

## Supplementary information

### **Metabolite variations in sera of HIV+ patients after an oral administration of effervescent glutamine and in comparison to non-HIV individuals by NMR**

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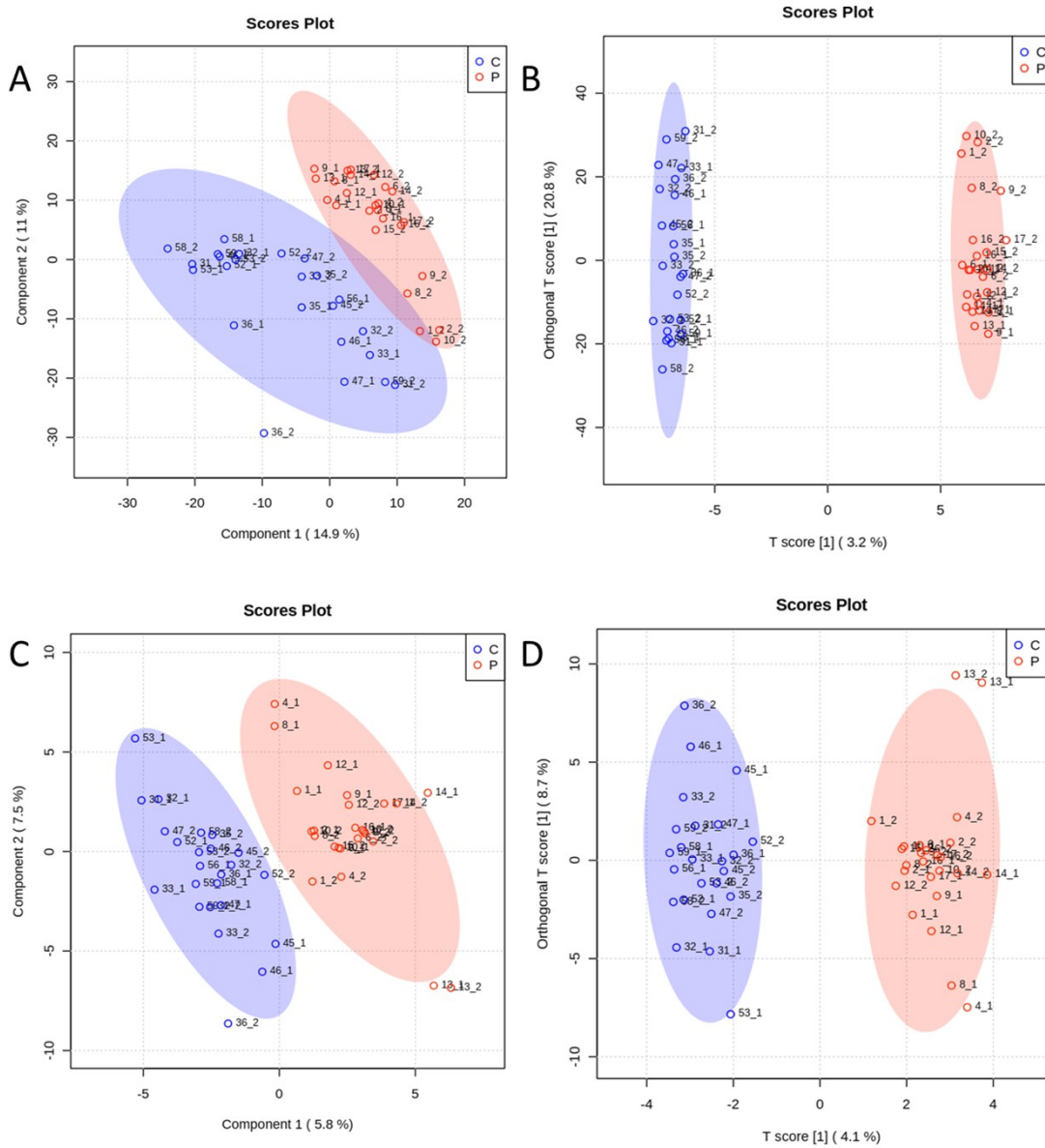
Table S1. Clinical characteristics\* of HIV+ patients

