

Table S1 List of experimental parameters for amino acids and internal standards used for quantification

Compound	Formula	Selected ion	Exact mass (m/z)	Mass window	IS used
Glycine	C ₂ H ₅ NO ₂	[M+H] ⁺	76.0393	76.03-76.05	¹⁵ N ₂ -glutamine
Serine	C ₃ H ₇ NO ₃	[M+H] ⁺	106.0499	106.04-106.06	¹⁵ N ₂ -glutamine
Alanine	C ₃ H ₇ NO ₂	[M+H] ⁺	90.0550	90.04-90.06	¹⁵ N ₂ -glutamine
Hydroxyproline	C ₅ H ₉ NO ₃	[M+H] ⁺	132.0655	132.06-132.08	¹⁵ N ₂ -glutamine
Threonine	C ₄ H ₉ NO ₃	[M+H] ⁺	120.0655	120.05-120.07	¹⁵ N ₂ -glutamine
Asparagine	C ₄ H ₈ N ₂ O ₃	[M+H] ⁺	133.0608	133.05-133.07	¹⁵ N ₂ -glutamine
Proline	C ₅ H ₉ NO ₂	[M+H] ⁺	116.0706	116.07-116.09	¹⁵ N ₂ -glutamine
Aspartic acid	C ₄ H ₇ NO ₄	[M+H] ⁺	134.0448	134.03-134.05	¹⁵ N ₂ -glutamine
Glutamine	C ₅ H ₁₀ N ₂ O ₃	[M+H] ⁺	147.0764	147.06-147.08	¹⁵ N ₂ -glutamine
¹⁵ N ₂ -glutamine	C ₅ H ₁₀ ¹⁵ N ₂ O ₃	[M+H] ⁺	149.0705	149.06-149.08	NA
Ornithine	C ₅ H ₁₂ N ₂ O ₂	[M+H] ⁺	133.0972	133.09-133.11	¹⁵ N ₂ -glutamine
Glutamic acid	C ₅ H ₉ NO ₄	[M+H] ⁺	148.0604	148.05-148.07	¹⁵ N ₂ -glutamine
Valine	C ₅ H ₁₁ NO ₂	[M+H] ⁺	118.0683	118.06-118.08	¹³ C ₆ -lysine
Citrulline	C ₆ H ₁₃ N ₃ O ₃	[M+H] ⁺	176.1030	176.10-176.11	¹³ C ₆ -lysine
Leucine	C ₆ H ₁₃ NO ₂	[M+H] ⁺	132.1019	132.09-132.11	¹³ C ₆ -lysine
Lysine	C ₆ H ₁₄ N ₂ O ₂	[M+H] ⁺	147.1128	147.11-147.13	¹³ C ₆ -lysine
¹³ C ₆ -lysine	¹³ C ₆ H ₁₄ N ₂ O ₂	[M+H] ⁺	153.1329	153.13-153.15	NA
Methionine	C ₅ H ₁₁ NO ₂ S	[M+H] ⁺	150.0583	150.05-150.07	¹³ C ₆ -lysine

Isoleucine	C ₆ H ₁₃ NO ₂	[M+H] ⁺	132.1019	132.09-132.11	¹³ C6-lysine
Histidine	C ₆ H ₉ N ₃ O ₂	[M+H] ⁺	156.0768	156.07-156.08	¹⁵ N4-arginine
Arginine	C ₆ H ₁₄ N ₄ O ₂	[M+H] ⁺	175.1190	175.11-175.13	¹⁵ N4-arginine
¹⁵ N4-arginine	C ₆ H ₁₄ ¹⁵ N ₄ O ₂	[M+H] ⁺	179.1071	179.10-179.12	NA
Phenylalanine	C ₉ H ₁₁ NO ₂	[M+H] ⁺	166.0863	166.07-166.09	¹⁵ N4-arginine
Tyrosine	C ₉ H ₁₁ NO ₃	[M+H] ⁺	182.0812	182.07-182.09	¹⁵ N4-arginine
Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	[M+H] ⁺	205.0972	205.09-205.11	¹⁵ N4-arginine

Table S2. The primers used in this study

Primer name	Sequence
P1	TTGTTTTACCTGCCCAAAT
P2	CACGGCGCGCCTAGCAGCGGGGTGGTTTTAGTGTTTGGTGA
P3	GTCAGCGGCCGCATCCTGCGTTGTGCAAGTACTAAATTCAATGG
P4	TTTCGTCAACCAACCAACAA
P5	CCGCTGCTAGGCGCGCCGTGAGCTCGGATCCACTAGTAACG
P6	GCAGGGATGCGGCCGCTGACGCCAGTGTGATGGATATCTGC

