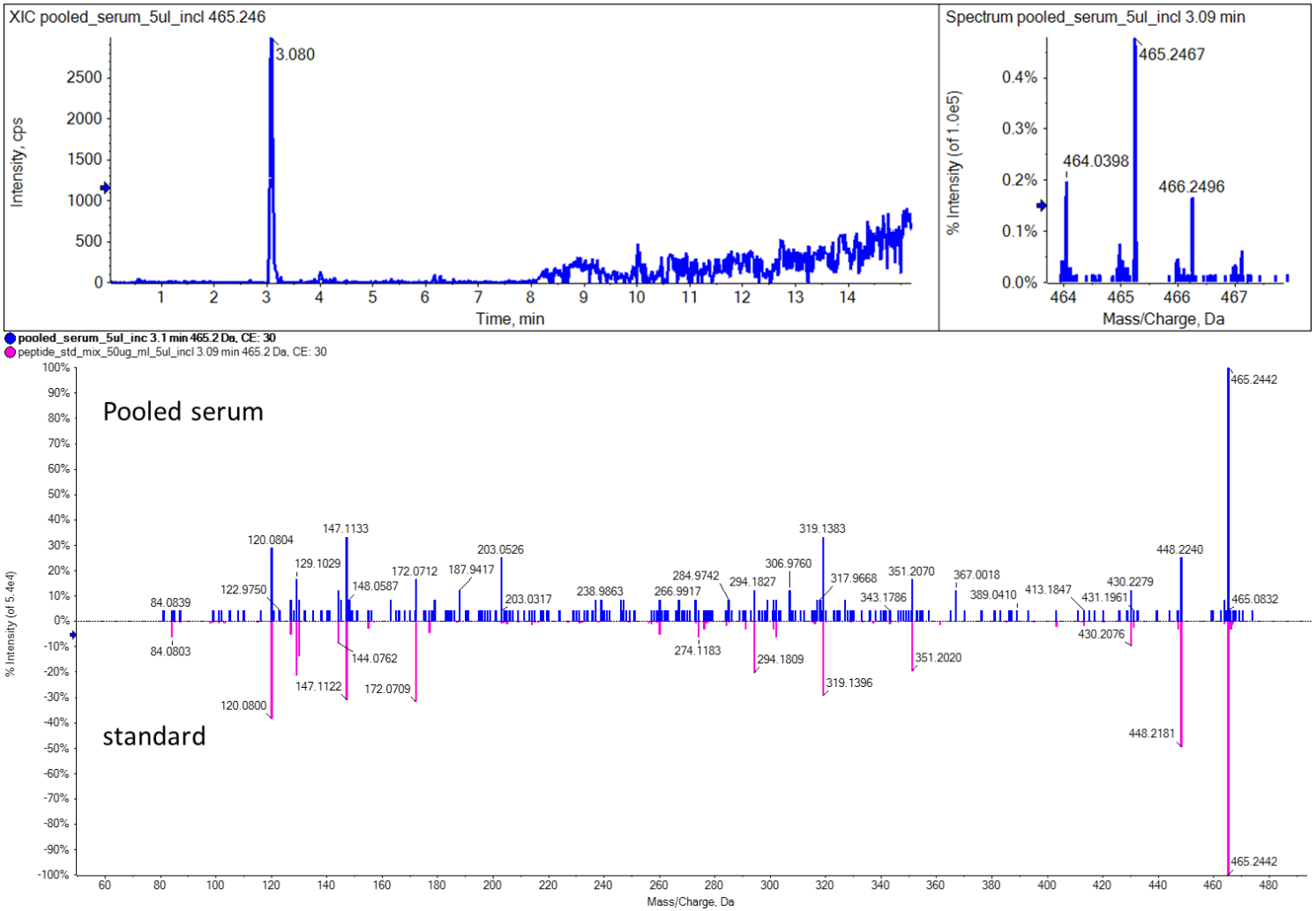
(d)