<b>Time of infection</b>	6.3 ± 1.8 (range of 2 to 17).
	1 to 5 years (58.3%)
	5 to 10 years ( 16.6%)
	10 to 15 years (25.0%)
<b>Time of starting current ART</b>	2.4 ± 0.6 years (range of 1 to 7)
<b>Antiviral therapy</b>	3TC, TDF, EFZ (41.7%)
	3TC, AZT, LPV/r (16.7%)
	3TC, AZT, RTV (8.3%)
	3TC, TDF, ATV, RTV (8.3%)
	3TC, TDF, EFZ, DRV, RTV(8.3%)
	3TC, TDF, ATV/r (8.3%)
	3TC, TDF, LPV/r (8.3%)

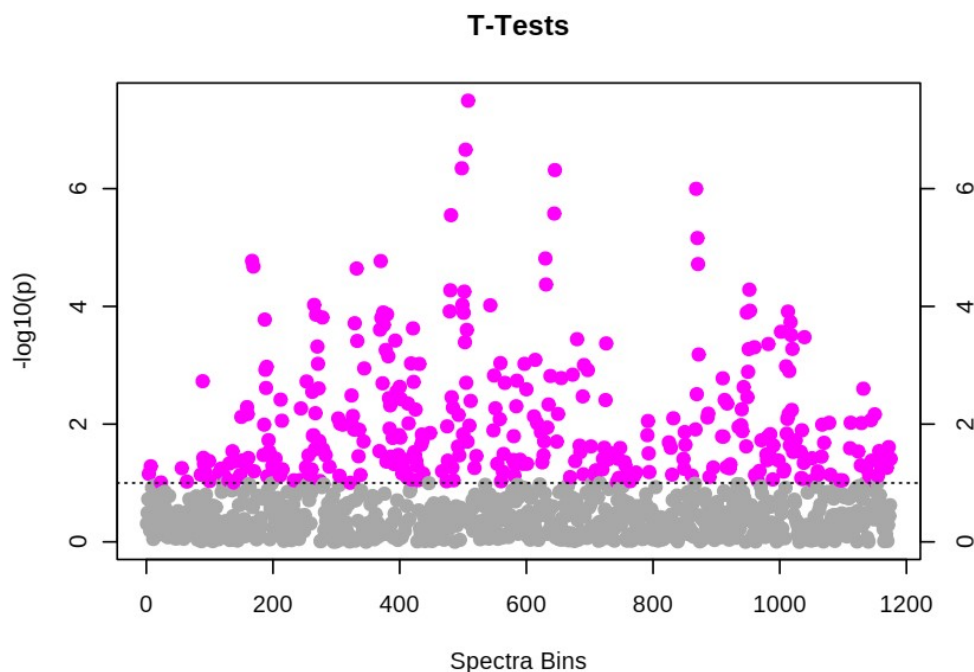
**Key:** Antiretroviral therapy (ART), Lamivudine (3TC), tenofovir (TDF), efavirenz (EFZ), zidovudine (AZT), lopinavir/ritonavir (LPV/r), atazanavir (ATV), ritonavir (RTV), darunavir (DRV), atazanavir/ritonavir (ATV/r).

The values are expressed as mean ±standard deviation.

\* Information previously published in *J. Med. Food* v. 23, p. 485-490, 2019.



