

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

The raw proteomics data from all DIA methods was processed using DIA-NN, 1.8.1 obtained from GitHub, detailed pipeline and raw data were provided in Mendeley
Zeno MRM-HR data were processed using Skyline (64-bit, v.23.1.0.268), output are provided in supplementary table

Data analysis

Wilcoxon signed-rank test

To assess whether the relative abundance rankings of peptides differ across blood matrices, we performed pairwise Wilcoxon signed-rank tests for each peptide. For each comparison, peptide intensity values from matched samples (same donor measured across matrices) were tested using the `wilcox.test()` function in R (v4.2.2) with `paired = TRUE`. All six pairwise matrix combinations were tested independently. To account for multiple hypothesis testing, p-values were adjusted using the Benjamini-Hochberg (BH) procedure with the `p.adjust(method = "BH")` function. The resulting adjusted p-values were compiled into a long-format data table with peptide identifiers and matrix comparison labels. Peptides showing significant rank shifts (adjusted $p < 0.05$) were flagged for further interpretation.

coefficient of variation (CV)

To assess intra-group variability of peptide precursors, we calculated the coefficient of variation (CV) using two methods. The primary method employed a robust measure, defined as the median absolute deviation (MAD) divided by the median of intensity values, expressed as a percentage ($CV = MAD / Median \times 100$). This approach was implemented using the `mad()` function from the R stats package (v4.2.2), offering robustness to outliers and suitability for skewed or heteroscedastic data commonly observed in proteomics. For comparison, we also computed CV using the conventional formula based on standard deviation divided by the mean ($CV = SD / Mean \times 100$). Both measures are reported where relevant to provide complementary insights into variability.

Kendall's tau (KT) trend test

Significance testing of the trend between peptide quantities (normalized endogenous quantity or ratio) and the ordinal classification as provided by the WHO disease severity (levels as indicated) was performed using Kendall's tau (KT) statistics as implemented in the "EnvStats v2.8.1" R package "kendallTrendTest" function. For the clinical cohort, the KT statistics were calculated as the trend of absolute peptide concentrations against the following WHO groups: 0, 3, 4, 5, 6, and 7; selected peptides in each comparison were used for data analysis, without imputation. Where indicated, multiple testing correction was performed by controlling for false discovery rate using the Benjamini-Hochberg procedure 1 as provided by the R package "stats v4.2.2" - "p.adjust" function. A full summary of these statistical test results is provided in the respective supplementary tables. (Adjusted) p-values were considered significant when $p < 0.05$.

Ordinal logistic regression

Significance testing of the association between absolute peptide concentrations and clinical disease severity was performed using ordinal logistic regression, as implemented in the MASS v7.3-61 R package via the polr (proportional odds logistic regression) function. For clinical cohort analysis, disease severity was modeled as an ordered categorical variable, based on WHO clinical classification grouped into four categories: healthy, mild, moderate, and critical. These categories were derived from WHO levels as follows: healthy (WHO 0), mild (WHO 3), moderate (WHO 4–5), and critical (WHO 6–7). The model estimated the association between absolute peptide concentrations and increasing disease severity under the proportional odds assumption. No imputation was applied; only peptides with observed intensity values were included. Where applicable, p-values were adjusted for multiple testing using the Benjamini-Hochberg procedure to control the false discovery rate, as implemented in the stats v4.2.2 R package via the p.adjust function. A full summary of model coefficients and statistical test results is provided in the respective Supplementary Tables. Adjusted p-values were considered significant when $p < 0.05$.

Variance Partitioning Analysis

To assess the contribution of technical and biological factors to peptide-level variability, we used the variancePartition R package. A linear mixed-effects model was applied with MS platform (MS) and disease severity (Severity) as random effects, using the formula:

expression $\sim (1 | MS) + (1 | Severity)$

Missing values are imputed with zero. The model was fitted using fitExtractVarPartModel() from the variancePartition v1.36.2 R package, and the proportion of variance explained by each factor was computed for each peptide. The results were summarized to quantify the overall impact of technical and biological variance in the dataset.