Figure S2. LC-MS/MS confirmation of Chagas disease biomarkers in human

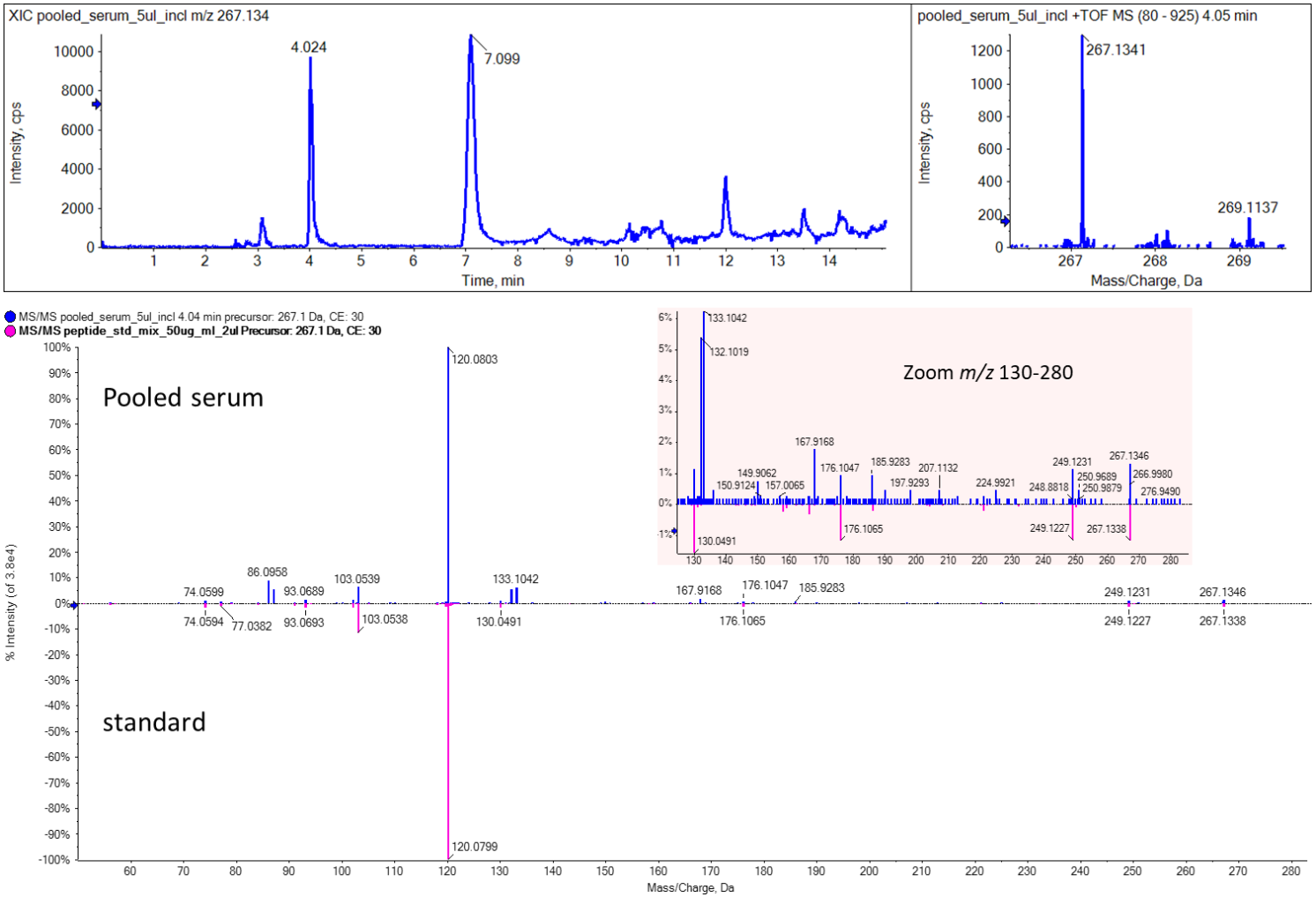