Table S3 Retention time, linearity of calibration and limits of detection and quantification determined by the established LC-TOF/MS method (1 μ L injection)

Compound	Retention time	Linearity			LOQ (ng mL ⁻¹)	LOD (ng mL ⁻¹)
		Regression equation	Range (μ g mL ⁻¹)	R ²		
Glycine	2.356	y=70.512x-0.1849	0.132-26.4	0.9994	75	20
Serine	2.606	y=43.628x-0.2825	0.10625-42.5	0.9978	30	9
Alanine	3.472	y=18.717x-0.0017	0.06575-26.3	0.9997	26	10
Hydroxyproline	4.618	y=6.5624x+0.128	0.02925-11.7	0.9990	29	8
Threonine	5.062	y=6486x+0.1833	0.06325-25.3	0.9996	42	12
Asparagine	6.954	y=11.741x+0.264	0.06275-25.1	0.9991	13	4
Proline	7.272	y=9.4874x+0.135	0.0555-22.2	0.9994	37	10
Aspartic acid	7.297	y=17.808x-1.4117	0.29125-116.5	0.9972	23	7
Glutamine	8.629	y=16.534x-0.2103	0.09325-37.3	0.9998	16	5
Ornithine	9.857	y=14.481x+0.4363	0.18325-73.3	0.9996	52	15
Glutamic acid	10.032	y=21.094x-0.6104	0.34825-69.65	0.9981	20	6
Valine	10.582	y=2.1155x+0.0224	0.21675-21.675	0.9993	173	50
Citrulline	12.628	y=6.515x+0.3297	0.2875-57.5	0.9992	15	4
Leucine	13.225	y=1.1891x-0.1812	0.14025-28.05	0.9972	15	5
Lysine	12.57	y=12.277x-0.4584	0.2075-83	0.9997	34	10
Methionine	14.125	y=3.114x-0.0371	0.02975-11.9	0.9993	6	2

Isoleucine	14.382	$y=1.1626x-0.0053$	0.14475-28.95	0.9993	19	5
Histidine	16.838	$y=23.752x-1.0172$	0.19925-79.7	0.9982	17	5
Arginine	18.815	$y=20.454x-0.3498$	0.129875-51.95	0.9998	17	5
Phenylalanine	23.387	$y=7.1124x-0.3228$	0.128-25.6	0.9986	11	3
Tyrosine	23.960	$y=15.11x-0.0781$	0.097-38.8	0.9996	13	4
Tryptophan	30.287	$y=27.753x+0.085$	0.065-13	0.9991	40	12

Table S4 Intra- and inter-day precisions of 22 amino acids standard solutions at three different concentration levels

Compound	$\mu\text{g mL}^{-1}$			Intra-day (n=3, RSD%)			Inter-day (n=3, RSD%)		
	high	middle	low	high	middle	low	high	middle	low
Glycine	13.20	2.640	0.6600	1.22	0.98	0.42	2.15	1.23	1.01
Serine	10.62	2.125	0.5312	0.68	0.06	0.30	0.55	1.33	0.89
Alanine	6.575	1.315	0.3287	2.06	0.09	0.28	1.77	1.28	0.67
Hydroxyproline	2.925	0.585	0.1462	0.29	0.04	0.10	0.83	0.56	0.69
Threonine	6.325	1.265	0.3162	2.72	0.24	0.34	0.24	1.57	1.66
Asparagine	6.275	1.255	0.3137	0.75	0.10	0.12	0.33	0.48	0.90
Proline	5.550	1.110	0.2775	0.85	0.10	0.07	0.98	1.45	2.23
Aspartic acid	29.12	5.825	1.456	0.38	0.23	0.66	0.56	1.67	1.18
Glutamine	9.321	1.865	0.4662	2.99	0.25	0.09	0.35	0.83	0.72
Ornithine	18.32	3.665	0.9162	1.47	0.15	0.24	2.97	0.45	0.67

