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BRIEF COMMUNICATION

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MassIVE.quant: a community resource of quantitative mass spectrometry–based proteomics datasets

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MassIVE.quant is a repository infrastructure and data resource for reproducible quantitative mass spectrometry–based proteomics, which is compatible with all mass spectrometry data acquisition types and computational analysis tools. A branch structure enables MassIVE.quant to systematically store raw experimental data, metadata of the experimental design, scripts of the quantitative analysis workflow, intermediate input and output files, as well as alternative reanalyses of the same dataset.

Quantitative mass spectrometry data analysis currently has multiple unmet reproducibility goals[1]. At the minimum, the mass spectrometry–based workflows must provide enough information to enable its full independent replication[2]. Beyond that, conclusions of data analysis should not be dependent on particular tuning parameters or software tools. Data analysis should demonstrate that alternative and equally appropriate parameter settings or software lead to qualitatively similar conclusions.

In mass spectrometry–based proteomics, data analysis is broadly categorized into peptide ion identification and quantification. Much progress in identification has been made in terms of open availability of tools and transparency of their algorithms. Archival resources MassIVE, PRIDE[3], Panorama[4,5], the PASSEL[6] component of Peptide Atlas, and jPOST in ProteomeXchange[7,8] store raw data, peak lists, search engine output, identification results and corresponding mass spectra.

Unfortunately, reproducibility and transparency of data analysis for relative protein quantification is less satisfactory. First, given the great diversity of biological objectives and experiments, quantitative analyses require richer metadata describing experimental design and biological samples. Second, quantitative experiments require many data processing steps, which are distinct from similar steps in quantitative transcriptomic investigations. These include detection and identification of chromatographic peaks and reporter ions, and propagating those identities across multiple runs. Finally, existing analysis tools (such as Skyline[9], MaxQuant[10], OpenMS[11], OpenSWATH[12], DIA-Umpire[13], Proteome Discoverer or Spectronaut[14]) integrate, in their own unique ways, diverse functionalities for identification and quantification. These tools offer various parameters and options, and output different details in various storage formats. Many tools also offer graphical user interfaces, for which analyses are difficult to document.

Benchmarking of individual analysis strategies and tools for quantitative proteomics workflows has become increasingly prevalent[15], but lack infrastructure to store, document, annotate and reanalyze the full diversity of analyses.

To meet these reproducibility needs, we implemented MassIVE.quant, an infrastructure that supports quantitative mass spectrometry–based proteomics experiments. MassIVE.quant is integrated with an existing repository, the mass spectrometry interactive virtual environment (MassIVE).

MassIVE.quant systematically stores the intermediate output files of every tool and workflow in a way that allows the user to easily inspect, reproduce or modify any component of the workflow, beginning with well-defined intermediate files. To accomplish this, we first developed a series of steps that represent a quantitative proteomics experiment with any experimental design, data acquisition and data analysis tools (Fig. 1). These steps consist of (1) annotations of experimental design; in particular, descriptors of biological samples and conditions; (2) strategies of sample preparation and data acquisition; (3) peptide ions identification; (4) quantification and (5) statistical analysis. At each step, MassIVE.quant provides the infrastructure to store all intermediate descriptions, annotations, analysis scripts and results.

MassIVE.quant does not prescribe a standard format, but meets scientists where they are by directly accommodating the diverse nature of existing workflows. Each dataset contains links to the original publications or to metadata, which can be used to gain deeper insight into the biological context of the experiment. While

