Supporting Information:

Quantitative Proteomics Using Ultralow Flow Capillary Electrophoresis - Mass Spectrometry

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BIOLOGICAL RELEVANCE

In a separate manuscript we used the novel quantitative proteomics approach presented here in combination with more common proteomic approaches and transcriptomics, to comprehensively define the physiological function of the multivesicular body (MVB) pathway. Using these approaches we were able to show that the multivesicular body pathway regulates cellular amino acid homeostasis required for cell growth and survival during starvation (Martin Müller, Oliver Schmidt, Mihaela Angelova, Marietta Brunner, Klaus Faserl, Daniel Bindreither, Johannes Rainer, Leopold Kremser, Thaddäus Pfaffenwimmer, Claudine Kraft, Zlatko Trajanoski, Herbert Lindner and David Teis, manuscript submitted)

MATERIALS AND METHODS

Cell culture, protein extraction. For quantitative proteomics yeast was grown in complex synthetic medium (CSM -His, -Arg, -Lys, complemented with Arginine and Lysine) (SunriseScience Products) to midlog phase, diluted back and again grown to midlog phase. Cells were washed with their corresponding labeling medium and then used to inoculate an appropriate volume of labeling medium and kept in log-growth phase for 10 generations with either $^{13}C_6{}^{15}N_2$-L-Lysine or unlabeled $^{12}C_6{}^{14}N_2$-L-Lysine. Cells were harvested by centrifugation and labeled and unlabeled cells were mixed in a 1:1 ratio according to their OD_{600nm} and mechanically disrupted with glass beads at 4°C in PBS containing protease inhibitors (Aprotinin 10µg/ml; Pepstatin 1µg/ml, Leupeptin 10µg/ml, Pefablock SC 100µg/ml). Cell lysates were cleared by centrifugation (5 min. 1500rpm, 4°C).

Data Analysis and Protein quantification. For database search Proteome Discoverer and MaxQuant software were used.

Proteome Discoverer. For database searching using Proteome Discoverer the three search engines were used in parallel mode in a single workflow: Mascot (version 2.4.1; Matrix Science), Sequest HT and Spectra ST. Only spectra from ions with a precursor mass of less than 6,500 Da and charge state 2+ and 3+ were selected for a database search. Mascot and Sequest searches were performed using a precursor mass tolerance of 10 ppm and a fragment mass tolerance of 0.5 Da; the enzyme for protein cleavage was endoproteinase Lys-C; two missed cleavages were allowed. The fixed modification used was cysteine carbamidomethylation and oxidized methionine and SILAC labeled lysine were specified as variable modifications. For a Mascot search additional variable modifications were also applied: Protein N-terminal acetylation, protein N-terminal methionine loss, and a combination of both. For a Sequest search the minimum peptide length was set to 6 amino acids. Spectra which did not yield high confidence peptide identifications were re-evaluated using Sequest HT allowing semi-specific enzyme cleavage of Lys-C (unspecific cleavage allowed at one peptide terminus). For Spectra ST a precursor mass tolerance of 1 Da was applied. The searched peptides were further validated using target decoy (Spectra ST) and percolator search (Mascot, Sequest HT), both based on the q-value. Only peptides with a false discovery rate of less than 1% and rank 1 were accepted. SILAC based quantification of peptides and proteins was performed using

Proteome Discoverer. The mass precision was set to 2 ppm with 1 single missing channel allowed, and the label set to $^{13}C_6{}^{15}N_2$ lysine (Lys8).

Maxquant. For database search using MaxQuant with the Andromeda search engine, default settings for parameters were used with the following adjustments: Endoproteinase Lys-C was defined as enzyme for protein cleavage with two missed cleavages allowed. Acceptable charge states were 2+ and 3+, while the required minimum peptides length was set to 6 amino acid residues. The fixed modification was carbamidomethylation of cysteine, whereas protein N-terminal acetylation, protein N-terminal methionine loss, a combination of both, and oxidized methionine were specified as variable modifications. Again, only those peptides with a false discovery rate of less than 1% and rank 1 were accepted. SILAC based quantification of peptides and proteins was performed using Maxquant default settings and a peptide label set to $^{13}C_6{}^{15}N_2$ lysine (Lys8).

Phosphopeptides. Phosphorylation at serine, threonine and tyrosine was used as an additional variable modification. Proteome Discoverer database searches were performed using Mascot and Sequest HT in a separate manner, semi-specific cleaved peptides were not searched for, and Spectra ST was not used. The algorithm Phospho RS embedded in Proteome Discoverer was used for phosphorylation site localization. Phosphorylation sites were accepted when localized with at least 95% confidence.

RESULTS AND DISCUSSION

Table S-1: *Database search results giving an overview about peptides and proteins identified and quantified by CE-MS. Database searches were performed with Proteome Discoverer and MaxQuant.*

Database Search Result	Proteome Discoverer	MaxQuant
Peptide sequences identified	33,854	30,394
Peptide sequences quantified	28,538	29,090
Peptide H/L ratios (Quantification events)	79,240	88,364
Proteins identified		
1 unique Peptide	4,164	3,885
1 unique Peptide + 1 razor Peptide		3,597
2 unique Peptides	3,429	3,583
Proteins quantified		
1 unique Peptide / 2 Ratio H/L counts	3,408	3,622
1 unique peptide / 3 Ratio H/L counts	3,019	3,273
1 unique pep. + 1 razor pep. / 2 h/l counts		3,534
1 unique pep. + 1 razor pep. / 3 h/l counts		3,241
2 unique peptide / 2 Ratio H/L counts	3,272	3,520
2 unique peptide / 3 Ratio H/L counts	2,981	3,229

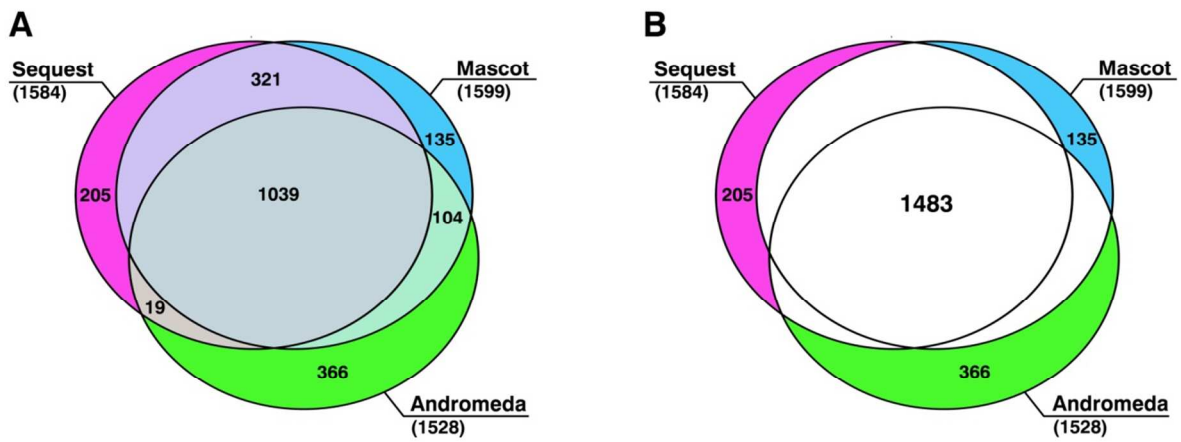

Figure S-1: ***(A)*** *Venn diagram showing the absolute numbers and the overlap of high confidence phosphopeptides identified in the CE-MS dataset by three different algorithms, Sequest and Mascot implemented in Proteome Discoverer and Andromeda being part of MaxQuant software.* ***(B)*** *Those 1,483 peptides located in the overlap areas were taken into account for subsequent investigations.*

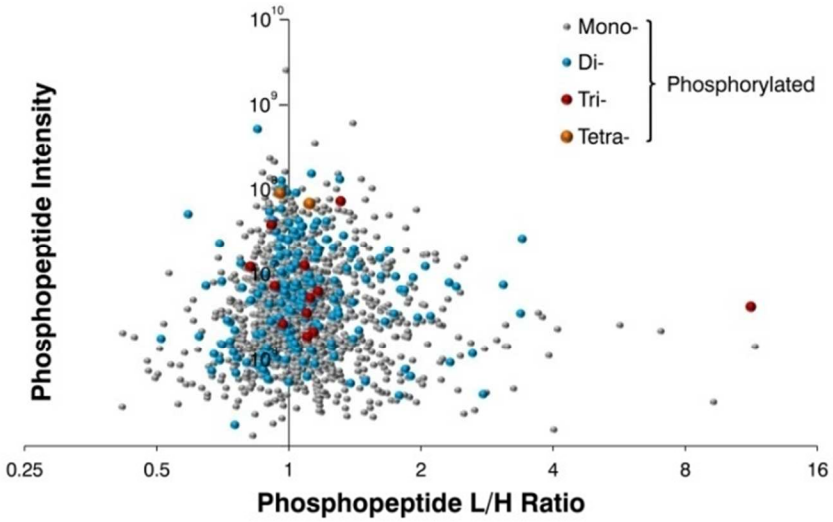

Figure S-2: *Phosphopeptide intensities and Light/Heavy ratios obtained by CE-MS. Each dot represents one phosphorylated peptide which is labeled according to the number of phosphate groups attached.*