Gut microbiota dysbiosis is associated with altered tryptophan metabolism and dysregulated inflammatory response in severe COVID-19

Supplemental Information

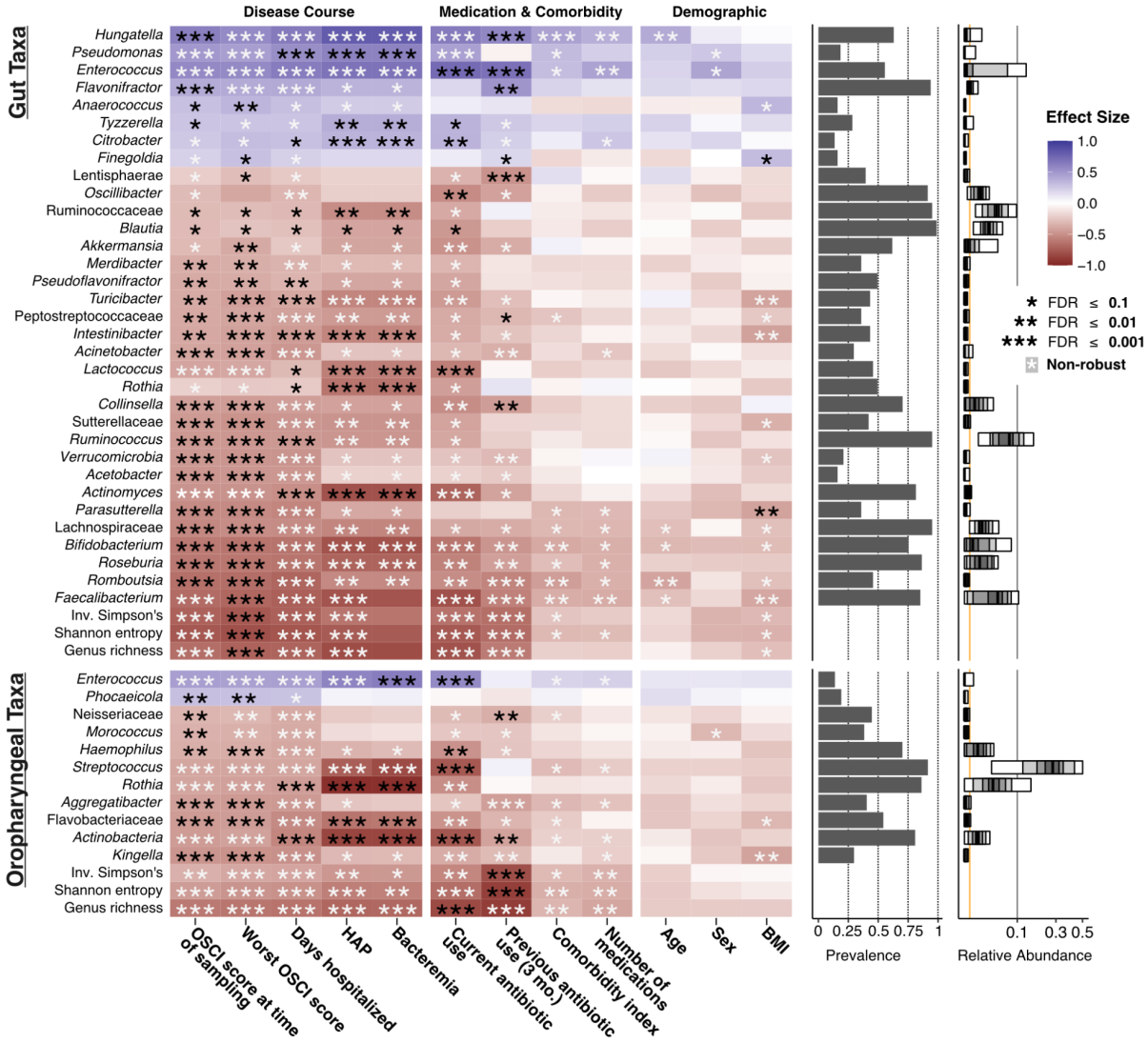

Figure S1: Severity-associated microbiota in hospitalized COVID-19 patients and controls, related to Figure 2
To correlate bacterial taxa with clinical variables (see Table S1 for full list), standardized, non-parametric effect sizes were calculated and tested for significance (shown in the heatmap, either Spearman for continuous variables or Cliff's delta/MWU for binary variables). Summary alpha diversity metrics and (naïve) severity-associated taxa of the gut and oropharynx are depicted. In a second step, feature-wise iterative models were built and integrated to determine the robustness of clinical associations, including disease severity, and identify potential confounders (see Methods). If a naïve association retained significance across all possible covariate models, its significance is denoted in black in the heatmap and it may be considered robust. White stars denote instances in which naive associations were reducible to one or more other covariates and therefore are not considered robust. Multiple columns of robust associations for a given feature indicate variables which captured similar variation, e.g. hospitalization-associated variables (length of stay, HAP, bacteremia). Taxa prevalences and square root-transformed relative abundance quantile plots are shown on the right; the yellow line indicates a relative abundance of 0.001, which was used as a cutoff to determine a robust OSCI-associated subset of microbiota features for later integration with host metabolites and immune response features. See also main text Fig. 1. HAP: hospital-acquired pneumonia; OSCI: ordinal scale for clinical improvement

Figure S2: Dominant respiratory taxa in hospital-acquired pneumonia (HAP) patients, related to Figure 2
A) Oropharyngeal samples from the five patients with nosocomial pneumonia infections; all were ventilated except for P03. **B)** Tracheobronchial sputum samples obtained from ventilated patients.