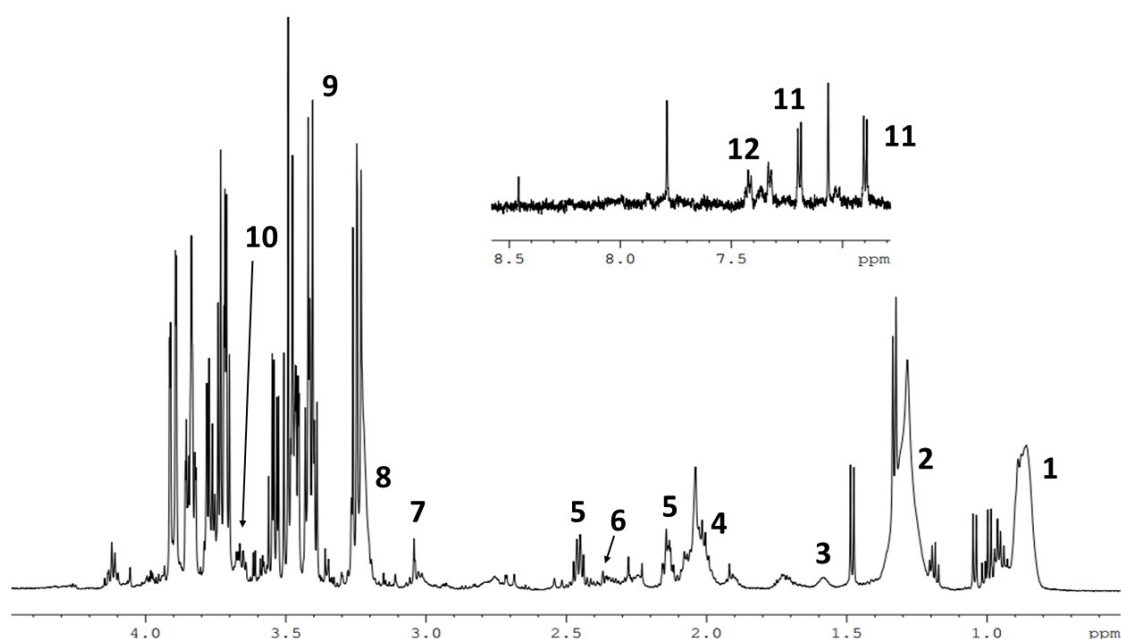
**Figure S1.** Multivariate analyses were performed on serum  $^1\text{H-NMR}$  data of HIV+ patients (P) and control donors (C). **A.** PLS-DA 2D scores from  $^1\text{H-NMR}$  data with water elimination pulse sequence (WATERGATE; Accuracy: 0.840;  $R^2$ : 0.974 and  $Q^2$ : 0.344). **B.** OPLS-DA 2D scores from  $^1\text{H-NMR}$  data with water elimination pulse sequence (WATERGATE). **C.** PLS-DA 2D scores from  $^1\text{H-NMR}$  edited by diffusion (stebpgp1s191d; Accuracy: 0.940;  $R^2$ : 0.924 and  $Q^2$ : 0.550). **D.** OPLS-DA 2D scores from  $^1\text{H-NMR}$  edited by diffusion (stebpgp1s191d).



**Figure S2.** Important features selected by Univariate Analysis (t-tests) with threshold 0.1. They are responsible for summarizing  $^1\text{H-NMR}$  CPMG data from the serum of patients living with HIV (P), and control donors (C).

**Table S2.** Important metabolites selected by Univariate Analysis (t-tests, identified as the metabolites with the lowest p-value in the  $^1\text{H-NMR}$  CPMG serum data in patients living with HIV (P), and control donors (C)

Name	t.stat	p.value	$-\log_{10}(p)$	FDR
Tyrosine	5.6381	$1.01 \times 10^{-6}$	5.9974	$23.6 \times 10^{-5}$
Threonine	-4.8331	$15.4 \times 10^{-6}$	4.8137	$181 \times 10^{-5}$
Pyruvate	4.1854	$127 \times 10^{-6}$	3.8965	$559 \times 10^{-5}$
Phenylalanine	-4.4625	$52.0 \times 10^{-6}$	4.2839	$366 \times 10^{-5}$
Lysine	6.0824	$21.7 \times 10^{-6}$	6.6618	$12.8 \times 10^{-5}$
Lipids	-4.8045	$16.9 \times 10^{-6}$	4.7724	$18.1 \times 10^{-5}$
Lactate	-4.7382	$21.05 \times 10^{-6}$	4.6768	$19.0 \times 10^{-5}$
Glutamine	-4.7149	$22.7 \times 10^{-6}$	4.6434	$19.1 \times 10^{-5}$
Glutamate	4.8029	$17.0 \times 10^{-6}$	4.7702	$18.1 \times 10^{-5}$
Glucose	-5.8515	$0.49 \times 10^{-6}$	6.3159	$14.1 \times 10^{-5}$
Creatine	6.6369	$319 \times 10^{-6}$	7.4951	$3.76 \times 10^{-5}$
Choline	4.2735	$958 \times 10^{-6}$	4.0187	$5.35 \times 10^{-5}$