PyroGlu-Gly MS and MS/MS



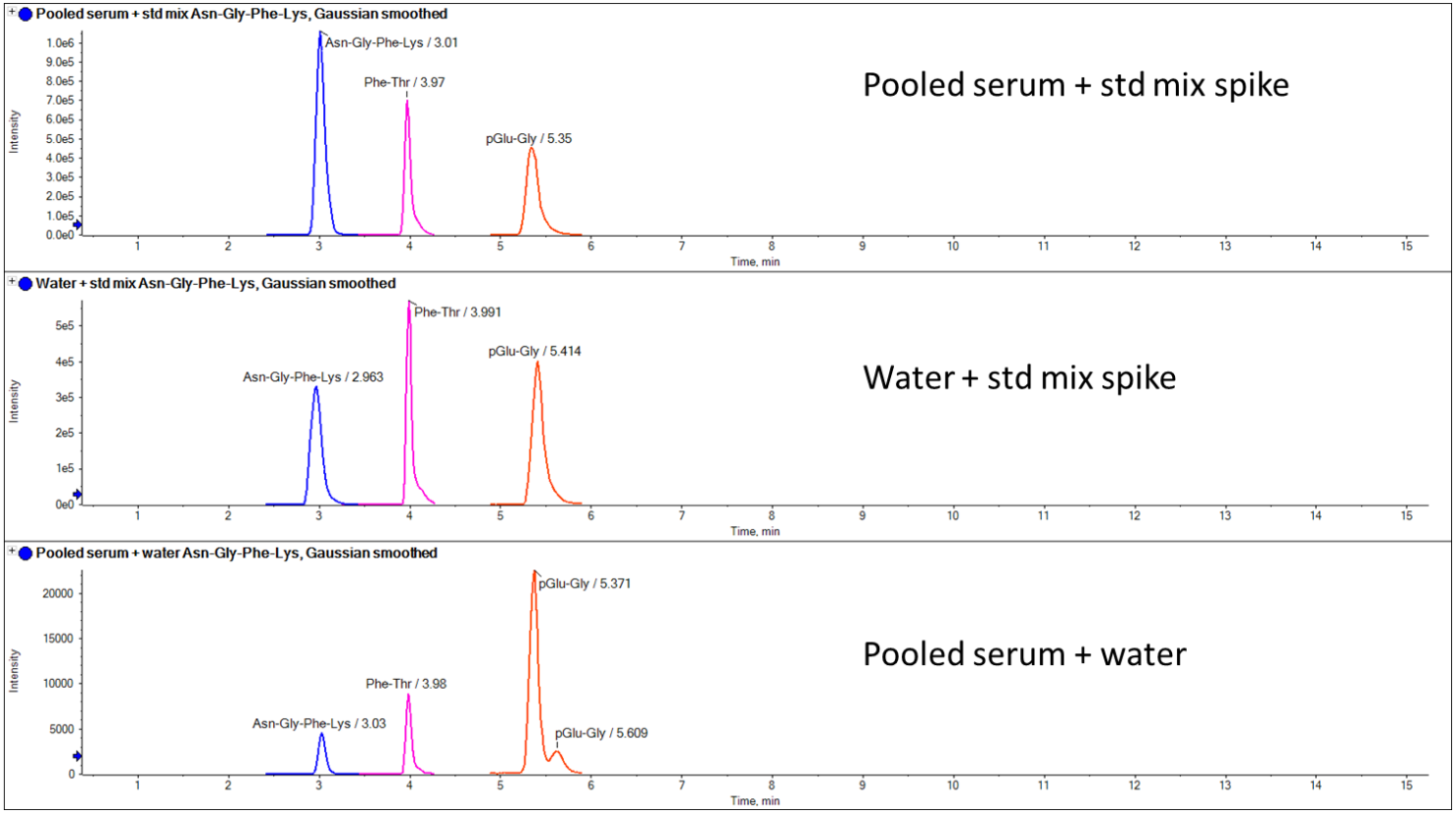
Asn-Gly-Phe-Lys MS and MS/MS



