Supporting Information

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Figure S2. The PCA score plots and Z-score heatmaps of clustering analysis of intra- and extra-cellular profiling of 40 amino acid and derivatives on cell lysates and cell culture media from the cell lines of normal and TNBC. Each square in the Z-score

heatmap represents the clustering value of a metabolite within the cohort. Red or green color indicates relatively up- or down-regulated expression of the metabolites in the samples between the two groups on the horizontal axis. A hierarchical classification of differential metabolites is shown on the vertical axis. **(a-b.)** Cell lysates; **(c-d.)** Cell culture media.

Table S1. Concentration of cell lysates and medium from normal breast cell of MCF10A, and concentration of blank medium before cell culture.

Analyte	Cell lysates of MCF10A (Normal) (mean ± SD, ng/mL) [a]	Medium from MCF10A (Normal) (mean ± SD, ng/mL)	Blank cell culture medium (mean ± SD, ng/mL)
N-acetyl-L-methionine	0.852±0.228	N.D.	N.D.
N-acetyl-L-tryptophan	N.D.	5.59±0.84	6.74±0.5
Beta-alanine	139±20.4	481±37	542±6.29
L-alanine	182±16.2	9808±240	8267±414
L-arginine	1000±294	162208±3022	178833±2898
L-asparagine	615±61.4	48833±719	51500±1887
L-aspartic acid	510±35.3	24908±739	22792±1561
Cyclo-leucine	BLOQ	N.D.	N.D.
L-cysteine	66.7±4.33	436±28.1	237±10.8
Glycine	713±84.5	18854±1897	18283±1891
Glycyl-glycine	1.76±0.278	15.3±0.68	15.6±0.57
Glycyl-L-proline	0.651±0	N.D.	N.D.
Glycyl-L-valine	N.D.	27.1±4.51	23.1±2.34
L-histidine	1297±82.6	16604±254	17258±213
L-isoleucine	396±70.7	51083±1021	54417±1258
L-lysine	397±73.2	37958±1018	39250±750
L-phenylalanine	272±31.7	19258±1102	20167±510
L-proline	159±18.2	21258±646	21408±528
L-pyroglutamic acid	378±87.8	67708±5243	41750±1750
L-sarcosine	1.47	N.D.	N.D.
L-serine	337±44.1	29667±1114	31750±1090
L-threonine	232±21	22413±382	23475±534
L-tryptophan	78.2±13.6	5204±372	5600±86.6
4-hydroxy-L-proline	308±33.4	22613±596	22675±195
L-tyrosine	87±15.4	19092±486	19517±984
Valine	89.7±15.6	22892±652	23850±331
L-citrulline	15.2±3.47	1880±157	1848±51.0
Glutathione	9500±224	N.D.	N.D.
Glycyl-L-leucine	0.762±0	7.27±0.6	4.42±0.41
N-phenylacetylglycine	11.5±3.26	1609±134	1543±85
N-acetyl-L-aspartyl-L-glutamic acid	3.08±2.42	N.D.	N.D.
N-acetyl-L-serine	16.4±2.73	66.8±10.5	71.8±4.13
N-alpha-acetyl-L-lysine	BLOQ	27.1±2.19	27.8±1.15
Cysteine-glutathione disulfide	67.5±12.1	4883±312	3700±363
Homocysteine	1.10±0.078	6.57±2.2	4.5±1.36

L-S-adenosyl-homocysteine	7.51±2.23	N.D.	N.D.
L-methionine	69.4±11.1	11229±232	12042±142
L-glutamine	2562±347	245375±6208	297500±4330
L-glutamic acid	3963±212	44292±749	35417±764
L-leucine	360±55.3	53542±1913	57083±764

[a] Concentration of cell lysates of MCF10A was cited from our previous methodology paper[1].

Table S2. The statistical analysis results of intra- and extra-cellular data among the three cell lines of TNBC, non-TNBC and normal breast epithelial cell.

Analyte	Cell (Intra-cellular) *p*-value [a]	Cell culture medium (Extra-cellular) *p*-value [a]
N-Acetylmethionine	0.001	-
N-Acetyltryptophan	-	-
β-Alanine	0.001	0.003
Alanine	0.003	-
Arginine	0.004	0.003
Asparagine	0.002	0.031
Aspartic aid	0.001	0.042
Cycloleucine	-	-
Cysteine	0.001	0.001
Glycine	0.003	-
Glycylglycine	-	0.003
Glycylproline	-	-
Glycylvaline	-	0.003
Histidine	0.000	0.014
Isoleucine	0.003	-
Lysine	0.001	0.002
Phenylalanine	0.001	0.039
Proline	0.001	-
Pyroglutamic acid	0.002	0.001
Sarcosine	-	-
Serine	0.001	0.001
Threonine	0.001	0.008
Tryptophan	0.001	-
trans-4-Hydroxyproline	0.002	-
Tyrosine	0.005	0.022
Valine	0.003	0.005
Citrulline	0.008	-
Glutathione	0.001	-
Glycylleucine	-	0.003
N-phenylacetylglycine	0.001	-
N-Acetyl-Asp-Glu	0.001	-
N-Acetylserine	0.001	-
N-Acetyllysine	-	-
L-cysteine-glutathione	0.001	0.001
Homocysteine	-	-
S-Adenosylhomocysteine	0.004	0.005

Methionine	0.002	0.032
Glutamine	0.001	0.001
Glutamic acid	0.001	0.001
Leucine	0.001	-

[a] *p*-value obtained by Kruskal-Wallis test.