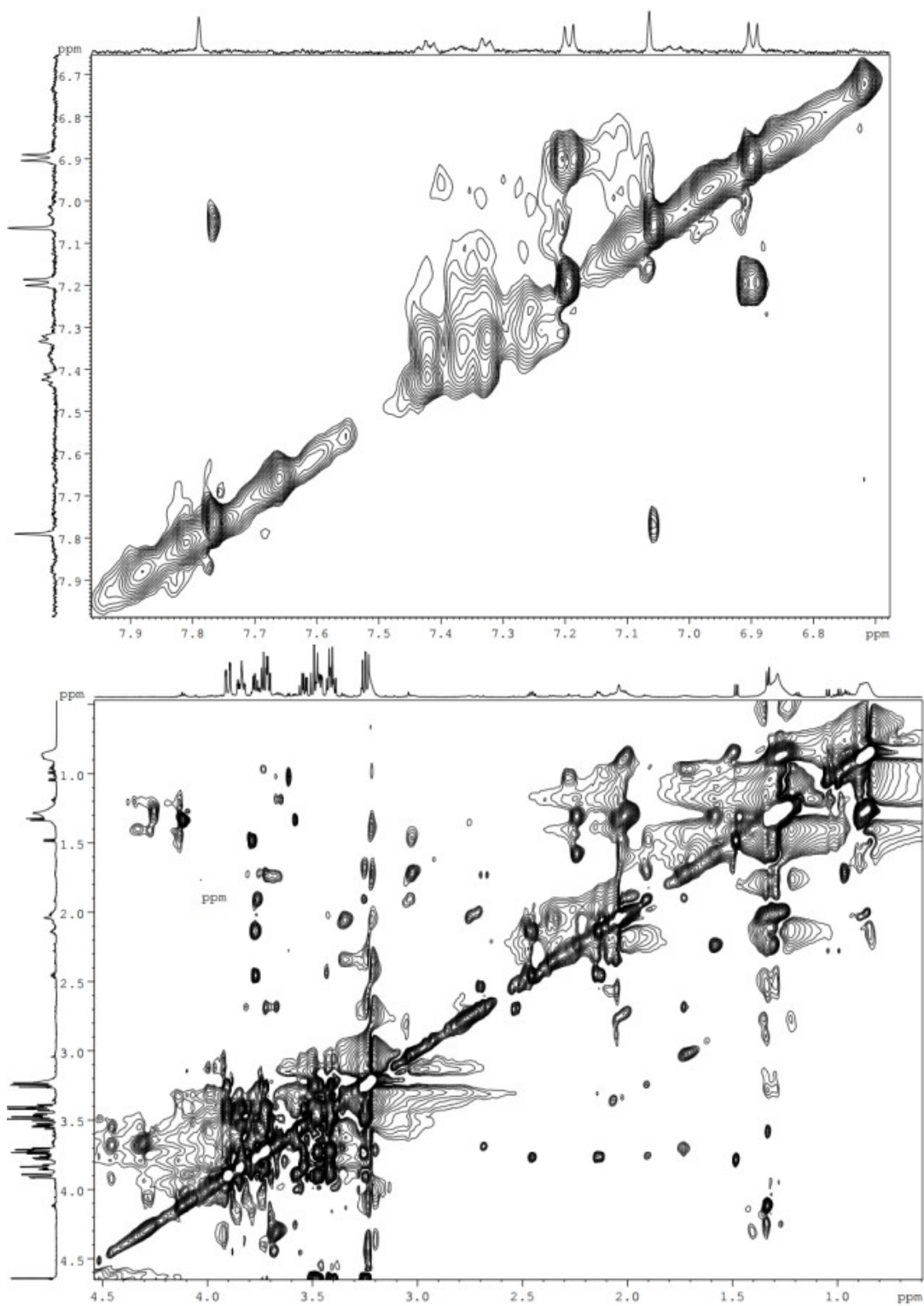


**Figure S3.** Examples of  $^1\text{H-NMR}$  spectral data (0.50 to 4.50 ppm) from serum from individuals living with HIV. The spectra were acquired on the Bruker AVANCE III 600 MHz at 25 °C, by a  $^1\text{H-NMR}$  pulse sequence with a  $T_2$  filter (cpmgrp1d). The numbers indicate the metabolites indicated as biomarkers in this study: 1,  $\text{CH}_3$  