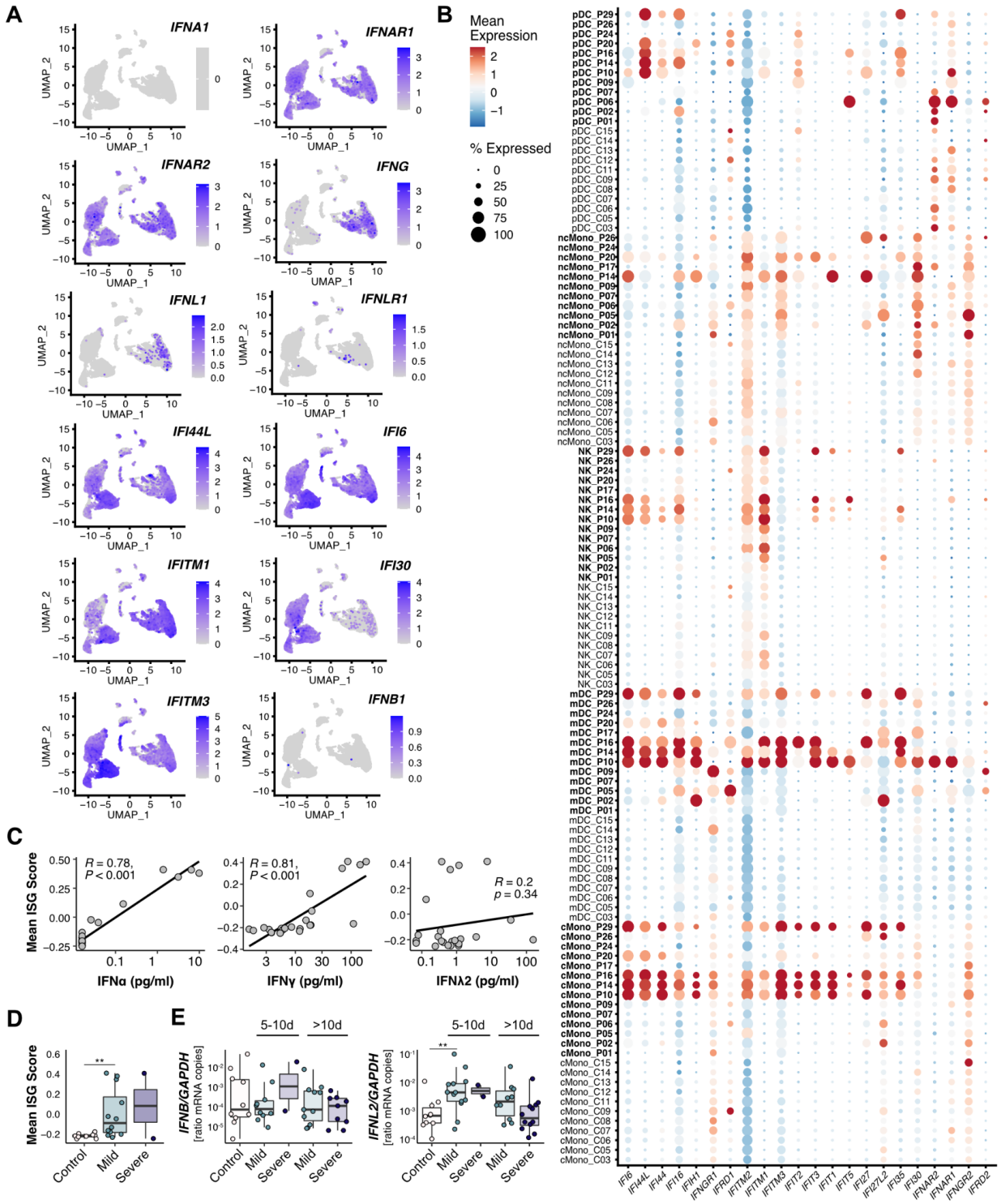

Figure S3: IFN response in COVID-19 patients, related to Figure 3

A) Expression of IFN-related genes projected onto the UMAP of cell (sub)types of PBMCs identified by scRNAseq from 14 COVID-19 patients and 11 controls. **B)** Dot plots indicating expression of IFN-related genes in different cell types of PBMCs from patients and controls. **C)** Linear regression analysis of the ISG score (as shown in D) versus levels of type I, II, and III IFN levels in plasma. **D)** ISG score (calculated by applying Seurat function *AddModuleScore* to IFN-stimulated genes) in PBMCs from controls and patients with mild and severe COVID-19. **E)** Relative oropharyngeal expression of type I and III IFNs in controls and patients with mild and severe COVID-19 at the early (<10 d after symptom onset) and late (>10 d after symptom onset) phase of infection. See also main text Fig. 3.