Phe-Thr MS and MS/MS



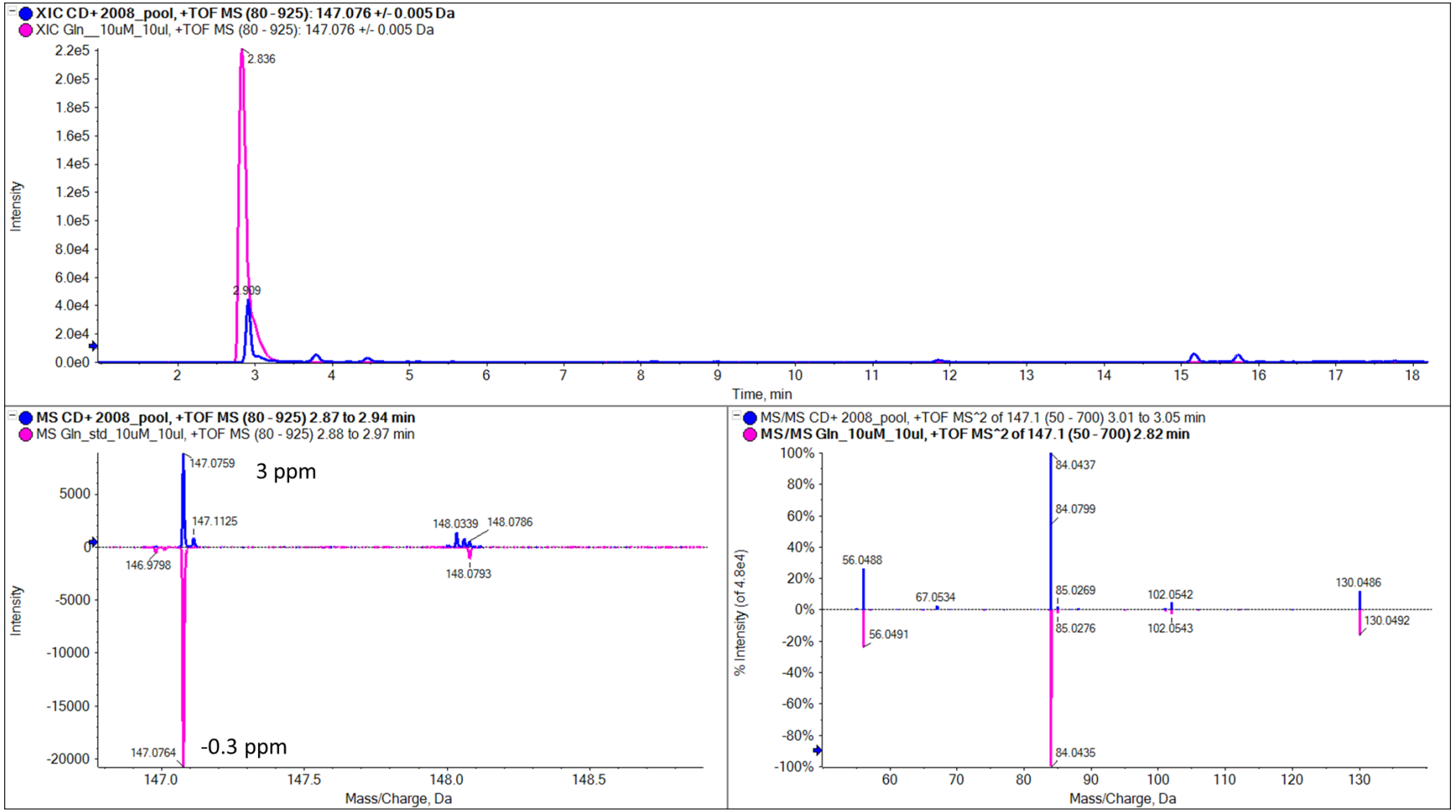
XIC of 3 peptides in 3 serum-spiked samples