from lipids (LDL, and VLDL); 2,  $\text{CH}_2$  from lipids; 3, lysine; 4, glutamate; 5, glutamine; 6, pyruvate; 7, creatine; 8, choline; 9, glucose; 10, threonine; 11, tyrosine; 12, phenylalanine.

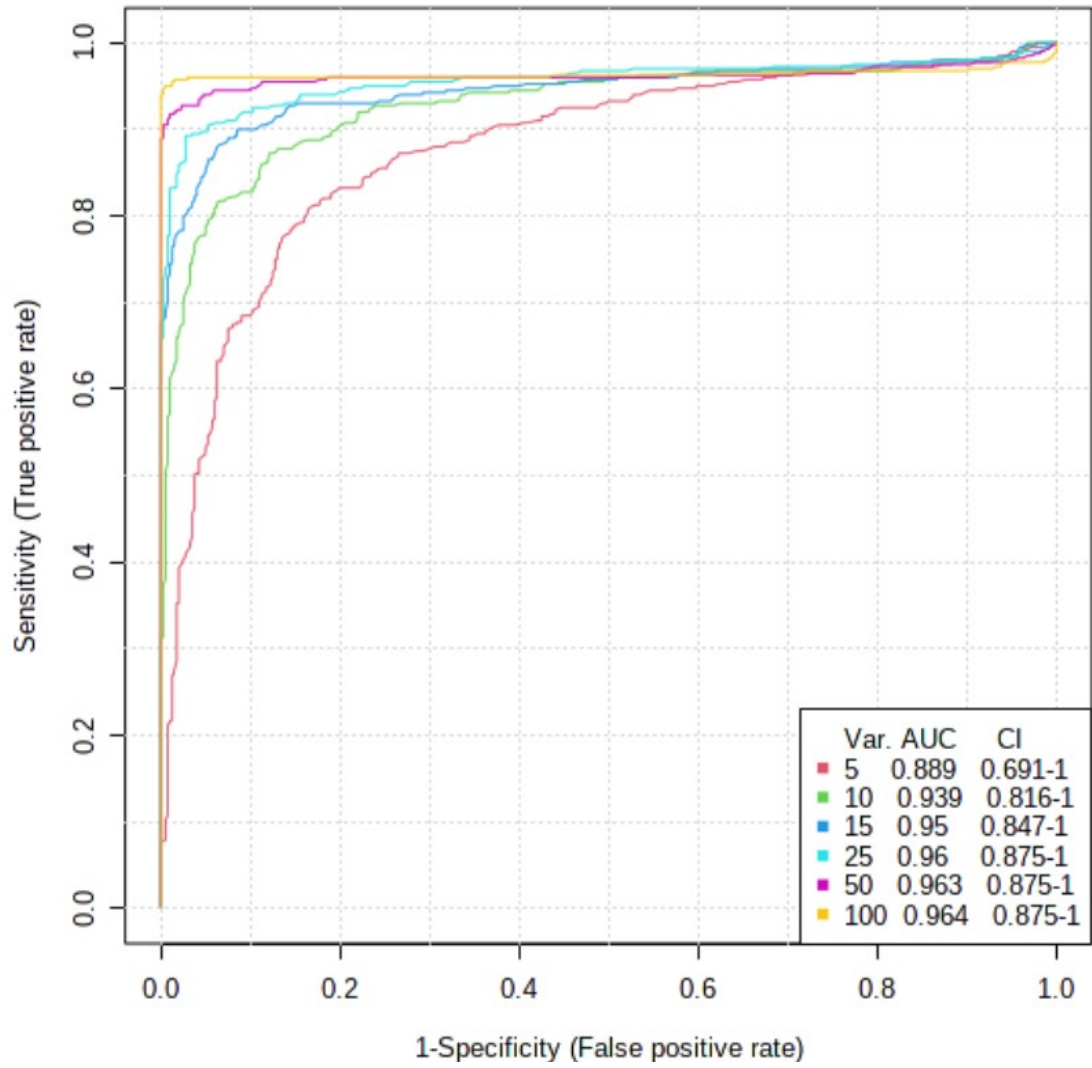
**Table S3.** Chemical shifts, spectral peaks' multiplicities, and coupling constants of some of the most important biomarkers (1-12) as depicted in Figure S3