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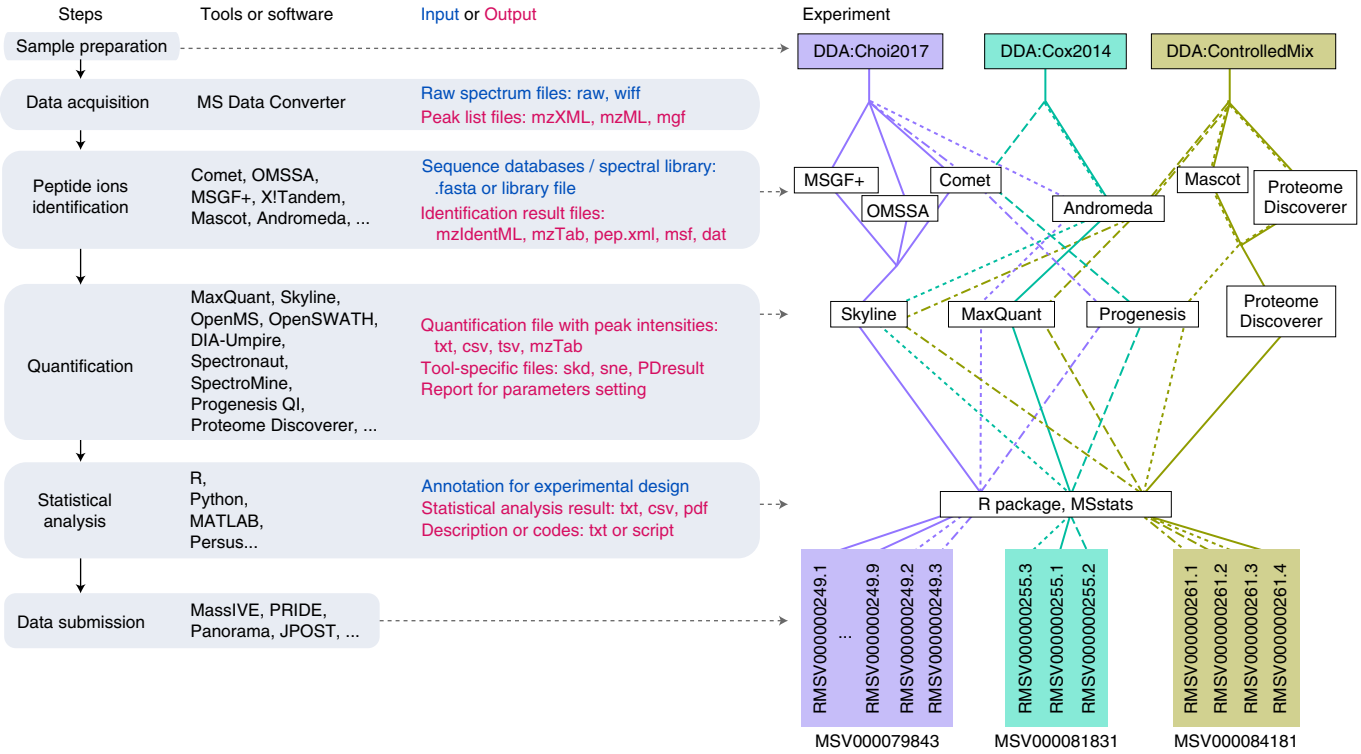


Fig. 1 | Outline of MassIVE.quant repository structure and reanalysis of three DDA-based experiments. Each step can be performed with multiple algorithms and software tools, generating tool-specific files in diverse formats. For the experiments in the figure, MassIVE.quant stores the intermediate outputs from combinations of algorithms and tools for peptide ion identification and quantification. For example, DDA:Choi2017 was processed with eight combinations of parameter settings in Skyline. Each reanalysis is saved with a unique reanalysis ID, prefixed by RMSV, under the experiment repository prefixed by MSV in MassIVE.quant.

analyte identification is represented by existing standard formats (such as mzTab, thousands of examples available for MassIVE datasets) and some of these can represent some quantitative information (such as the quant section in mzTab files, for example, the mzTab file for RMSV000000249.18), the output files produced by quantitative analyses tools can be of any nature. None of the formats mentioned before support reporting of the results of statistical analyses of quantitative data. We chose a tabular format (that is, .csv) as a common representation of the output of quantitative and statistical analyses for all tools. This format emphasizes biologically relevant aspects of the output, such as the identity of differentially abundant peptides or proteins, the magnitudes of fold changes and the associated variation.