Glutamic acid	34.82	6.965	1.741	3.15	0.40	2.32	1.63	2.09	0.45
Valine	21.67	4.335	1.084	0.93	1.83	2.72	0.15	0.50	0.78
Citrulline	28.75	5.750	1.437	2.19	0.32	0.89	1.40	0.29	0.22
Leucine	14.02	2.805	0.7012	2.59	1.62	0.91	1.12	0.26	0.69
Lysine	20.75	4.150	1.037	1.40	0.54	3.67	0.79	0.26	0.93
Methionine	2.975	0.595	0.1487	1.97	0.15	1.38	1.12	1.02	0.83
Isoleucine	14.47	2.895	0.7237	2.14	0.81	0.85	0.79	0.15	0.83
Histidine	19.92	3.985	0.9962	1.24	0.15	1.14	0.65	0.76	0.09
Arginine	12.99	2.595	0.6494	1.12	0.22	1.47	0.37	0.11	0.98
Phenylalanine	12.82	2.560	0.6400	1.21	0.05	1.36	0.95	0.59	0.08
Tyrosine	9.701	1.940	0.4850	0.98	0.10	1.18	0.89	1.32	1.11
Tryptophan	3.252	0.650	0.1625	0.40	0.11	0.22	0.52	0.43	0.34

Table S5 Recovery experiment of the analytical method for 22 amino acids

Compounds	Sample ($\mu\text{g mg}^{-1}$)	Spiked ($\mu\text{g mg}^{-1}$)	Sample+spiked ($\mu\text{g mg}^{-1}$)	Recovery(%)
Glycine	2.242	1.201	3.499	104.7
Serine	1.504	1.932	3.470	101.8
Alanine	22.25	1.195	23.50	104.6
Hydroxyproline	0.571	0.532	1.025	85.34
Threonine	1.780	1.150	2.916	98.78
Asparagine	2.670	1.141	3.787	97.90
Proline	4.635	1.010	5.587	94.26
Aspartic acid	1.602	5.295	7.351	108.6
Glutamine	14.52	3.390	18.17	107.7
Ornithine	4.228	3.332	7.330	93.10
Glutamic acid	48.77	6.332	55.30	103.1
Valine	1.502	3.941	5.082	90.84
Citrulline	1.321	5.227	5.856	86.76
Leucine	1.469	2.550	3.734	88.82
Lysine	11.63	3.503	15.26	103.6
Methionine	0.133	0.541	0.690	103.0
Isoleucine	2.555	2.632	5.111	97.11
Histidine	1.815	3.623	5.597	104.4
Arginine	2.132	4.723	6.591	94.41

Phenylalanine	0.121	2.327	2.678	109.9
Tyrosine	1.700	1.764	3.163	82.94
Tryptophan	0.558	0.590	1.082	88.81

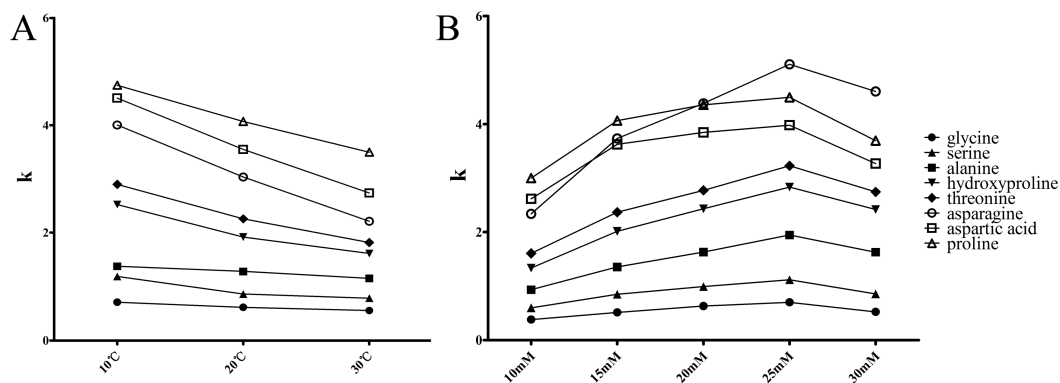


Fig. S1 (A) Influence of the column temperature using 15 mM NFA as IP buffer, and (B) the NFA concentration at 10 °C column temperature on the retention factor (k) of the first eight eluted underivatized amino acids.