Metabolite	N <sup>o</sup>	Chemical shift (ppm), spectral peaks multiplicities and coupling constant
Lipids ( $-\text{CH}_3$ )	1	0.83 m
Lipids ( $-\text{CH}_2-$ )	2	1.28 m
Lysine	3	1.46 m; 1.71 m; 1.89 m; 3.02 t; 3.74 t ( $J = 6.09$ Hz)
Glutamate	4	2.04 m; 2.12 m; 2.34 m; 3.75 dd ( $J = 7.19, 4.72$ Hz)
Glutamine	5	2.13 m; 2.45 m; 3.77 t ( $J = 6.18$ Hz)

<b>Pyruvate</b>	6	2.36 s
<b>Creatine</b>	7	3.02 s; 3.92 s
<b>Choline</b>	8	3.19 s; 3.51 dd ( $J = 5.82, 4.16$ Hz); 4.06 ddd
<b>Glucose</b>	9	3.23 dd ( $J = 9.41, 7.98$ Hz); 3.40 m; 3.46 m; 3.52 dd ( $J = 9.82, 3.77$ ); 3.73 m; 3.82 m; 3.88 dd ( $J = 12.30, 2.23$ Hz); 4.63 d ( $J = 7.98$ ); 5.22 d ( $J = 3.80$ )
<b>Threonine</b>	10	1.33 d ( $J = 5.95$ Hz); 3.66 d ( $J = 6.05$ Hz); 4.30 m
<b>Tyrosine</b>	11	3.03 dd ( $J = 14.55, 8.01$ Hz); 3.34 dd ( $J =$ 14.53, 4.68 Hz); 4.04 dd ( $J = 8.03, 4.68$ Hz); 6.94 m; 7.20 dd ( $J = 7.95, 1.51$ Hz); 7.24 td ( $J = 7.76, 1.71$ Hz)
<b>Phenylalanine</b>	12	3.19 m; 3.98 dd ( $J = 7.88, 5.31$ Hz); 7.32 d ( $J$ $= 6.96$ Hz); 7.34 m; 7.42 m