Next, at each step, a branch structure enables the user to view reanalyses of each experiment. The reanalyses can be performed by the user offline with any combination of software tools and settings. MassIVE.quant stores the intermediate files and allows the user to check for the presence of script files, accuracy of parameters and completeness of documentation.

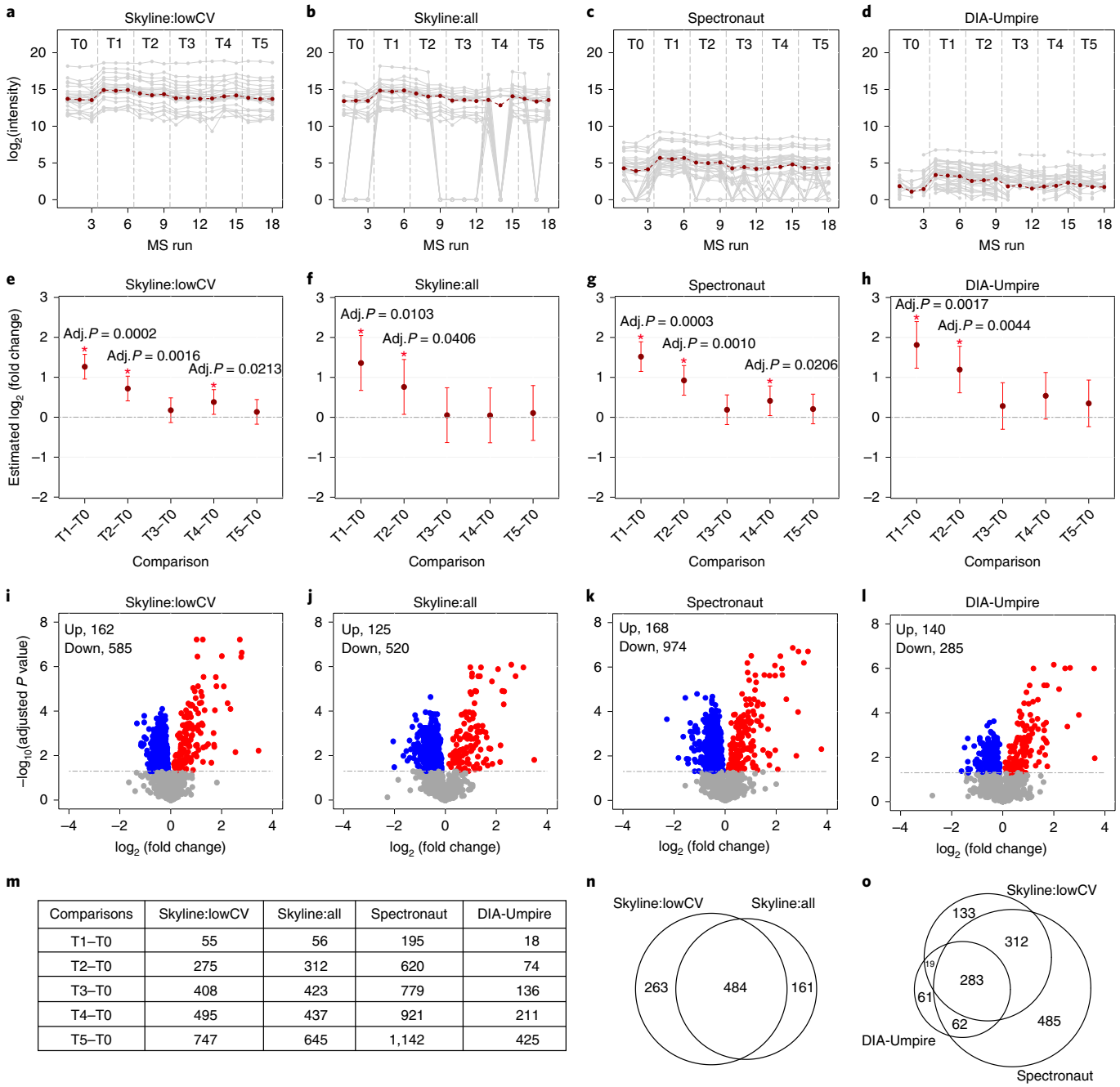
To scale the submission procedure and to ensure the reproducibility of a quantitative workflow, MassIVE.quant maintains datasets with four levels of curation (bronze, silver, gold and platinum), reflecting the documentation and the reproducibility of the quantitative workflow. During the submission, the infrastructure checks whether the submission of the dataset or reanalysis meets the minimal requirements for the entry level of curation. The submitter can then request the advanced review to level up.

