Supplementary Information

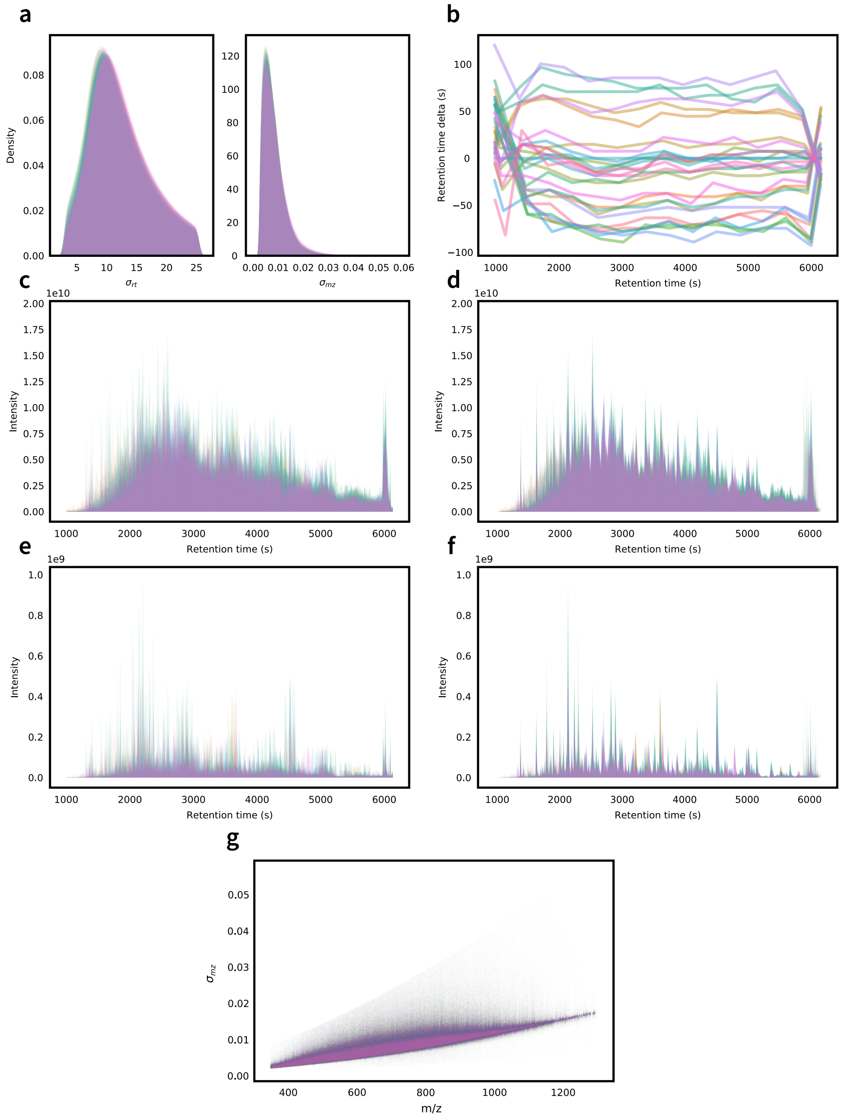

Figure S1: Example of automatically generated QC plots for the HYE dataset. (a) Distribution of detected isotopic peak widths in the m/z and retention time dimensions. (b) Extent of retention time shift (retention time delta). (c,d) Extracted ion chromatogram (XIC) before (c) and after (d) alignment. (e,f) Base peak chromatogram before (e) and after (f) alignment. (g) Scatterplot of peak widths across the m/z range.