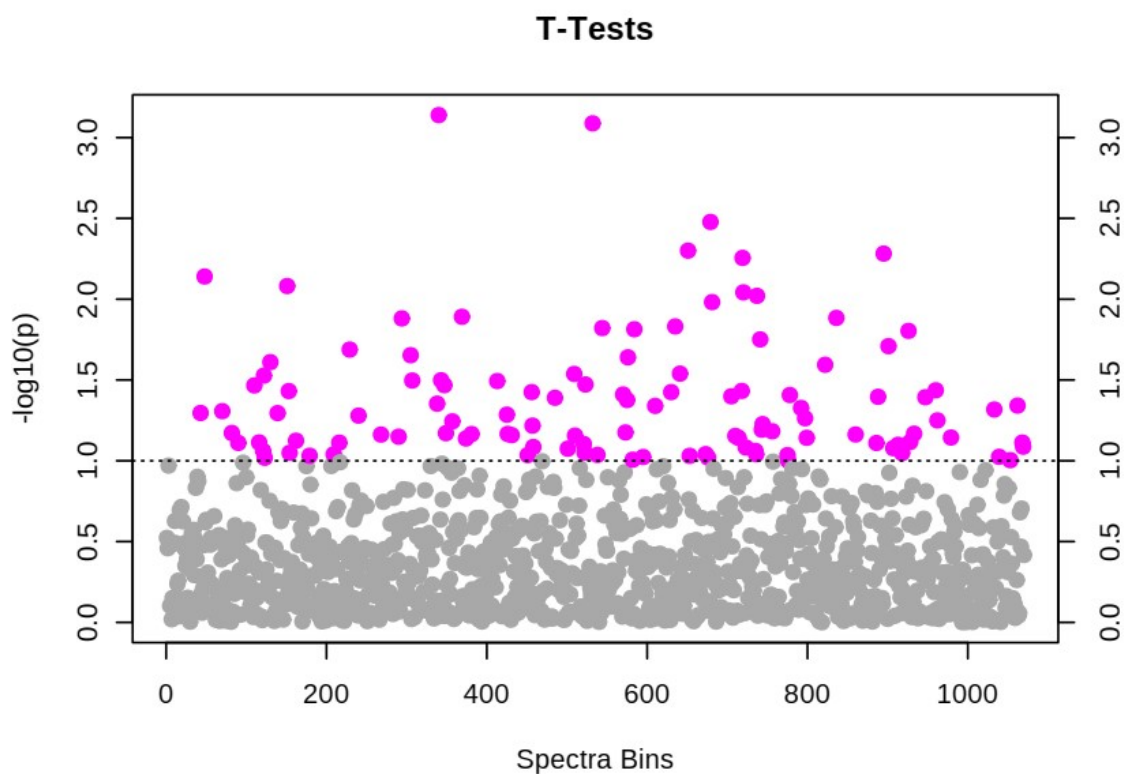


**Figure S4.**  $^1\text{H}$ - $^1\text{H}$  NMR TOCSY of a sample from the HIV cohort. The spectrum was acquired on the Bruker AVANCE III 600 MHz at 25 °C. On the bottom is shown the aliphatic spectral region (0.50 to 4.50 ppm), and on the upper panel is shown the aromatic spectral region.



**Figure S5.** Comparisons of differential metabolites between patients (P) and control donors (C) based on multivariate ROC curve analysis. Var. (variables) indicate the number of selected features.