MassIVE.quant further automates the statistical analysis of quantified proteins with an online MSstats workflow accessible with a user-friendly interface. This workflow can be used to reproduce the statistical analysis steps in MassIVE.quant reanalyses, as well as to analyze new private or public datasets. The MSstats Comparison workflow in MassIVE.quant automatically compares MSstats outputs across alternative reanalyses, and produces figures such as Fig. 2o. This enables the user to evaluate the implications of alternative reanalyses on conclusions regarding differential protein abundance.

The online user documentation clearly describes the structure and the vocabulary used by MassIVE.quant, and provides detailed instructions for contributing data, reanalyses and comparisons (Supplementary Note 1). Supplementary Figs. 1 and 2 and Supplementary Table 1 describe the infrastructure of MassIVE.quant and give an example of the reanalysis submission workflow.

The impact of the choice between various analysis options is best understood in the presence of some notion of ground truth. Therefore, we populated MassIVE.quant with a collection of ten datasets with controlled mixtures with known changes in protein abundance. These include three datasets collected using label-free data-dependent acquisition (DDA), four datasets collected using data-independent acquisition (DIA), one dataset collected using selective reaction monitoring (SRM) with heavy labeled isotope peptides and two DDA dataset collected using chemical labeling (TMT) (Supplementary Table 2). These datasets vary in background proteomes, number of conditions and replicates, and type and number of differentially abundant proteins.

We also populated MassIVE.quant with 95 reanalyses of these ten controlled datasets using multiple software tools, performed by the developers of the tools or by expert users. All the DDA experiments in Fig. 1 were processed with up to six tools for identification and four tools for quantification. For example, data from



Comparisons	Skyline:lowCV	Skyline:all	Spectronaut	DIA-Umpire
T1–T0	55	56	195	18
T2–T0	275	312	620	74
T3–T0	408	423	779	136
T4–T0	495	437	921	211
T5–T0	747	645	1,142	425

Fig. 2 | Reanalyses of DIA:Selevsek2015, profiling changes in proteome abundance of *Saccharomyces cerevisiae* over six time points: T0 (0 min), T1 (15 min), T2 (30 min), T3 (60 min), T4 (90 min) and T5 (120 min), $n = 3$ biologically independent samples per each time point, in response to osmotic stress (RMSV000000251). a–d, Discrepancies of quantification of protein YKL096W across data processing tools. Gray lines, fragments reported by each tool. Red lines, protein quantification summarized by MSstats. **a**, Skyline:lowCV used Skyline to quantify a subset of the fragments with low coefficient of variation. **b**, Skyline:all used Skyline to quantify all detectable peptides, with a maximum of six fragments each. **c**, Data processed by Spectronaut. **d**, Data processed by DIA-Umpire. **e–h**, Discrepancies in detecting differential abundance for protein YKL096W across data processing tools, with statistical analysis by MSstats: Skyline:lowCV (**e**), Skyline:all (**f**), Spectronaut (**g**) and DIA-Umpire (**h**). Dark red dot, center for error bars, model-based estimates of $\log_2$(fold change) of protein abundance, as determined by MSstats. Error bars, 95% confidence intervals for the $\log_2$(fold change), as determined by MSstats. *Adjusted $P < 0.05$. **i–l**, Volcano plots, summarizing differential abundance between T5 and T0: Skyline:lowCV (**i**), Skyline:all (**j**), Spectronaut (**k**) and DIA-Umpire (**l**). Dashed line, FDR = 0.05; blue dots, significantly down-regulated proteins; red dots, significantly up-regulated proteins (counts are shown at the top left corner; other time points are shown in Supplementary Fig. 3). **m**, Number of differentially abundant proteins across all time points and all tools, FDR = 0.05. **n**, Venn diagram of differentially abundant proteins between two processing approaches by Skyline, comparing T5 versus T0. **o**, Venn diagram of differentially abundant proteins across all tools, comparing T5 versus T0 (other time points are shown in Supplementary Fig. 4).