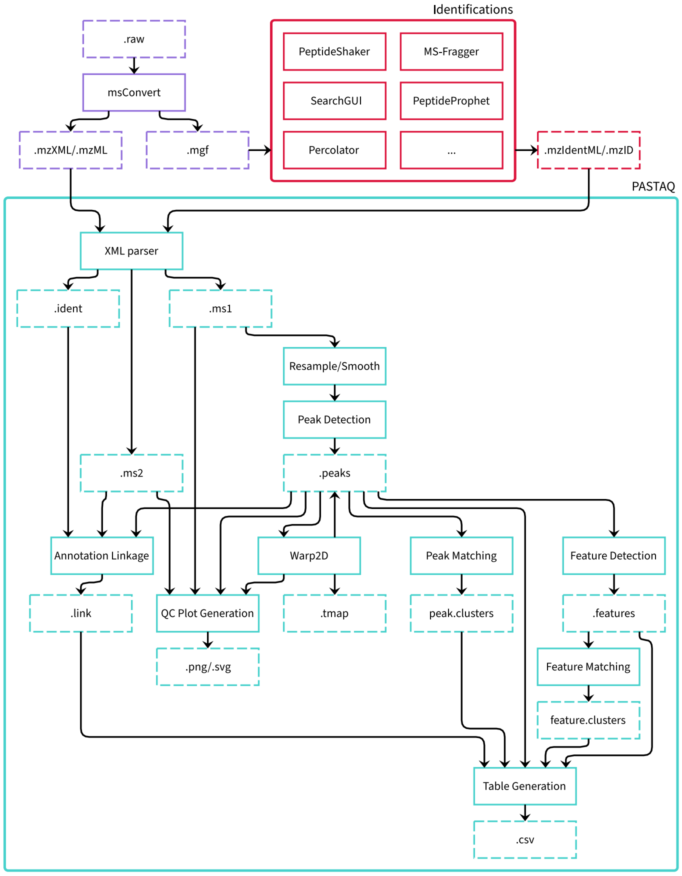

Figure S2: Detailed diagram of the main modules in PASTAQ's default DDA pipeline. The dashed lines represent input/output files. Most of the intermediate binary output files can be safely removed after the pipeline has finished running, but PASTAQ provides functions to read these files for further exploration and analysis.

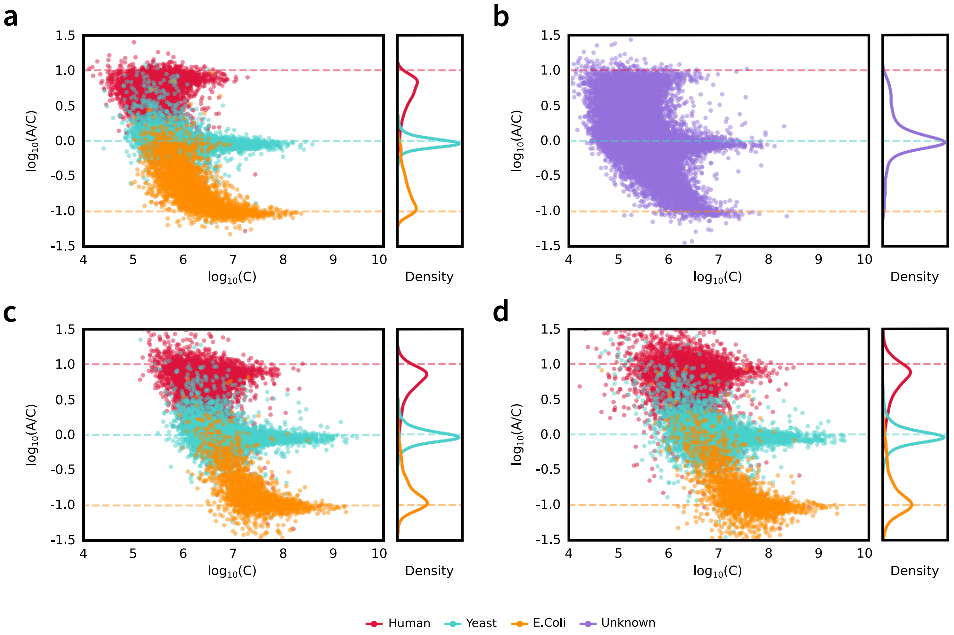

Figure S3: Scatterplots of matched features in the HYE dataset showing $\log_{10}$ ratios of sample groups A and C versus $\log_{10}$ intensity of sample group C. The density plot on the right side shows the overall distribution of the ratios. Expected values based on the known mixing ratios are represented as dashed lines: (a) PeptideShaker identifications linked to quantified PASTAQ features using theoretical $^{m}/_{z}$. (b) Quantified PASTAQ features from (a) without linked identifications passing a 1% FDR. (c) MaxQuant identifications from the evidence.txt output file. (d) MaxQuant identifications from the peptides.txt output file.