Conclusions:

- Retention times of standards spiked in serum and water ctl are same
- Retention time of endogenous metabolites in pooled serum same as spiked serum
- Intensity highest for spiked serum, then water spiked, then endogenous serum

Glutamine: 14.0769 m/z, 3.1 min, fold-change = -2.1, p = 2.2e-5



Taurine: 126.0219 m/z, 3.1 min, fold-change = -1.5, p = 3.3e-4

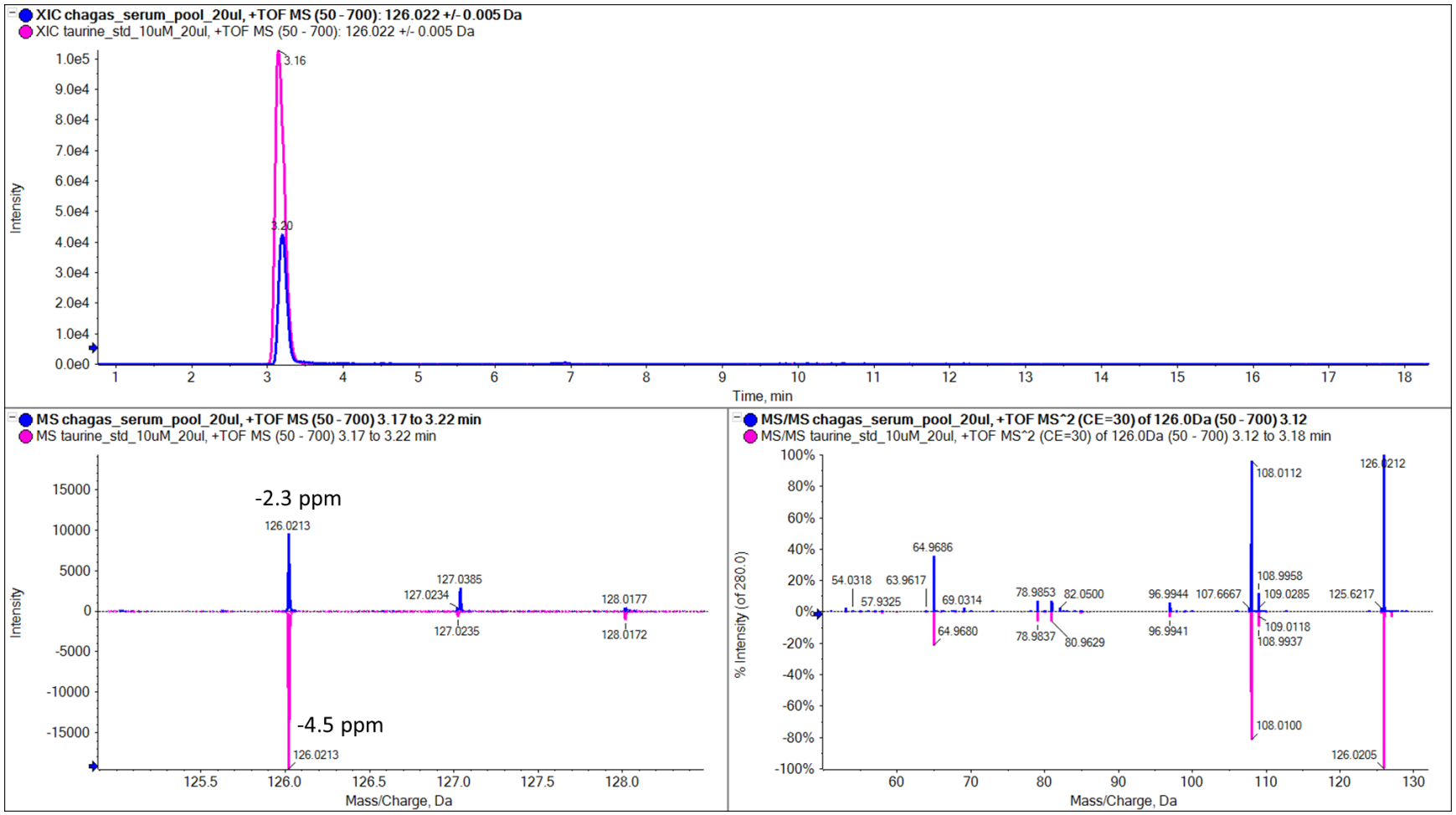
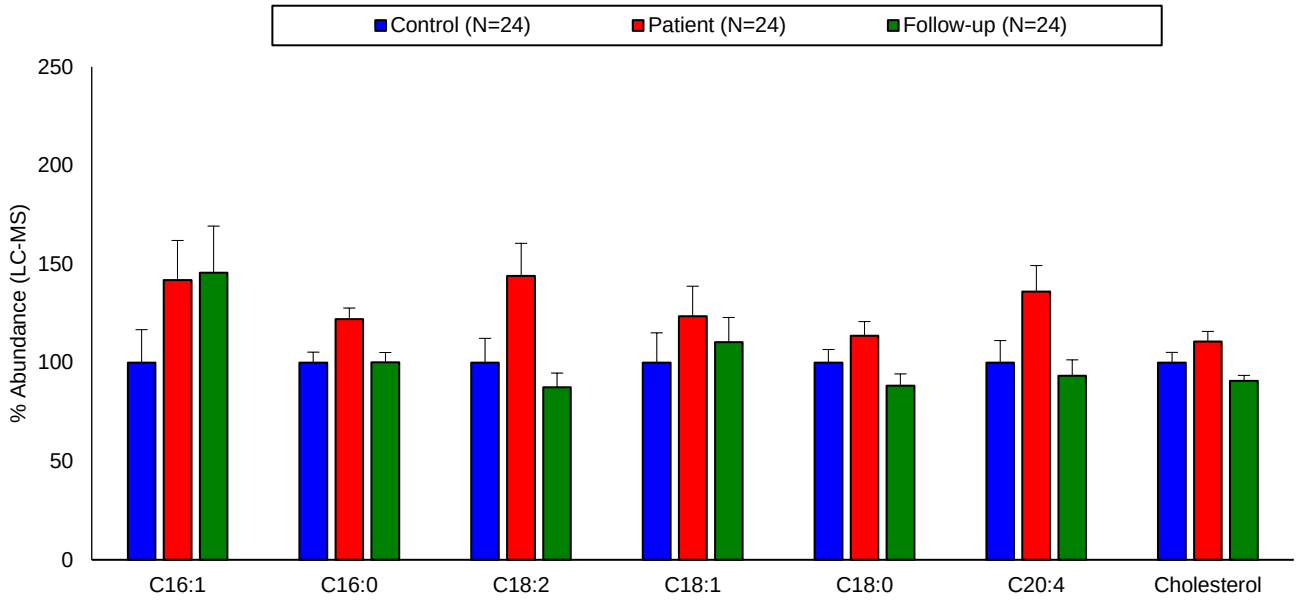


Figure S3. Relative abundances of selected metabolites in Chagas disease patients, post-treatment follow-up samples and demographically matched negative controls. Patient and follow-up values were normalised to the controls. Error bars represent mean ± standard error of mean.



	C16:1	C16:0	C18:2	C18:1	C18:0	C20:4	Cholesterol
p (ANOVA)	0.22	0.0057	0.0059	0.51	0.032	0.017	0.010

Figure S4. PCR results from the testes of uninfected (A1-A3), and T. cruzi infected mice not subjected to (B1-B4) and subjected to (C1-C4) nifurtimox treatment. Control negative (**-CO**) and positive DNA samples (**+CO**) showing bands at 195 bp, 360 bp, and 550 bp respectively, were included in the analysis to verify that carryover DNA contamination had not occurred.