Choi et al.[16] (DDA:Choi2017) were processed with eight different combinations of parameter settings in Skyline. The combinations of algorithms, tools and settings generated ten distinct quantification reports. Finally, up to five different types of downstream statistical analysis per dataset using MSstats[17] generated 22 distinct tests for differential protein abundance for DDA:Choi2017.

To demonstrate the use of documentation, reanalysis and curation in basic biology investigations, we further populated MassIVE.quant with a collection of biological datasets, at the time of publication, including eight DIA/SWATH, seven SRM, 12 DDA and six experiments collected DDA-TMT acquisition, analyzed with multiple tools; 25 datasets with platinum level of curation and 18 datasets with gold (Supplementary Table 3). For example, the DIA experiment by Selevsek et al.[18] (DIA:Selevsek2015) was reanalyzed four times using different analysis strategies and different processing tools and parameter settings. Figure 2, Supplementary Table 4 and Supplementary Figs. 3 and 4 illustrate how changes in data processing propagated themselves into discrepancies in the number of quantified proteins, frequency of missing values and lists of differentially abundant proteins. Figure 2a–d illustrates these discrepancies in the special case of one protein. The analysis strategies and processing tools affected protein-level summaries in terms of scale, variation and patterns of missing values. This in turn affected the estimates of fold changes (Fig. 2e–h) and tests for differential abundance (Fig. 2i–l). Analysis with filtering in Skyline, applied to limit the DIA features to those known to be informative a priori (Skyline:lowCV, RMSV000000251.1) detected a smoother, and therefore more biologically plausible, pattern of differential abundance in time (Fig. 2m). While the true differential abundance is unknown, changes identified by most tools are more likely to be real (Fig. 2n–o). Such comparisons help curate the results of biological investigations.

To summarize, MassIVE.quant provides an opportunity for large-scale deposition of heterogeneous experimental datasets and facilitates a community-wide conversation about the benefits of its use. We hope that the community will find the resource useful and welcome user-driven submissions of both new datasets and documented reanalyses of the existing datasets.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41592-020-0955-0.

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Methods

Manual and tutorials. MassIVE.quant user manuals and video tutorials explain how to (1) create an account in MassIVE.quant, (2) upload files to MassIVE account via FTP, (3) submit quantification reanalysis to MassIVE.quant, (4) access reanalyses, (5) run MSstats workflow in MassIVE.quant and (6) compare the results of statistical analysis by MSstats. User manuals are available at MassIVE.quant section in https://ccms-ucsd.github.io/MassIVEDocumentation/. The video tutorial is available in https://www.youtube.com/channel/UCPeNhYFItiabsoOITPZBc5Q.