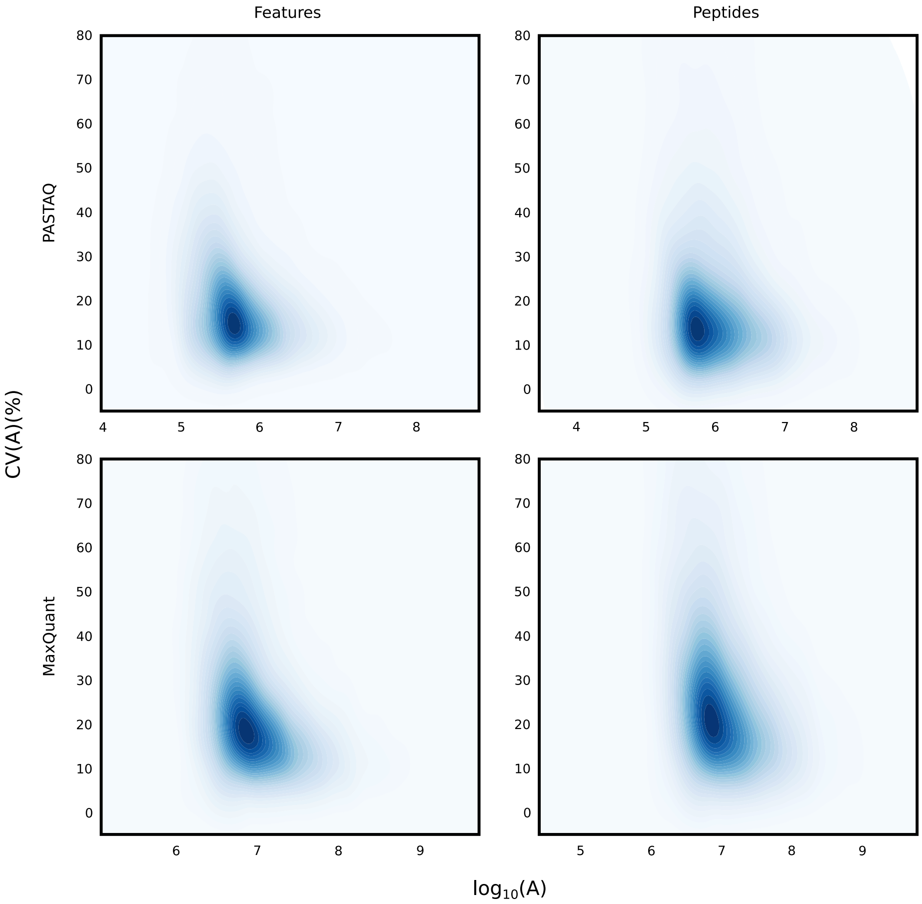

Figure S4: 2D density distribution of CVs versus intensity for the sample group A from the HYE dataset.

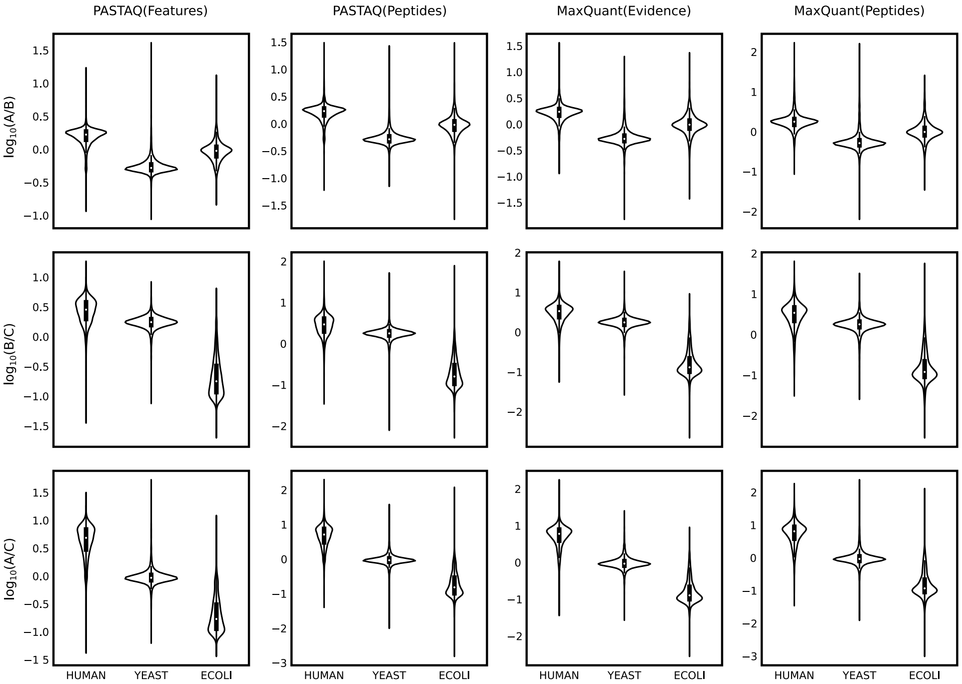

Figure S5: Distribution of $\log_{10}$ intensity ratios per proteome.