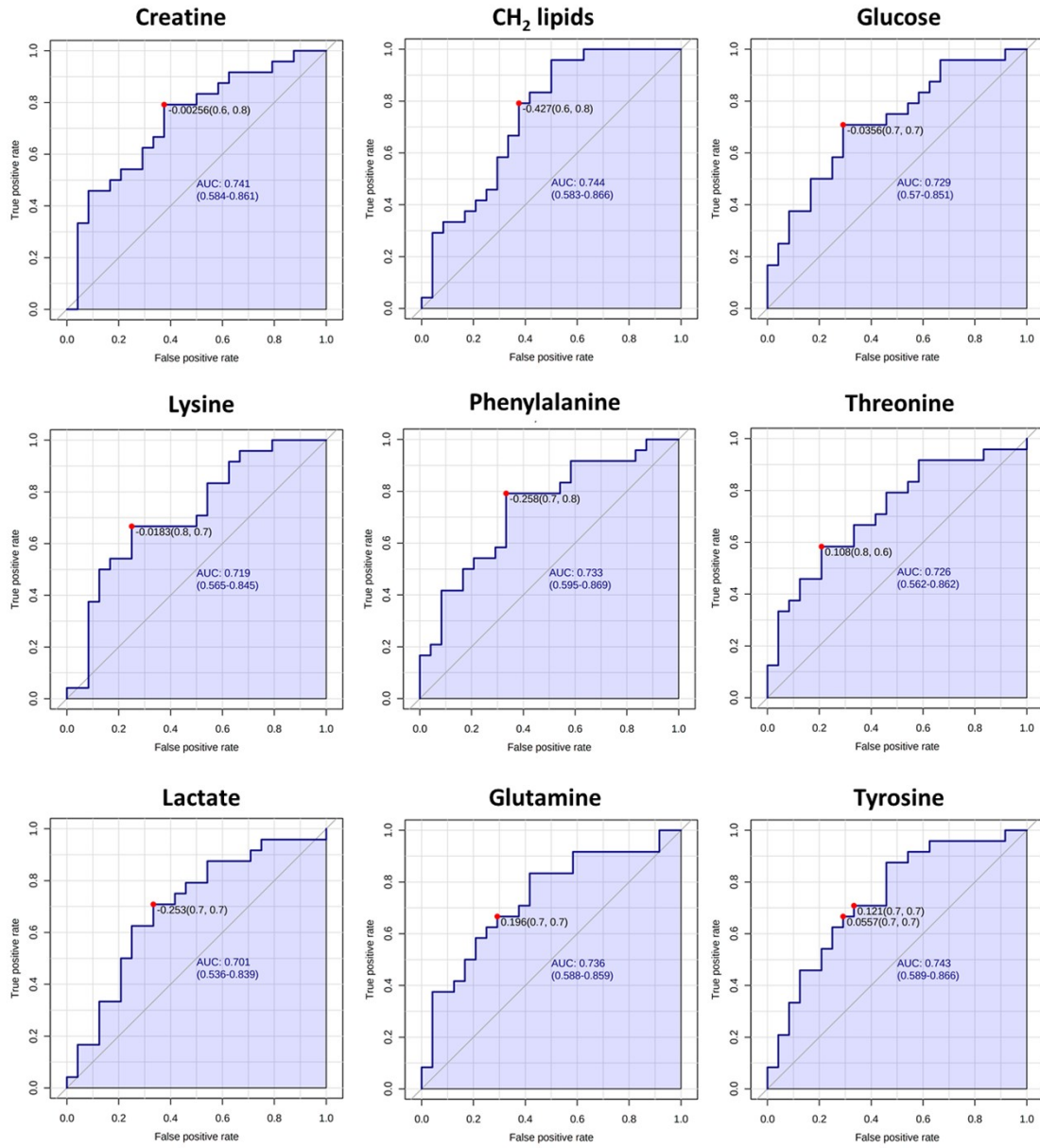




**Figure S6.** Important features selected by Univariate Analysis (t-tests) with threshold 0.1, which point to the most important  $^1\text{H-NMR}$  CPMG features of cohort before (BT), and after EG administration (AT).

**Table S4.** Important metabolites selected by Univariate Analysis (t-tests, identified as the lowest p-value metabolites in the  $^1\text{H-NMR}$  CPMG data, patients before (BT), and after administration (AT) of EG

Name	t.stat	p.value	$-\log_{10}(p)$	FDR
Tyrosine	2.7389	0.00887	2.0522	0.72673
Threonine	-2.9655	0.00487	2.3127	0.59094
Phenylalanine	-2.9577	0.00497	2.3036	0.59094
Lysine	-2.9790	0.00469	2.3285	0.59094
Lactate	2.7221	0.00926	2.0333	0.72673
Glutamine	3.0391	0.00398	2.3997	0.59094
Glucose	-3.3415	0.00171	2.7677	0.59094
Creatine	-2.6598	0.01087	1.9639	0.72673
$\text{CH}_2$ lipids	-2.4923	0.01653	1.7817	0.80588



**Figure S7.** Univariate ROC curve analysis was performed on biomarkers identified as discriminatory for HIV patients before and after 30 days of treatment.