Data analysis for DIA:Selevsek2015. For Skyline:All, all files for ProteomeXchange ID PXD001010 were downloaded, along with the forward and reversed FASTA and mzXML files ($n=46$) used in the library peptide search (requested from the authors). Using Skyline (daily v.3.7.1.11571), first a spectral library was built using the iProphet score cut-off 0.0242 suggested in the paper as achieving 1% false discovery rate (FDR) at the peptide-spectrum matches (PSM) level, with 1,031 ambiguously matched peptides excluded, resulting in 82,439 unique peptides (104,993 entries). Because the Biognosys iRT standard peptides used were not included in the search, an iRT library was created from these files by adding all detected peptides with the iRT standards added as targets in Skyline and importing the mzXML files for MS1 extraction. The iRT values were then calculated using both the extracted peaks for the iRT standards and target peptide MS1 peaks where the peak contained a matching tandem mass spectrometry ID, because the runs used fractionated samples and all peptides were not expected in all runs. For DIA, allowing unique peptides of length 7–45 amino acids resulting from semitryptic cleavage with up to two missed cleavages, with Carbamidomethyl (C) and optionally Oxidation (M), precursors of charge states 2, 3 or 4, from 400–1,200 *m/z* (the range covered by the DIA method), with six product transitions found in the library, of y or b type and 1 or 2 charge state (excluding y1, y2, b1 and b2). Chromatogram extraction was set to use time of flight extraction at 18,000 resolving power with high-selectivity extraction applying to all tandem mass spectra within 10 min of predicted retention times using the iRT library. Importing the FASTA file and then removing duplicate peptides and empty proteins resulted in targets for 4,603 proteins, 68,910 peptides, 87,042 precursors and 522,252 fragment ions, at 32% protein, 2.7% unique peptide FDR by reversed sequence decoy counting (decoys/targets). The protein FDR is likely overstated because the FASTA file contains only 6,717 protein sequences, which means as many as two-thirds of false peptides can be expected to occur in a true protein, while the same is not true for detections of reversed peptides. Even at 10% protein FDR, however, this target set seemed to contain a higher error rate than we felt desirable. For these experiments, we decided to rebuild the library using the iProphet score cut-off 0.9, with 361 ambiguously matched peptides excluded, resulting in 64,501 unique peptides (84,245 entries). For our most inclusive method, we chose to include only fully tryptic peptides and no variable modifications (dropping oxidation (M)), which resulted in targets for 4,152 proteins, 36,889 peptides, 48,082 precursors and 288,492 fragment ions, at 2.6% protein, 0.29% unique peptide FDR by reversed sequence decoy counting. An equal number of shuffled sequence decoys were generated for mProphet model generation. The 18 runs were then imported into the template an mProphet model trained and applied. The MSstats report was exported for further analysis.

For Skyline:lowCV, using Skyline (daily v.3.7.1.11571) and starting from the settings for the broadly inclusive test, we restored semitryptic cleavage and the variable modification oxidation (M), expecting unstable peptides to be filtered by our first experiment. Importing the forward and reversed FASTA file resulted in targets for 4,246 proteins, 58,168 peptides, 74,314 precursors and 445,884 fragment ions, at 4.5% protein, 0.34% unique peptide FDR by reversed sequence decoy counting. Reversed sequences, accounting for 193 proteins and 200 peptides, were left in the targets list to be carried through the subsequent 'reproducibility' experiment. An equal number of shuffled sequence decoys were generated for mProphet model generation. The runs ($n=8$ reported as $n=4$ in the paper) acquired for the reproducibility experiment were then imported into the template, an mProphet model trained and applied. Next, the targets were filtered for peptides detected at q values less than 0.01 in at least four (of eight) runs, and where the CV of the detected peak areas, median normalized, was less than 20%, resulting in targets for 2,212 proteins, 13,744 peptides, 18,910 precursors and 113,460 fragment ions, at 1% protein, 0.18% unique peptide FDR by reversed sequence decoy counting. The remaining peptides comprised 249 of 2,367–10.5% (oxidation (M), 679 of 18,479–3.7%) unmodified semitryptic and 12,816 of 37,322–34.3% unmodified tryptic peptides. As expected, oxidation (M) and semitryptic peptides made it through this filter at much lower rates than unmodified fully tryptic peptides. An equal number of shuffled sequence decoys of matching types were generated for mProphet model generation. The 18 runs were then imported into the template an mProphet model trained and applied. The MSstats report was exported for further analysis.