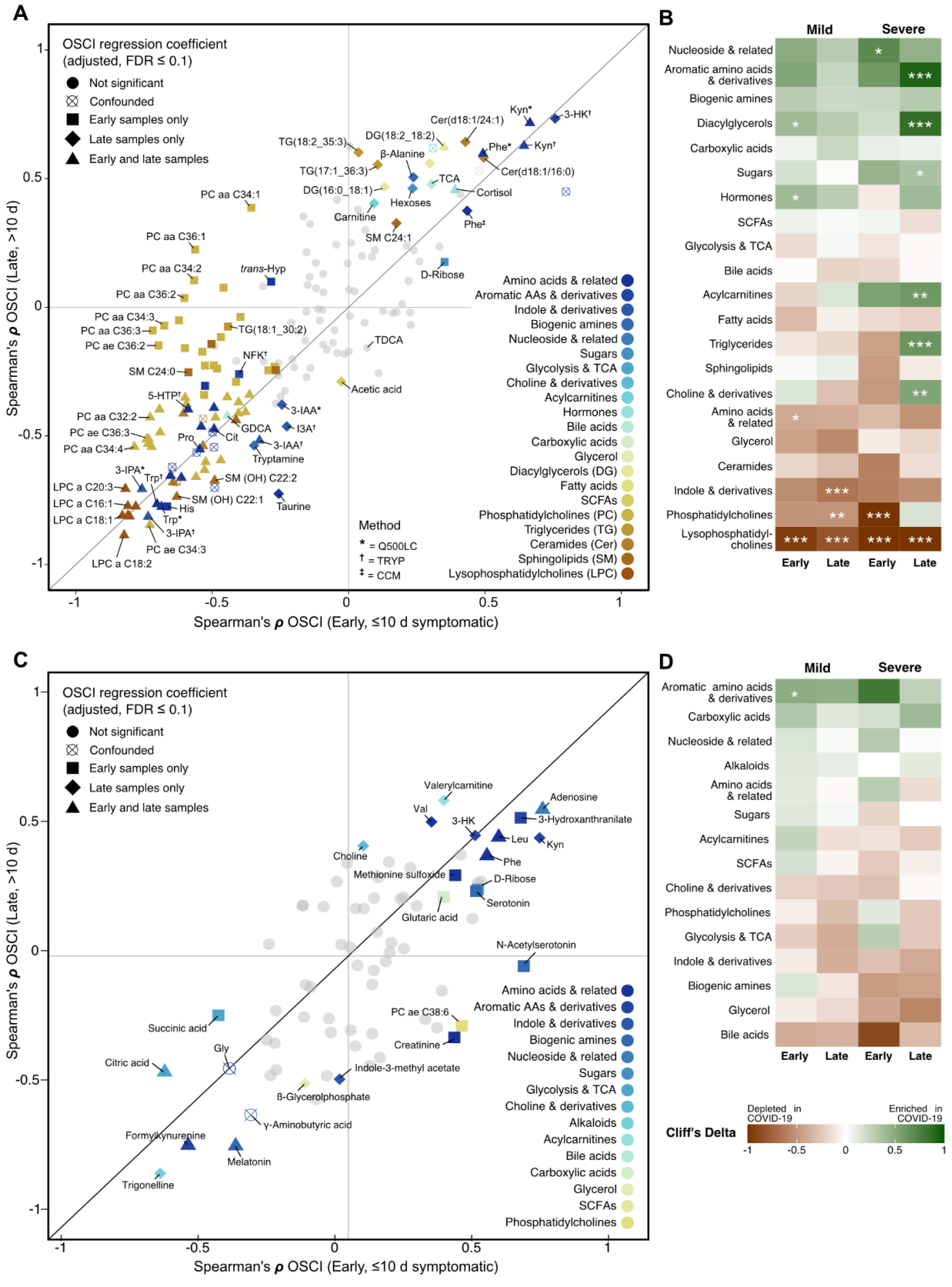
A
OSCI regression coefficient
(adjusted, FDR ≤ 0.1)
Not significant
Confounded
Early samples only
Late samples only
Early and late samples
Spearman's ρ OSCI (Late, >10 d)
Spearman's ρ OSCI (Early, ≤10 d symptomatic)
Method
* = Q500LC
† = TRYP
‡ = CCM
Amino acids & related
Aromatic AAs & derivatives
Indole & derivatives
Biogenic amines
Nucleoside & related
Sugars
Glycolysis & TCA
Choline & derivatives
Acylcarnitines
Hormones
Bile acids
Carboxylic acids
Glycerol
Diacylglycerols (DG)
Fatty acids
SCFAs
Phosphatidylcholines (PC)
Triglycerides (TG)
Ceramides (Cer)
Sphingolipids (SM)
Lysophosphatidylcholines (LPC)
B
Mild
Severe
Early
Late
C
D
Cliff's Delta
Depleted in COVID-19
Enriched in COVID-19