Table S3. The statistical analysis results of intra- and extra-cellular data between the non-TNBC and normal breast cells.

Analyte	Cell lysates of HCC 202 cell (Non-TNBC) vs. MCF10A cell (Normal)			Cell culture medium from HCC 202 (Non-TNBC) vs. MCF10A (Normal)		
	p-value [a]	VIP value	Fold change	*p*-value [a]	VIP value	Fold change
N-Acetylmethionine	0.028	-	-	-	-	-
N-Acetyltryptophan	-	-	-	0.041	-	0.12
β-Alanine	0.002	1.1	0.17	0.026	1.1	0.26
Alanine	0.002	1.1	1.61	-	-	-
Arginine	0.004	-	1.95	0.002	1.4	0.49
Asparagine	0.002	1.2	2.05	0.041	1.2	0.19
Aspartic aid	0.005	1.1	0.63	-	-	-
Cycloleucine	-	-	-	-	-	-
Cysteine	0.002	1.1	0.68	0.002	1.6	0.19
Glycine	0.005	1.2	2.26	-	-	-
Glycylglycine	-	-	-	0.002	1.5	-15.08
Glycylproline	-	-	-	-	-	-
Glycylvaline	-	-	-	0.002	1.5	-3.09
Histidine	0.005	1.1	1.41	-	-	-
Isoleucine	0.002	1.1	1.73	0.041	-	0.46
Lysine	0.002	-	1.69	-	-	-
Phenylalanine	0.002	1.1	1.80	-	-	-
Proline	0.005	1.1	1.47	-	-	-
Pyroglutamic acid	0.002	1.1	2.33	0.026	1.4	0.63
Sarcosine	-	-	-	-	-	-
Serine	0.002	1.2	2.44	0.026	1.2	-0.06
Threonine	0.005	1.2	2.29	-	-	-
Tryptophan	0.002	1.1	1.89	-	-	-
trans-4-Hydroxyproline	0.002	1.1	1.79	-	-	-
Tyrosine	0.008	-	1.51	-	-	-
Valine	0.005	1.1	1.69	-	-	-
Citrulline	0.009	-	1.51	-	-	-
Glutathione	0.002	1.2	0.48	-	-	-
Glycylleucine	-	-	-	0.002	1.6	-0.23
N-phenylacetylglycine	0.002	1.1	2.65	-	-	-
N-Acetyl-Asp-Glu	-	-	-	-	-	-
N-Acetylserine	0.015	-	0.79	-	-	-
N-Acetyllysine	-	-	-	-	-	-
L-cysteine-glutathione	0.003	-	-	0.002	1.6	-2.50
Homocysteine	-	-	-	-	-	-
S-Adenosylhomocysteine	0.008	-	0.40	-	-	-

Methionine	0.002	1.1	1.76	0.015	1.1	0.49
Glutamine	0.002	1.2	2.49	0.002	1.5	0.41
Glutamic acid	0.002	1.2	0.28	0.002	1.6	0.21
Leucine	0.005	1.1	1.74	-	-	-

[a] *p*-value obtained by Mann-Whitney U test.

Table S4. The statistical analysis results of intra- and extra-cellular data between the TNBC and normal breast cells.