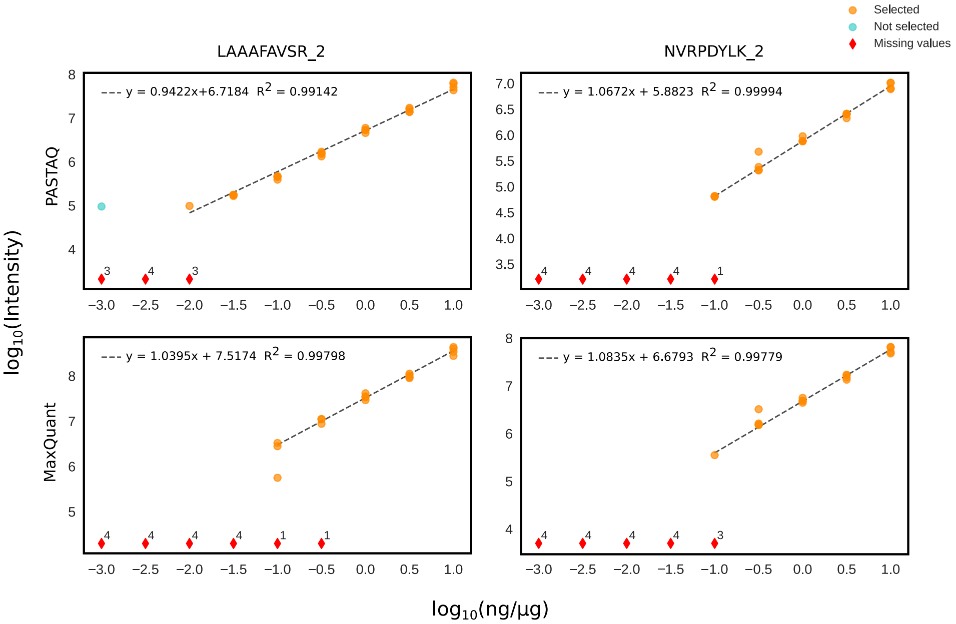

Figure S6: Example of linear fit for two different QconCAT features.

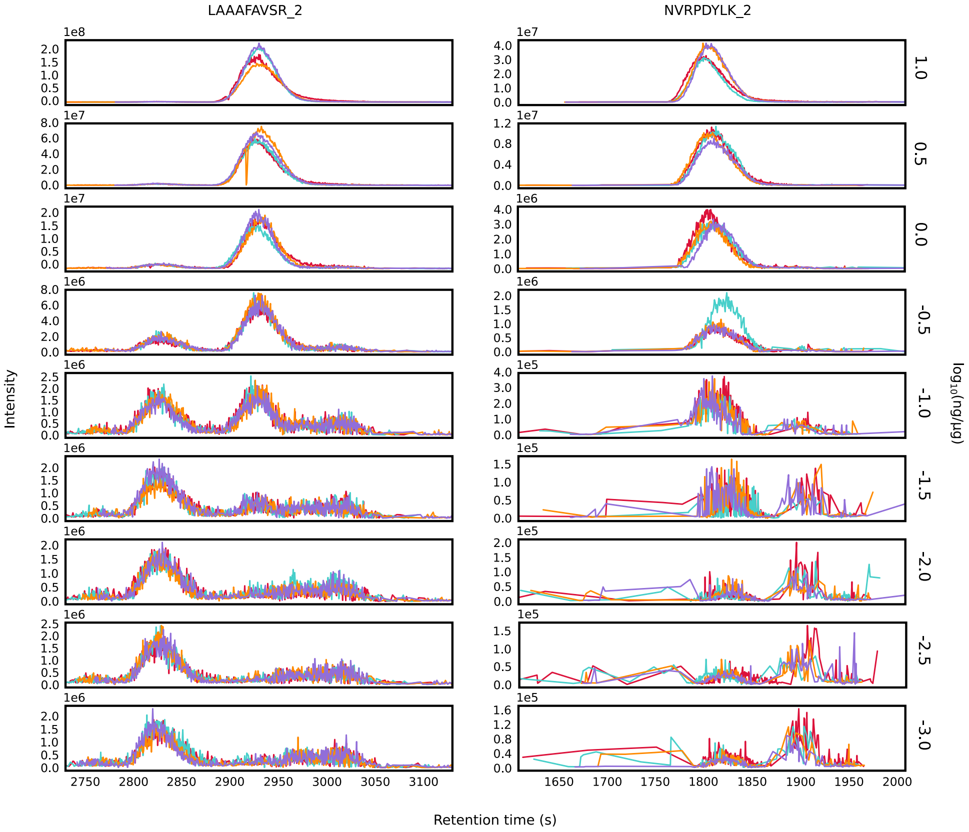

Figure S7: Extracted ion chromatograms (XIC) after retention time alignment of the monoisotopic peak of QconCAT features LAAAFAVSR_2 (rt = 2930 s, $^m/_z$ = 456.26–456.28) and NVRPDYLK_2 (rt = 1808 s, $^m/_z$ = 508.79–508.81).

Pipeline	Num. Protein Groups	Median CV (%)	Mean CV (%)	Stdev CV (%)
PASTAQ	448	14.6453	18.1029	10.8133
MaxQuant	278	16.9106	25.2668	23.9102
MaxQuant (LFQ)	277	12.4435	19.8053	19.4572

Table S1: Number of quantified protein groups and distribution of CV for technical replicates in the biological data. PASTAQ + MSFragger is compared with the original MaxQuant results from Geyer et al. [18].