Figure S4: Metabolite associations with disease severity at both early and late timepoints, related to Figure 4
A) Spearman correlations between plasma metabolite concentrations and OSCI scores at the time of sampling were calculated using either early (N=26) or late (N=40) subsets of COVID-19 patient samples and healthy controls (N=30), and are shown contrasted with one another. Metabolites are annotated to show their biochemical class and analytical method. Shapes were assigned according to the integrated output from our modeling with clinical variables in each subset (early and late) separately, after adjusting for case-control disease status. Compounds closer to the diagonal line displayed consistent severity correlations regardless of sample timepoint, while levels of several phosphatidylcholines were negatively correlated with disease severity in early samples only. **B)** Cliff's delta effect sizes for different subsets of COVID-19 patients compared to controls for each chemical class. Adjusted significances from Mann-Whitney U tests which were not confounded from our modeling analysis with clinical covariates are depicted (FDR<0.1=*, FDR<0.01=**, FDR<0.001=***). **C-D)** The same as in A) and B) except for urine metabolites. See also main text Fig. 4. Phe: phenylalanine, Pro: proline, Cit: citrullin, *trans*-Hyp: *trans*-4-hydroxyproline, Trp: tryptophan, Kyn: kynurenine, 5-HTP: 5-hydroxytryptophan, 3-HK: 3-hydroxykynurenine, NFK: N-Formylkynurenine, TDCA: taurodeoxycholic acid, GDCA: glycodeoxycholic acid, TCA: taurocholic acid, 3-IPA: indole-3-propionic acid, 3-IAA: indole-3-acetic acid, I3A: indole-3-carboxaldehyde; HAP: hospital-acquired pneumonia

The following tables will be uploaded separately for publication but are currently available at www.github.com/sxmorgan/pa-covid-multi-omics.

Table S1: Cohort characteristics, related to Table 1

Table S2: Clinical lab parameters, related to Figure 1

Table S3: Taxonomic profiles, related to Figures 2, S2

Table S4: Immune response profiles, related to Figures 3, S3

Table S5: Metabolite profiles, related to Figures 4, S4

Table S6: Results from statistical analysis with clinical variables, related to Figures 2-4, S2-S4

Table S7: Integrated statistical analysis results, related to Figure 5

Table S8: Quality Control reporting of metabolomics data according to mQACC standards

Table S9: Ultra-high performance liquid chromatography (UHPLC) gradient for LC part

	LC1				LC2			
No	Time [min]	Flow [mL/min]	A [%]	B [%]	Time [min]	Flow [mL/min]	A [%]	B [%]
1	0.00	0.8	100	0	0.00	0.8	100	0
2	0.25	0.8	100	0	0.25	0.8	100	0
3	1.50	0.8	88	12	0.50	0.8	75	25
4	2.70	0.8	82.5	17.5	2.00	0.8	50	50
5	4.00	0.8	50	50	3.00	0.8	25	75
6	4.50	0.8	0	100	3.50	0.8	0	100
7	4.70	1.0	0	100	4.70	1.0	0	100
8	5.00	1.0	0	100	5.00	1.0	0	100
9	5.10	1.0	100	0	5.10	1.0	100	0
10	5.80	1.0	100	0	5.80	1.0	100	0