Analyte	Cell lysates of HCC1143 cell (TNBC) vs. MCF10A cell (Normal)			□	□Cell culture medium from HCC1143 cell (TNBC) vs. MCF10A cell (Normal)		
	p-value [a]	VIP value	Fold change		*p*-value [a]	VIP value	Fold change
N-Acetylmethionine	0.005	1.2	2.46		-	-	-
N-Acetyltryptophan	-	-	-		-	-	-
β-Alanine	0.002	1.2	3.04		0.041	1.1	1.89
Alanine	0.002	1.2	1.64		-	-	-
Arginine	0.004	-	1.68		0.015	1.1	0.71
Asparagine	0.005	1.2	1.87		-	-	-
Aspartic aid	0.005	1.2	2.77		0.026	-	0.43
Cycloleucine	-	-	-		-	-	-
Cysteine	0.002	1.2	0.45		0.002	1.5	0.41
Glycine	0.005	1.2	2.27		-	-	-
Glycylglycine	0.015	-	1.36		-	-	-
Glycylproline	-	-	-		-	-	-
Glycylvaline	-	-	-		0.002	1.5	-3.24
Histidine	0.005	-	1.20		0.016	1.2	1.85
Isoleucine	0.002	1.1	1.63		-	-	-
Lysine	0.015	-	1.32		0.002	1.3	2.42
Phenylalanine	0.008	1.1	1.45		0.093	-	2.09
Proline	0.005	1.2	1.91		-	-	-
Pyroglutamic acid	-	-	-		0.002	1.4	0.46
Sarcosine	-	-	-		-	-	-
Serine	0.002	1.2	1.88		0.004	1.3	2.11
Threonine	0.005	1.1	1.74		0.002	1.3	2.64
Tryptophan	0.004	-	1.42		-	-	-
trans-4-Hydroxyproline	0.002	1.2	1.65		-	-	-
Tyrosine	0.002	1.1	1.51		0.026	1.2	3.05
Valine	0.004	-	1.58		0.009	1.2	2.19
Citrulline	0.008	-	1.54		-	-	-
Glutathione	0.005	1.2	1.70		-	-	-
Glycylleucine	-	-	-		0.002	1.6	-0.23
N-phenylacetylglycine	0.002	1.1	2.04		-	-	-
N-Acetyl-Asp-Glu	0.002	1.1	5.47		-	-	-
N-Acetylserine	0.002	1.1	1.92		-	-	-
N-Acetyllysine	-	-	-		-	-	-

L-cysteine-glutathione	0.041	-	0.25	0.002	1.5	2.57
Homocysteine	-	-	-	-	-	-
S-Adenosylhomocysteine	-	-	-	0.007	1.5	-
Methionine	0.002	1.1	1.57	-	-	-
Glutamine	0.002	1.1	1.86	0.041	-	1.16
Glutamic acid	0.002	1.2	2.35	0.002	1.5	1.53
Leucine	0.004	1.1	1.56	-	-	-

[a] *p*-value obtained by Mann-Whitney U test.

Table S5. Parameters of PCA and OPLS-DA models among the three cell lines.

	PCA		OPLS-DA		
	R2X (P1)	R2X (P2)	R2X	R2Y	Q2
Cell					
Normal, Non-TNBC and TNBC	0.568	0.236			
Non-TNBC vs. Normal	0.732	0.079	0.71	0.965	0.952
TNBC vs. Normal	0.757	0.085	0.69	0.915	0.895
TNBC vs. Non-TNBC	0.589	0.219	0.51	0.906	0.887
Cell culture medium					
Normal, Non-TNBC and TNBC	0.345	0.199			
Non-TNBC vs. Normal	0.406	0.155	0.372	0.931	0.873
TNBC vs. Normal	0.401	0.191	0.381	0.961	0.918
TNBC vs. Non-TNBC	0.46	0.119	0.415	0.919	0.849