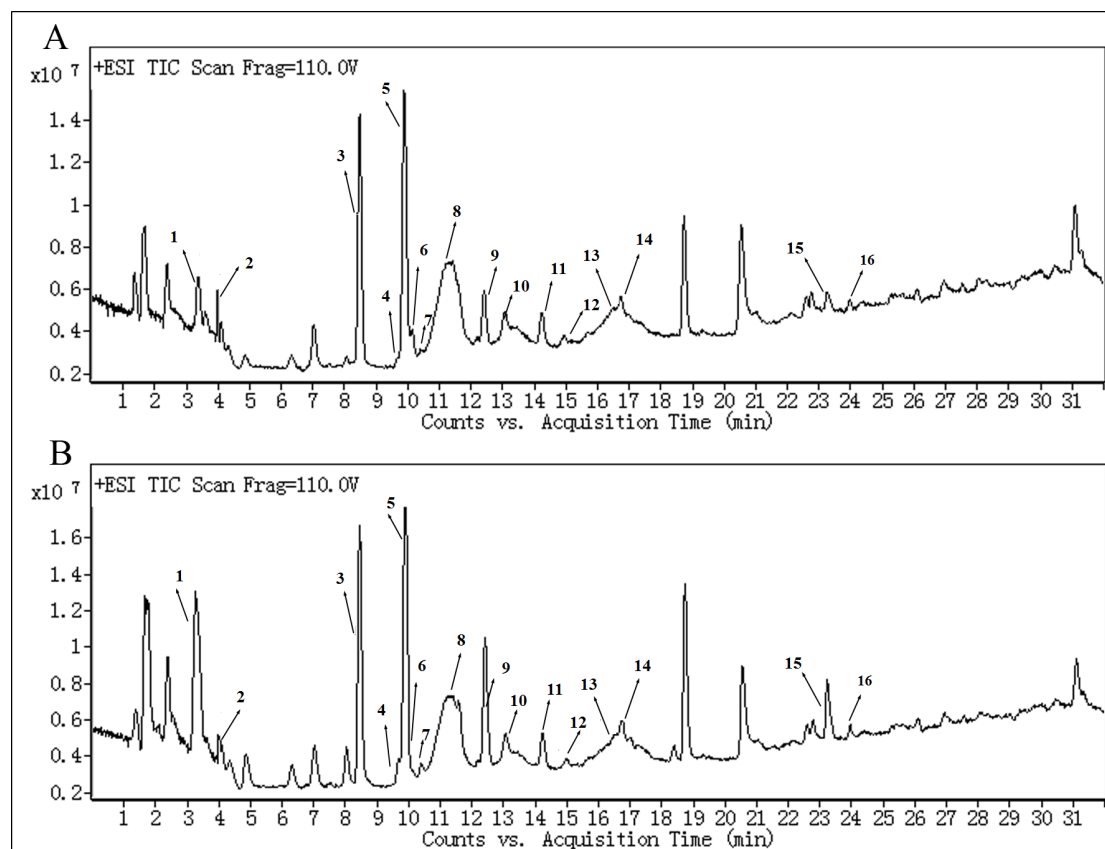


Fig. S2 Typical IP-PGC-TOF/MS total ion chromatograms (TIC) of *C. albicans* SC5314 (A) planktonic and (B) biofilm cells.

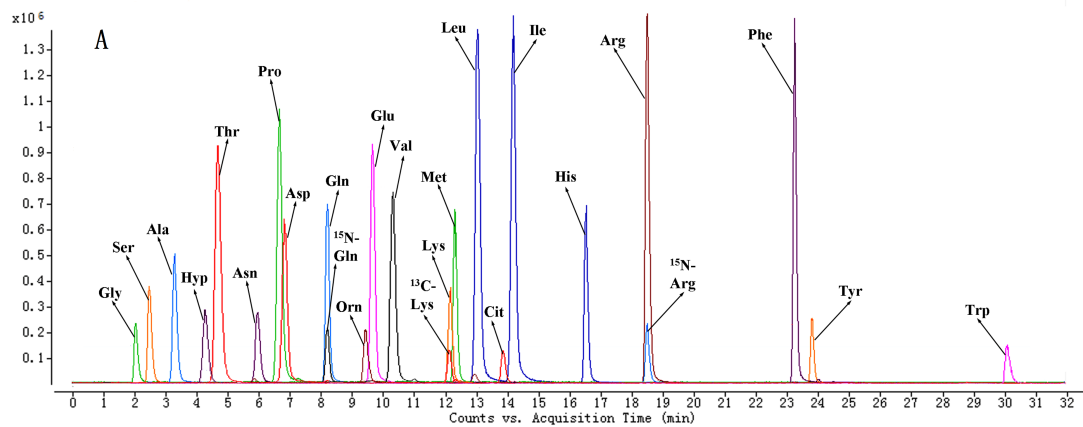


Fig. S3 Extracted ion chromatography (EIC) of 22 targeted amino acids and 3 internal standards.