Table S10: Ultra-high performance liquid chromatography (UHPLC) gradient for flow injection (FIA) part

No	Time [min]	Flow [mL/min]	A [%]	B [%]
1	0.0	0.03	0	100
2	1.6	0.03	0	100
3	2.4	0.20	0	100
4	2.8	0.20	0	100
5	3.0	0.03	0	100

Table S11: Acquisition method parameters for liquid chromatography (LC) and flow injection analysis (FIA)

Option	Parameter	LC1	LC2	FIA1	FIA2
MS	Scan type	MRM	MRM	MRM	MRM
	Polarity	Positive	Negative	Positive	Negative
	MRM detection window (sec)	30	30	-	-
	Duration (min)	5.45	5.45	2.95	2.95
	Delay time (sec)	0	0	0	0
	Cycle (sec)	0.25	0.15	N/A	N/A
Advanced MS	Resolution Q1	Unit	Unit	Unit	Unit
	Resolution Q3	Unit	Unit	Unit	Unit
	Intensity threshold	0	0	0	0
	Setting time (ms)	0	0	0	0
	Pause between mass ranges (ms)	2	2	5.007	3
Source/ gas	Curtain gas	45	20	20	10
	Collision gas	9	8	9	9
	Ion spray voltage	5500	-4500	5500	5500
	Temperature	500	650	200	350
	Ion source gas 1	60	40	40	30
	Ion source gas 2	70	40	50	85

MS: mass spectrometry

Table S12: List of metabolite derivatives and their biological groups used for reference search

Group	Metabolite	Abbreviation	Detected as
AA	Alanine	Ala	3TMS
			2TMS
AA	Asparagine	Asn	2TMS
AA	Aspartic acid	Asp	2TMS
			3TMS
AA	Cysteine	Cys	3TMS
AA	Glycine	Gly	2TMS
			3TMS
AA	Isoleucine	Ile	1TMS
			2TMS
AA	Leucine	Leu	1TMS
			2TMS
AA	Lysine	Lys	3TMS
AA	Methionine	Met	1TMS
			2TMS
AA	Phenylalanine	Phe	1TMS
			2TMS
AA	Proline	Pro	1TMS
			2TMS
AA	Serine	Ser	2TMS
			3TMS
			4TMS
AA	Threonine	Thr	2TMS
			3TMS
AA	Tryptophan	Trp	2TMS
AA	Tyrosine	Tyr	3TMS
AA	Valine	Val	1TMS
			2TMS
Glycerol	Dihydroxyacetone phosphate	DHAP	1MeOX 3TMS
Glycerol	Glycerol	Glyc	3TMS
Glycerol	Glycerol-3-phosphate	Glyc3P	4TMS
Glycolysis	Fructose-6-phosphate	F6P	1MeOX 6TMS

Glycolysis	Glucose-6-phosphate	G6P	1MeOX 6TMS
Glycolysis	Glyceric acid-3-phosphate	GA3P	4TMS
Glycolysis	Lactic acid	Lac	2TMS
Glycolysis	Phosphoenolpyruvic acid	PEP	3TMS
Glycolysis	Pyruvic acid	Pyr	1MeOX 1TMS
Nucleobase	Adenine	Adenine	2TMS
Nucleobase	Uracil	Uracil	2TMS
Nucleoside	Adenosine	Adenosine	3TMS
			4TMS
Nucleoside	Cytosine	Cytosine	2TMS
Others	Butanoic acid, 3-hydroxy-	But3h	2TMS
Others	Butanoic acid, 4-amino-	But4am	3TMS
Others	Erythritol	Ery	4TMS
Others	Glutaric acid	Glut	2TMS
Others	Glyceric acid	Glyc	3TMS
PPP	Ribose-5-phosphate	R5P	1MeOX 5TMS
PPP	Ribose	Ribose	1MeOX 4TMS
TCA	Citric acid	Cit	4TMS
TCA	Fumaric acid	Fum	2TMS
TCA	Glutaric acid, 2-hydroxy-	2HG	3TMS
TCA	Glutaric acid, 2-oxo-	aKG	1MeOX 2TMS
TCA	Malic acid	Mal	3TMS
TCA	Succinic acid	Suc	2TMS

AA: Amino acids. PPP: Pentose phosphate pathway. TCA: Tricarboxylic acid cycle. TMS: Trimethylsilyl derivatives. MeOX: Methoxyamine hydrochloride