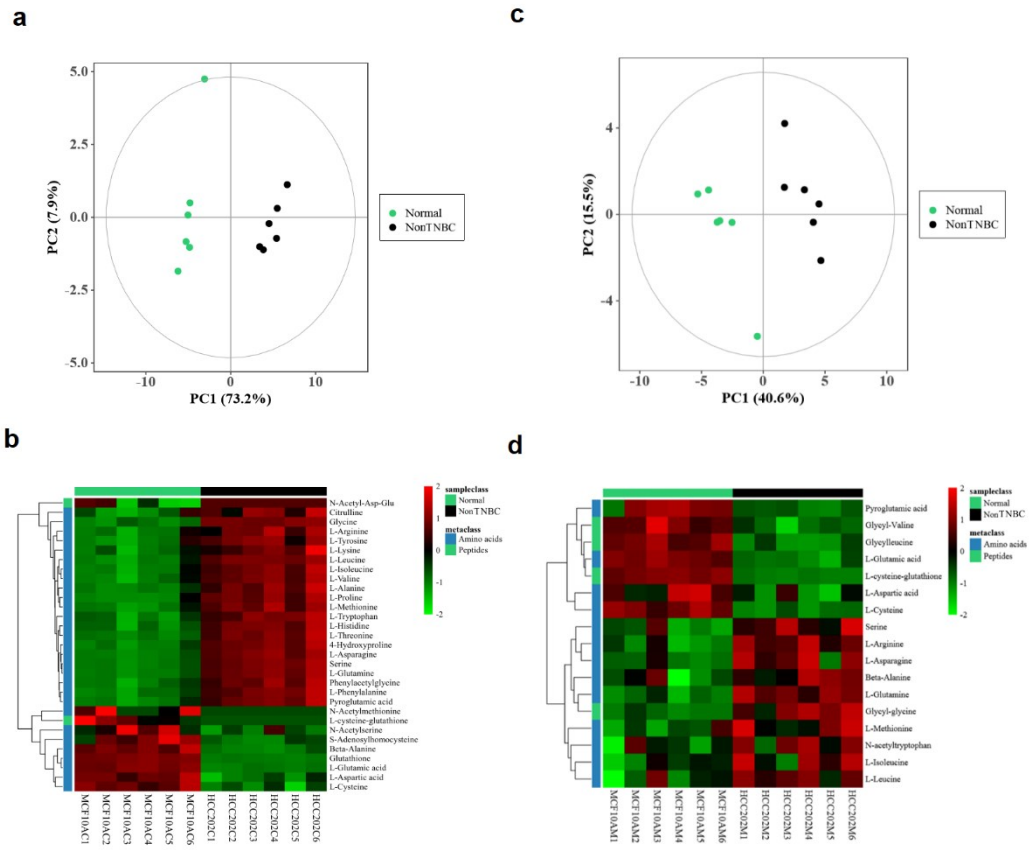

Figure S1. The PCA score plots and Z-score heatmaps of clustering analysis of intra- and extra-cellular profiling of 40 amino acid and derivatives on cell lysates and cell culture media from the cell lines of normal and non-TNBC. Each square in the Z-score heatmap represents the clustering value of a metabolite within the cohort. Red or green color indicates relatively up- or down-regulated expression of the metabolites in the samples between the two groups on the horizontal axis. A hierarchical classification of differential metabolites is shown on the vertical axis. **(a-b.)** Cell lysates; **(c-d.)** Cell culture media.

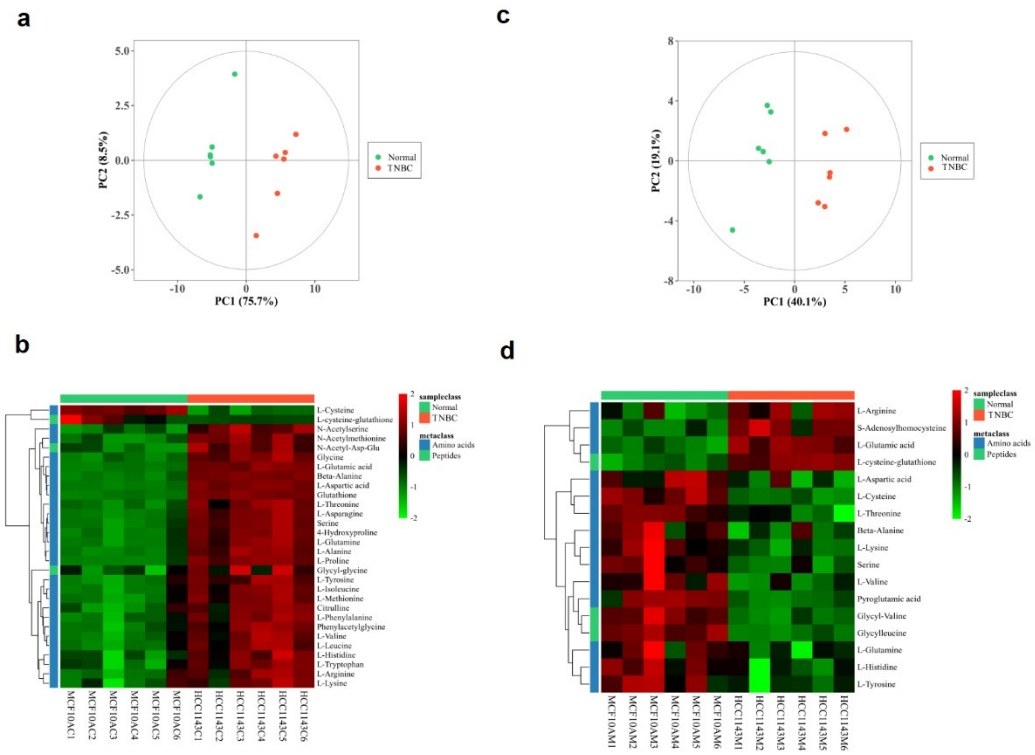

Figure S2. The PCA score plots and Z-score heatmaps of clustering analysis of intra- and extra-cellular profiling of 40 amino acid and derivatives on cell lysates and cell culture media from the cell lines of normal and TNBC. Each square in the Z-score heatmap represents the clustering value of a metabolite within the cohort. Red or green color indicates relatively up- or down-regulated expression of the metabolites in the samples between the two groups on the horizontal axis. A hierarchical classification of differential metabolites is shown on the vertical axis. **(a-b.)** Cell lysates; **(c-d.)** Cell culture media.

Reference

[1] Zhu, B. et al. (2020). A simultaneously quantitative profiling method for 40 endogenous amino acids and derivatives in cell lines using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry. Talanta 207, 120256.