Bland-Altman Analysis

To assess the agreement between different mass spectrometry acquisition methods, we performed Bland-Altman analyses at the peptide level (using ratios in two datasets). For each experimental condition, peptides were filtered to exclude infinite values and missing data. For every peptide detected under each condition, the mean and difference of the ratios from two datasets (e.g., hZsSWATH dataset and the composite dataset (not including any data from hZsSWATH dataset to avoid data leakage)) were calculated. The bias (mean difference), standard deviation (SD), and limits of agreement (LoA, calculated as $\text{bias} \pm 1.96 \times \text{SD}$) were determined. Confidence intervals (95%) for the bias and LoA were estimated using Student's t-distribution and the standard errors derived from the sample variance and size. Bland-Altman plots were generated for each peptide, visualizing the agreement between methods along with the calculated LoA and confidence intervals. The corresponding summary statistics were compiled into Supplementary table 7. All calculations and visualizations were performed in R using custom scripts based on the tidyverse (v 2.0.0) package for data handling and base R graphics functions for plotting.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw proteomics data generated in this study have been deposited in ProteomeXchange with identifier PXD070765 (<https://www.ebi.ac.uk/pride/archive/projects/PXD070765>). The processed proteomics data are available at Mendeley - Proteomics Data (DIA-NN & skyline). The data generated in this study are provided in the Supplementary Data.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

No sex or gender specific analyses were performed as part of this technical validation study.

Reporting on race, ethnicity, or other socially relevant groupings

The cohort has been described before (Wang et al 2022, <https://doi.org/10.1016/j.eclinm.2022.101495>). This analysis includes no data on race, ethnicity, or other socially relevant groupings.

Population characteristics

The cohort has been described before The cohort has been described before (Wang et al 2022, <https://doi.org/10.1016/j.eclinm.2022.101495>). In brief, the cohort comprised 10/30 (33%) women and the overall median age was 62 years (IQR: 50-37).

Recruitment

Patients were recruited within the Pa-COVID-19 study conducted at Charité - Universitätsmedizin Berlin, a prospective observational cohort study on the pathophysiology of COVID-19. All patients with PCR-confirmed SARS-CoV-2 infection were eligible for inclusion. Refusal to provide informed consent by the patient or a legal representative and any condition prohibiting supplemental blood collection for serial biosampling were exclusion criteria. Patients were treated according to current national and international guidelines. The cohort has been described before (Wang et al 2022, <https://doi.org/10.1016/j.eclinm.2022.101495>).

Ethics oversight

The COVID-19 cohort is a subcohort of the Pa-COVID-19 study conducted at Charité - Universitätsmedizin Berlin, Germany, and the matrix test cohort is part of prospective observational cohort study. Both studies were carried out in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice (ICH 1996), where applicable, and were approved by the ethics committee of Charité – Universitätsmedizin Berlin (EA2/066/20, EA4/245/20). Written informed consent was obtained from all participants or their legal guardians before initiation of study procedures. All data have been de-identified and presented in aggregate, with age reported as ranges to prevent identification, while maintaining sex/gender reporting in accordance with journal policy.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	45 human samples were used for the study
Data exclusions	The completeness of data for each peptide was evaluated based on its frequency of detection across all biological samples. Peptides were considered if they were detected in more than 66.7% (%) of the samples. We calculated the percentage of each peptide measured on each LC-MS platform/method and exclude the peptides with a completeness value less than 66.7% for subsequent analysis.
Replication	plasma samples were measured one time on each platform, quality control samples (pool of all studies) were measured 3 times on each platform. for calibration curves to check LOQs, each calibration curves were measured 3 times on each platform
Randomization	the samples were analysed in a fully randomised way on each LC-MS platform
Blinding	blinding is applied during data acquisition, process and pre-processing of data (data exclusion and batch correction)

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.