For Spectronaut, a spectral library was generated using all available raw files from the original publication using the Pulsar search engine integrated in Spectronaut 11(11.0.15038) with default settings. The uniprot yeast reference proteome was used for the spectrum centric data analysis for library generation. DIA data were then analyzed using default settings and exported using the built in MSstats Report (v.3.7.3) export schema.

For DIA-Umpire, the raw files were converted to mzXML files with centroiding. The resulting mzXML files were processed by the signal extraction module of DIA-Umpire to generate pseudo-tandem mass spectra. The generated pseudo-MS/MS spectra were searched using X! Tandem, Comet and MSGF+ search engines. The output files from the search engines were further analyzed by PeptideProphet and combined by iProphet. FDR filtering was done with PeptideProphet and ProteinProphet. DIA-Umpire's Quant module was for the quantification analysis. The outputs for all-level quantification (FragSummary, PeptideSummary, ProtSummary) were used in further analysis.

Statistical analysis for DIA:Selevsek2015. R package MSstats v.3.10.6 was used to preprocess the output from Skyline, Spectronaut and DIA-Umpire before statistical analysis, to have protein quantification and to perform differential abundance analysis. MSstats estimated $\log_2$(fold change) and the standard error by linear mixed effect model for each protein. To test two-sided null hypothesis of no changes in abundance, the model-based test statistics were compared to the Student t-test distribution with the degrees of freedom appropriate for each protein and each dataset. The resulting P values were adjusted to control the FDR with the method by Benjamini–Hochberg. Parameter settings as well as the R code used to analyze DIA:Selevsek2015 are available in reanalysis container, RMSV000000251 in MassIVE.quant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All the datasets that support this study are publicly available in MassIVE.quant (https://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp) with MassIVE and ProteomeXchange identifiers. Additionally, identifiers for all the datasets are listed in Supplementary Tables 2, 3 and 5.

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Author contributions

M.C., J.C., N.B. and O.V. designed the research. M.C. and T.H. collected datasets and performed statistical analysis. J.C. and B.P. implemented MassIVE.quant. T.-H.T. performed statistical analysis. C.C., E.S. and M.T. experimented with new controlled mixtures. C.C., M.T., R.H., G.C.T., Y.P-R., J.M., M.M., S.G., M.P., E.V., B.W., O.M.B., A.I.N., L.R., T.D. and E.S. analyzed data up to quantification. M.C., N.B. and O.V. wrote the manuscript, with input from all authors.

Competing interests

O.M.B., J.M. and L.R. are employees of Biognosys AG. Spectronaut is a trademark of Biognosys AG. M.T. and T.D. are employees of Hoffmann-La Roche Ltd. All other authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41592-020-0955-0.

Correspondence and requests for materials should be addressed to N.B. or O.V.

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Software and code

Policy information about availability of computer code

Data collection	This manuscript does not introduce new experiments, but analyses publicly available data. Descriptions of all the data acquisition steps corresponding to each dataset are available in reanalysis repository for each experiment. The list of links are available in MassIVE.quant (https://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp).
Data analysis	Currently MassIVE.quant has 180 reanalyses, each with its own identifier. It is not practical to list them here. Instead we provided this information in the supplementary tables, and pointed to it from the 'Data Availability' section. All this information is available in MassIVE.quant (https://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp).

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All the datasets that support this study are available in MassIVE. Identifiers for all the datasets are listed in Supplementary Table 2, 3, and 5, and MassIVE.quant (https://massive.ucsd.edu/ProteoSAFe/static/massive-quant.jsp).

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Sample size	NA (This manuscript does not introduce new experiments, but analyses publicly available data.)
Data exclusions	NA
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Blinding	NA (This manuscript does not introduce new experiments, but analyses publicly available data.)

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
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging