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A Powerful Tool for Targeted Proteomics

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Technical Note

Simple Targeted Assays for Metabolic Pathways and Signaling: A Powerful Tool for Targeted Proteomics

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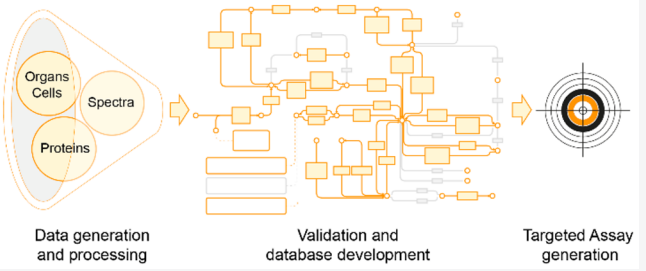
ABSTRACT: We introduce STAMPS, a pathway-centric web service for the development of targeted proteomics assays. STAMPS guides the user by providing several intuitive interfaces for a rapid and simplified method design. Applying our curated framework to signaling and metabolic pathways, we reduced the average assay development time by a factor of ∼150 and revealed that the insulin signaling is actively controlled by protein abundance changes in insulin-sensitive and -resistance states. Although at the current state STAMPS primarily contains mouse data, it was designed for easy extension with additional organisms.



Targeted mass spectrometry (MS) with or without isotopic dilution approaches, such as selective or parallel reaction monitoring (SRM/PRM), has the capability to deliver reliable protein quantification and validation of experimental results. However, creating a specific assay by targeting multiple proteins in signaling and metabolic pathways remains a laborious and time-consuming task compared to the actual MS data acquisition time. It includes, for instance, the selection of unique and proteotypic peptides, transition refinement, and retention time scheduling. On the other hand, targeted methods benefit from high-quality and high-resolution reference data to facilitate the development of such targeted assays. Several protein databases have been published that partially address and improve these above-mentioned steps.[1−7] Still, available databases either provide only spectral information as unprocessed proprietary raw files or are already compiled as a spectral library containing all peptide spectrum matches (PSMs) of all proteins within a given proteome. None of them provides a graphical representation of metabolic and signaling pathways for easy protein queries if someone is interested in downloading data for a certain (partial) pathway at once. Furthermore, their batch modes for querying multiple proteins at once is restricted to only one feature, like prebuilt assays. Our "Simple targeted assays for metabolic pathways and signaling" (STAMPS) database was developed to provide an all-in-one framework as an open-access tool to develop pathway-centric targeted assays in proteomics.

The database merges a comprehensive set of ∼150 000 manually curated high-quality and high-resolution reference spectra from 10 different mouse tissues (Table S1). STAMPS runs natively on web browsers without Java or Flash plugins; hence, it is platform-independent. Its main feature is an interactive pathway interface (Figure 1a), enabling the user to browse a graph-based visualization of manually curated pathways based on the latest knowledge and state-of-the-art databases such as KEGG.[8] During the protein selection, STAMPS offers additional information about proteins and metabolites. Furthermore, STAMPS provides curated and customized selectable MS spectra for each selected protein to highlight its measurability. Currently, the database includes 56 metabolic and 49 signaling pathways (with the objective of expanding this content), 16 810 proteins, 11 6873 unique peptide sequences, and 152 000 high-resolution spectra (including multiple charge states). In addition, the database can be queried for proteins based on (i) their subcellular localization, (ii) EC number[9] grouping, as suggested by the Enzyme Commission, (iii) UniProt[10] accessions, and (iv) their genetic position within chromosomes (Figure 1b).

Having assembled all proteins of interest, the user can proceed to review the selected proteins/peptides/spectra. In this step, final quality control can be performed by deselecting potentially irrelevant unique peptides (Figure 1c). Several filters

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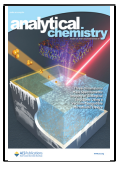

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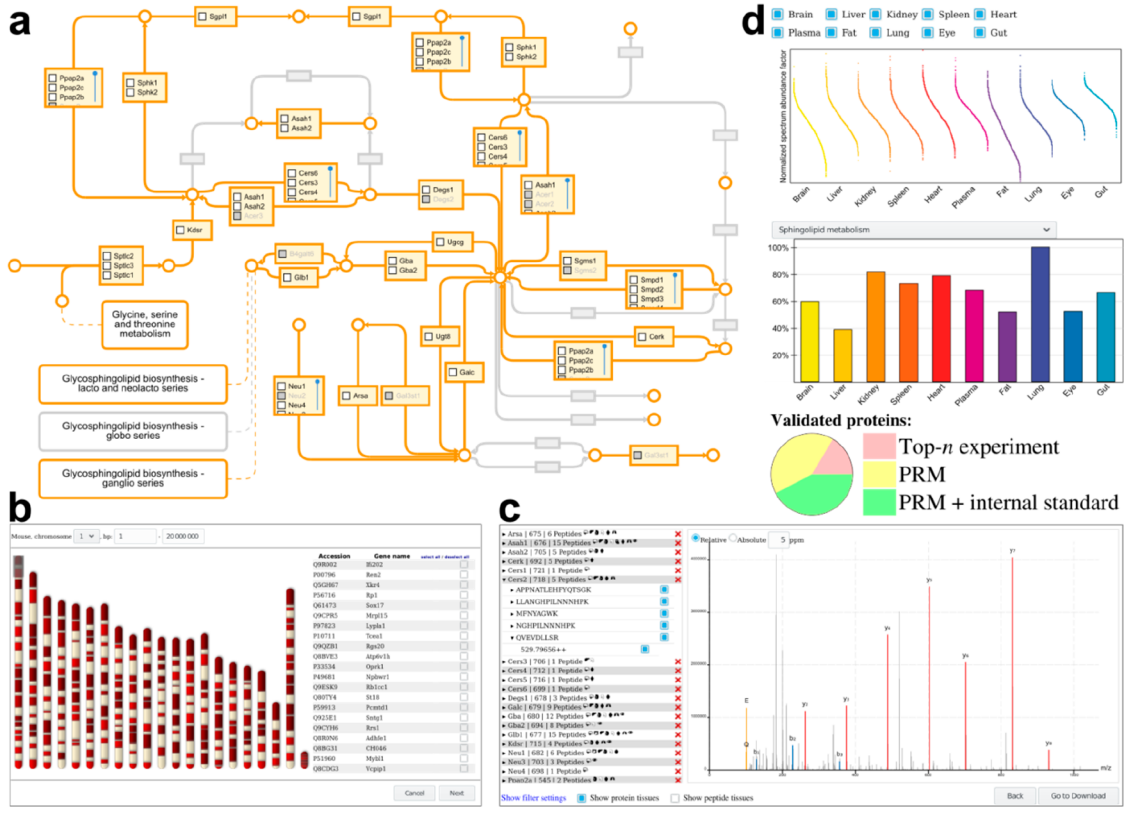

Figure 1. STAMPS in a nutshell. (a) Interactive graph-based pathway browser. Proteins are illustrated and grouped in pathways as known from literature (here: sphingolipid metabolism). The user can browse through these pathways, zoom in/out, mark preferable proteins (either in a single or multiple fashion), or access additional information. (b) Protein selection can be based on different criteria, for instance, using the chromosome browser. (c) After protein selection, all spectra/peptides/proteins can be reviewed and (de)selected as required. (d) Several measures are provided for quality control. For all pathways, protein abundance estimation is available, and proteins are validated according to three levels (Top-n, PRM/SRM, and PRM/SRM plus internal standard).

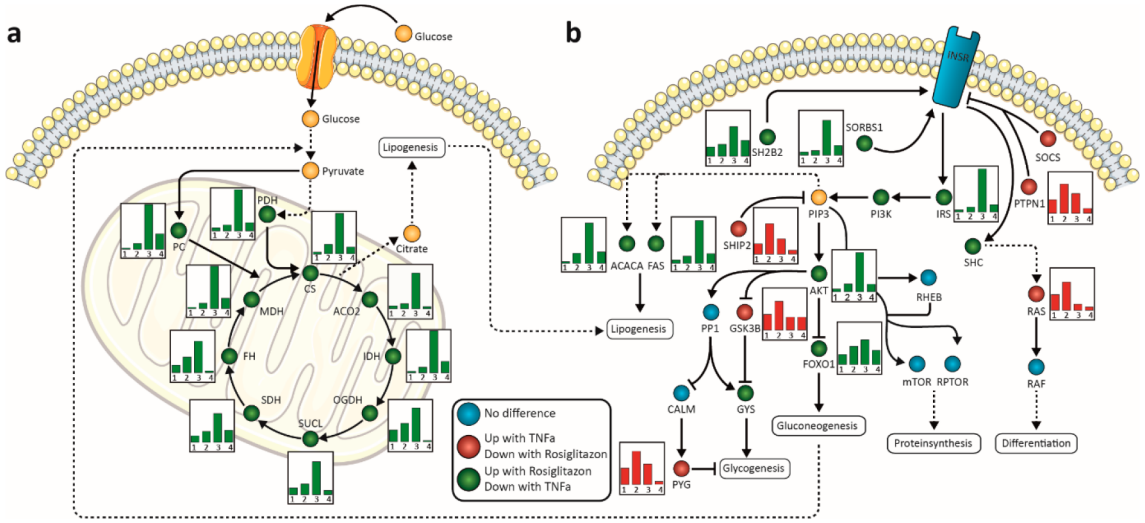

Figure 2. |Central metabolic pathways are driven not only by post-transcriptional changes but also by the adaptation of protein concentration upon the induction of insulin resistance. Here, (a) displays the tricarboxylic acid cycle and (b) the insulin signaling pathway in OP9 preadipocytes after a 24 h treatment of TNFα (10 ng/mL) (2), 48 h treatment of rosiglitazone (1 μM) (3), or an initial 48 h rosiglitazone plus a 24 h TNFα (4) treatment. DMSO (0.01%) serves as control (1). Bar graphs display n = 3 biological replicates; error distribution is provided in the Supporting Information (Figures S2 and S3).

are applicable, for example, min/max peptide length or min/max precursor charge. In addition, the database offers the unique filtering feature to store and display information about the tissue where the peptides of choice were identified (Figure 1d). After manual review, the assay can be downloaded and easily imported for further processing in tools such as Skyline.[11]

To keep all data and figures up-to-date, we provide several solutions. All figures and measures for assessment and quality

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control (Figure 1d) are computed in real time. The spectral library containing the peptide information is separate from the database containing the protein and pathway information. The peptide to protein mapping is computed using basic peptide mapping techniques.[12] This is especially beneficial when updating and adding peptides or proteins (e.g., from UniProt) to the database. For instance, the uniqueness of peptides is verified in real time during the mapping, and peptides failing this criterion are not reported.

An exceptional feature of STAMPS is the concept of human pathway managers, that is, domain experts curating the pathways. Currently, several managers are already assigned to 12 lipid biology and related pathways, keeping the pathway networks up-to-date (Table S3). Their task is supported by the development of a web-based pathway editor, allowing the intuitive and straightforward construction and updating of pathways (see Tutorial in the Supporting Information).

To determine the practicability of the workflow, we compared STAMPS with state-of-the-art targeted proteomics tools (Table S4). We measured the time to create a targeted assay for insulin signaling. Here, a time reduction of $\gtrsim$150-fold was achieved compared to the second fastest tool. A list of all features in comparison to other state-of-the-art tools is shown in Table S5.

Next, to confirm the applicability of STAMPS, we selected insulin signaling and the tricarboxylic acid cycle (TCA), two pathways important for the overall metabolism of higher eukaryotes. The quantitative SRM experiment revealed differential regulation of proteins across different treatments for both pathways (Figure 2).

It is known that mitochondrial function and the TCA cycle are impaired based on their interplay during insulin resistance, ultimately progressing to metabolic disease.[13,14] The strong coupling of insulin signaling to metabolism and the fact that insulin signaling is required for normal mitochondrial function[15,16] makes the TCA cycle an ideal target for the monitoring. Key proteins of these pathways were analyzed after inducing insulin resistance and sensitivity (Figures 2 and S1−S3).

To perturb the pathways, a preadipocyte cell model was treated with the insulin-sensitizing thiazolidinedione rosiglitazone, whereas insulin resistance was induced by using TNFα. As the insulin signaling is PPARγ-controlled at the transcriptional level[17] and due to the great influence of PPARγ on the overall metabolism, we expected strong changes of the targeted proteins during the induction of insulin sensitivity. On the other hand, we anticipated almost no change in the protein levels by the treatment with TNFα. We rather expected the TNFα treatment to mainly influence the regulation at the post-translational level involving tyrosine and serine/threonine phosphorylations, as they do not require a change in the protein abundance of the involved regulatory factors per se. However, the quantitative SRM experiment revealed differential regulation of proteins across different treatments for both pathways.

For the TCA cycle, an increase of almost all proteins was observed after the treatment with rosiglitazone (CS, ACOA2, IDH3a, SUCLA2, SDHB, and FH). For insulin signaling, downstream targets of the insulin receptor (INSR), such as IRS2, AKT2, FAS, and ARCA, were significantly upregulated, whereas proteins such as RASK and RASH were downregulated with rosiglitazone treatment. Upon induction of insulin resistance with TNFα, most of the proteins in the TCA cycle displayed no major changes whereas molecules involved in insulin signaling such as AKT1, PYGB, RASH and FOXO1 were significantly increased. Interestingly, some of those proteins (PYGB, RASK) were even further decreased in the treatment with rosiglitazone and TNFα, indicating a sensitization by PPARγ activation.

Based on the data, our assay clearly revealed that both pathways are indeed also protein-abundance-driven, indicating that there is an adaptive feedback regulation at the protein level in addition to the known control mechanisms at the post-translational and energy level.

Altogether, we provide a web interface for the most time-consuming step in targeted proteomics, the pathway-oriented assay development (a detailed tutorial is provided in the Supporting Information, section "Tutorials"). This will facilitate the investigation of biological and clinical questions. In addition, we proved its applicability for the investigation of signaling and metabolic pathways. With STAMPS, we add another resource helping to streamline the method development in the field of targeted proteomics. STAMPS provides a multitude of intuitive interfaces for fast protein selection, spectra reviewing, and assay building and, importantly, it is linked to Skyline.[11] At the same time, we ensure a top level of quality and sustainability, which is achieved, on the analytical level, by the provision of high-resolution spectra and, on the infrastructural level, by the German Network of Bioinformatics Infrastructure and expert pathway managers.

STAMPS is available as a web tool and can be accessed at https://stamps.isas.de.

EXPERIMENTAL SECTION

Data Acquisition. Spectral reference libraries, which are compilations of previously observed MS/MS spectra, play an important role as an information hub, enabling researchers to store, merge, retrieve, and share data. For building up a comprehensive spectral library, usable for targeted mass spectrometry, it is important to use as many resources as are available to detect as many proteins as possible. For this purpose, we used various tissue sources such as heart, liver, kidney, and spleen, together with neurons, stem cells, and differentiated cell types such as platelets and adipocytes, all derived from a mouse.

We started to build up STAMPS by gathering different organ tissues and cells and preparing them according to well established mass spectrometry workflows. See the sample preparation paragraph in the "Application" section of the Supporting Information for details.

All samples were analyzed using an Ultimate 3000 nano RSLC system coupled to a QExactive HF mass spectrometer (both Thermo Scientific). Peptides were preconcentrated on a C18 trapping column, followed by separation on a 75 μm × 50 cm C18 main column (both Pepmap, Thermo Scientific) with a 230 min LC gradient. MS survey scans were acquired on the QExactive HF from 300 to 1500 m/z at a resolution of 60 000 for MS^1 scans. The 15 most intense ions were subjected to higher collision-induced dissociation (HCD), taking into account a dynamic exclusion of 20 s. HCD spectra were acquired with a normalized collision energy of 27%. Automated gain control (AGC) target values were set to 10^6 for MS and 5 × 10^4 for ion trap MS^2 scans, and maximum injection times were set to 120 ms for full MS and 50 ms MS^2 scans.

To validate our data, we applied parallel reaction monitoring (PRM) for the first pathways of the energy and lipid

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metabolism, including glycolysis, pentose phosphate pathway, citric acid cycle, triacylglycerol pathway, sphingolipid pathway, fatty acid biosynthesis, glycerophospholipid pathway, and insulin signaling. Each pathway was acquired individually with the same PRM method on a Q Exactive Plus mass spectrometer coupled to an Ultimate 3000 nano RSLC system using a 70 min LC gradient. PRM scans were acquired on a Q Exactive Plus at a resolution of 17 500 for MS^2 scans. AGC target value was set to 10^6 with a maximum injection time of 50 ms and normalized collision energy of 35%.

Protein Identification and Quality Control. Peak lists obtained from MS^2 spectra were identified using Mascot version 2.4.1[18] and X! Tandem[19] (Vengeance (2015.12.15.2)). The search was conducted using SearchGUI.[20] Protein identification was conducted against a concatenated target/decoy[21] version of the *Mus musculus* complement of the UniProtKB[22] database (version of July 22nd, 2015 containing 16716 (target) sequences). The decoy sequences were created by reversing the target sequences in SearchGUI. The identification settings were as follows: Trypsin specific cleavages (allowing 1−2 missed cleavages), 5.0 ppm at MS^1 and 0.02 Da as MS^2 tolerances; fixed modification, carbamidomethylation of C (+57.0214 Da); variable modification, oxidation of M (+15.9949 Da). Fixed modification during the refinement procedure, carbamidomethylation of C (+57.0214 Da); variable modifications, acetylation of protein N-term (+42.0105 Da), pyrolidone from E (−18.0105 Da), pyrolidone from Q (−17.0265 Da), pyrolidone from carbamidomethylated C (−17.0265 Da). Peptides and proteins were inferred from the spectrum identification results using PeptideShaker[23] (1.16.3). Peptide spectrum matches (PSMs) and inferred peptides and proteins were validated at a 1.0% false discovery rate (FDR), estimated using the decoy hit distribution.

Peptide Selection and Filtering. We applied several filters on the PSMs. First, only peptides were kept that obeyed the tryptic cleavage rule, that is arginine or lysine as the last amino acid (AA) residue (exception at the end of the protein). Second, all miss cleaved peptides were removed. Additionally, we kept only peptides with a length of 8−30 AA residues and charges of +2 to +4. PSMs with less than three matched y-fragment ions within the 25% highest signals were discarded. When two (or more) PSMs were present from different measurements and referring to the same peptide, the PSM with the higher cumulative abundance of identified fragments was picked. Finally, we manually checked all remaining spectra that passed the filters, and about 152 000 spectra remained. Table S2 illustrates the distribution of identified peptides/spectra among the different tissues.

Peptide Completion. To complete the library, we additionally used mouse data from different publicly available projects. In particular, we took the brain samples measured by Sharma[24] (PXD001250), Rinschen[25] (PXD004040), and Zhao[26] (PXD003441). All data were processed as described in the previous section.

Statistical Methods. Several statistical measures, for example, normalized spectrum abundance factor,[27] ppm error histograms, normalized distribution of protein abundances per tissue, or correlation between tissue abundances are available via the web interface on several levels as pathway, protein, or spectrum level. All statistical figures within STAMPS are computed dynamically when calling the web site using the complete database and spectral library. For more details, please consider the section "Statistics" in the Supporting Information.

Code Availability. Currently, STAMPS is available as an executable web application. In the next release, a downloadable standalone version will be provided, which can be installed on each private system. The code will be published on github.

Data Availability. The data is accessible by ProteomeXchange (http://www.proteomexchange.org/) with the ID: PXD013343.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c02793.

> Current content of STAMPS; current curators of STAMPS; overview of assay development; feature comparison of different tools; application; tutorials; statistics (PDF)

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Author Contributions

D.K., A.H., and R.A. designed and D.K. implemented the database and Web site. A.H., C.C., and R.P.Z. acquired the raw data and conducted the wet-lab experiments for the application. N.H.S., T.H., C.E., D.M., N.M.H., O.S., H.-F.S., J.M., K.L., F.T., and B.B. curated several pathways and discussed the project. R.A. supervised and designed the project. A.H., D.K., and R.A. wrote the manuscript. All authors revised the manuscript.

Author Contributions

†D.K. and A.H. are cofirst authors.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Deutsch, E. W.; Lam, H.; Aebersold, R. *EMBO Rep.* **2008**, *9*, 429−434.
(2) Gaudet, P.; Argoud-Puy, G.; Cusin, I.; Duek, P.; Evalet, O.; Gateau, A.; Gleizes, A.; Pereira, M.; Zahn-Zabal, M.; Zwahlen, C.; Bairoch, A.; Lane, L. *J. Proteome Res.* **2013**, *12*, 293−298.
(3) Kusebauch, U.; Campbell, D. S.; Deutsch, E. W.; Chu, C. S.; Spicer, D. A.; Brusniak, M.-Y.; Slagel, J.; Sun, Z.; Stevens, J.; Grimes, B.; Shteynberg, D.; Hoopmann, M. R.; Blattmann, P.; Ratushny, A. V.; Rinner, O.; Picotti, P.; Carapito, C.; Huang, C.-Y.; Kapousouz, M.; Lam, H.; et al. *Cell* **2016**, *166*, 766−778.
(4) Lavallée-Adam, M.; Rauniyar, N.; McClatchy, D. B.; Yates, J. R. *J. Proteome Res.* **2014**, *13*, 5496−5509.
(5) Wilhelm, M.; Schlegl, J.; Hahne, H.; Gholami, A. M.; Lieberenz, M.; Savitski, M. M.; Ziegler, E.; Butzmann, L.; Gessulat, S.; Marx, H.; Mathieson, T.; Lemeer, S.; Schnatbaum, K.; Reimer, U.; Wenschuh, H.; Mollenhauer, M.; Slotta-Huspenina, J.; Boese, J.-H.; Bantscheff, M.; Gerstmair, A.; et al. *Nature* **2014**, *509*, 582−587.
(6) Lawrence, R. T.; Searle, B. C.; Llovet, A.; Villén, J. *Nat. Methods* **2016**, *13*, 431−434.
(7) Bollinger, J. G.; Stergachis, A. B.; Johnson, J. S.; Egertson, J. D.; MacCoss, M. J. *Methods Mol. Biol.* **2016**, *1410*, 207−221.
(8) Kanehisa, M.; Goto, S. *Nucleic Acids Res.* **2000**, *28*, 27−30.
(9) Webb, E. C. Enzyme nomenclature 1992. *Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes*; Elsevier, 1992.
(10) The UniProt Consortium. *Nucleic Acids Res.* **2017**, *45*, D158−D169.
(11) MacLean, B.; Tomazela, D. M.; Shulman, N.; Chambers, M.; Finney, G. L.; Frewen, B.; Kern, R.; Tabb, D. L.; Liebler, D. C.; MacCoss, M. J. *Bioinformatics* **2010**, *26*, 966−968.
(12) Kopczynski, D.; Barsnes, H.; Njolstad, P. R.; Sickmann, A.; Vaudel, M.; Ahrends, R. *Bioinformatics* **2017**, *33*, 2042−2044.
(13) Lowell, B. B.; Shulman, G. I. *Science* **2005**, *307*, 384−387.
(14) Petersen, K. F.; Dufour, S.; Befroy, D.; Garcia, R.; Shulman, G. I. *N. Engl. J. Med.* **2004**, *350*, 664−671.
(15) Liu, S.; Okada, T.; Assmann, A.; Soto, J.; Liew, C. W.; Bugger, H.; Shirihai, O. S.; Abel, E. D.; Kulkarni, R. N. *PLoS One* **2009**, *4*, No. e7983.
(16) Cheng, Z.; Guo, S.; Copps, K.; Dong, X.; Kollipara, R.; Rodgers, J. T.; Depinho, R. A.; Puigserver, P.; White, M. F. *Nat. Med.* **2009**, *15*, 1307−1311.
(17) Park, B. O.; Ahrends, R.; Teruel, M. N. *Cell Rep.* **2012**, *2*, 976−990.
(18) Perkins, D. N.; Pappin, D. J.; Creasy, D. M.; Cottrell, J. S. *Electrophoresis* **1999**, *20*, 3551−3567.
(19) Craig, R.; Beavis, R. C. *Bioinformatics* **2004**, *20*, 1466−1467.
(20) Vaudel, M.; Barsnes, H.; Berven, F. S.; Sickmann, A.; Martens, L. *Proteomics* **2011**, *11*, 996−999.
(21) Elias, J. E.; Gygi, S. P. *Methods Mol. Biol.* **2010**, *604*, 55−71.
(22) Apweiler, R.; Bairoch, A.; Wu, C. H.; Barker, W. C.; Boeckmann, B.; Ferro, S.; Gasteiger, E.; Huang, H.; Lopez, R.; Magrane, M.; Martin, M. J.; Natale, D. A.; O'Donovan, C.; Redaschi, N.; Yeh, L. S. *Nucleic Acids Res.* **2004**, *32*, 115D−119.
(23) Vaudel, M.; Burkhart, J. M.; Zahedi, R. P.; Oveland, E.; Berven, F. S.; Sickmann, A.; Martens, L.; Barsnes, H. *Nat. Biotechnol.* **2015**, *33*, 22−24.
(24) Sharma, K.; Schmitt, S.; Bergner, C. G.; Tyanova, S.; Kannaiyan, N.; Manrique-Hoyos, N.; Kongi, K.; Cantuti, L.; Hanisch, U.-K.; Philips, M.-A.; Rossner, M. J.; Mann, M.; Simons, M. *Nat. Neurosci.* **2015**, *18*, 1819−1831.
(25) Rinschen, M. M.; Schroeter, C. B.; Koehler, S.; Ising, C.; Schermer, B.; Kann, M.; Benzing, T.; Brinkkoetter, P. T. *American Journal of Physiology-Cell Physiology* **2016**, *311*, C404−417.
(26) Zhao, L.; Chen, Y.; Bajaj, A. O.; Eblimit, A.; Xu, M.; Soens, Z. T.; Wang, F.; Ge, Z.; Jung, S. Y.; He, F.; Li, Y.; Wensel, T. G.; Qin, J.; Chen, R. *Genome Res.* **2016**, *26*, 660−669.
(27) Zybailov, B.; Mosley, A. L.; Sardiu, M. E.; Coleman, M. K.; Florens, L.; Washburn, M. P. *J. Proteome Res.* **2006**, *5*, 2339−2347.