SUPPLEMENTAL INFORMATION

Supplemental Experimental Procedures

Peptide Selection

For every human protein annotated in the UniProtKB/Swiss-Prot database (release 2010-05) a set of PTPs was selected by mining the human PeptideAtlas (www.peptideatlas.org, build 2010-05 internal, PSM FDR 0.0003) for previously mass spectrometry (MS) observed peptides and supplemented by bioinformatic prediction using published and in-house algorithms (Braisted et al., 2008; Fusaro et al., 2009; Tang et al., 2006; Webb-Robertson et al., 2010) (Mallick et al., 2007). To provide SRM assays for new protein entries in the UniProtKB/Swiss-Prot release 2014-11 and 2015-08 additional peptides were selected by mining PeptideAtlas (build 2014-08 public, PSM FDR 0.0002). The UniProtKB/Swiss-Prot database is updated on a monthly basis and the number of proteins changes with every release depending on new, revised and erased entries as the human proteome is still refined. However, in large part the human proteome remained unchanged with 98% being consistent over the last five years. The UniProtKB/Swiss-Prot database lists 20,277 proteins in release 2010-05, 20,193 in release 2014-08 and 20,203 in 2015-08.

Each PeptideAtlas peptide sequence was given an empirical suitability score, each predicted sequence was given a predictive suitability score, and the PABST algorithm (Deutsch et al., in preparation) was used to calculate an adjusted suitability score to determine the best peptides with the following criteria: fully tryptic, no missed cleavage site, length of 7-30 amino acids (99.8% of peptides), sequence specific retention (SSRCalc) (Krokhin, 2006) of 10-46 as conservative rule (91.6% of peptides), allowing SSR 4-60 as relaxed rule and unique within the human proteome as far as possible. We considered only fully tryptic peptides because trypsin is the primary enzyme used in proteomics, and it generally provides peptides of suitable length. We avoided, as far as possible, sequences with consecutive arginine and/or lysine residues and arginine/lysine preceding proline as these can be problematic during the cleavage process (Olsen et al., 2004; Rodriguez et al., 2008). Preference was given to peptides with an expected charge state of 2 to 4.

Reactive amino acid residues susceptible to oxidation, cyclization, pyroglutamate formation and deamidation as the most prominent sample handling reactions were avoided as far as possible (Grant, 2002). Peptides containing more than three consecutive or more than 75% hydrophobic residues (C, F, I, L, M, V, W, Y) were avoided as a requirement for successful peptide synthesis. Peptides containing *N*-terminal glutamine were penalized. PABST parameters and weight factors are provided in Table S2. Peptide selection for spliced isoforms was based on UniProtKB/Swiss-Prot Varsplic (2010-06). The selection of peptides accounting for SNPs was based on the subset of NCBI dbSNP (build 131) entries annotated in the UniProt feature tables. *N*-glycosylated peptides are characterized by deamidated asparagine in the *N*-linked glycosylation NxS/T motif (N: asparagine, S/T: serine or threonine, x: any amino acid except proline).

Peptides were grouped into sets of 95 based on their *C*-terminal amino acid and length as part of the synthesis requirements, while at the same time the calculated SSRCalc value of each peptide was taken into account to support an evenly distributed chromatographic elution and to reduce concurrent MS events crowding small retention time windows. A single non-human QC peptide (HWYITTGPVREK) was included in each batch, totaling 96 peptides per pool.

Peptide Synthesis

Peptides were synthesized with FMOC-based chemistry using solid phase peptide synthesis and high-throughput 96 well synthesizers (Thermo-Fisher Scientific, PEPotec Grade 1, 0.1 mg per peptide) or on a microscale on cellulose membranes using SPOT technology (JPT Peptide Technologies, Micro-scale Peptides, 50 nmol per peptide). Peptides with length 7-20 amino acids were synthesized by either SPPS or SPOT technology, peptides >20 amino acids and with non-tryptic termini were synthesized by SPPS. All peptides were synthesized as free amine at the *N*-terminus and carboxylic acid at the *C*-terminus, and cysteine residues were introduced as carboxyamidomethylated cysteine building blocks. Peptides were recovered from their respective synthesis support as crude products, stored in 2D-barcoded 96-well plates and tracked with an in-house built laboratory information system. Peptides from both synthesis methods were detected at an equally good success rate.

Generation of Peptide Pools

Peptides were delivered and stored in 2D-barcoded tubes in 96-well Matrix Latch Racks (Thermo Scientific, Waltham, MA) or Lobo Racks (Micronic, Lelystad, The Netherlands). Each plate was labeled with a 1D barcode. Barcodes were scanned using a VisionMate High Speed 2D Barcode Reader with a VisionMate 1D Reader Adapter

(Thermo Scientific, Waltham, MA) to track each peptide with an in-house developed laboratory information system. Peptides were dissolved, pooled and mixed using a Freedom Evo liquid handling robotics platform (Tecan Systems Inc., San Jose, CA) equipped with a cooling block to keep the peptides at 4° C and a liquid handling arm with eight fixed coated pipetting tips. Gemini Software (version 4.2.8.301) was used to program the robot. The liquid handling arm aspirates column-wise each peptide and dispenses it in a single tube. The eight channels were washed twice with 10 mL deionized water between each step. The peptide pool was mixed by pipetting 0.5 mL of the peptide solution 10 times. 0.1 mg of each peptide generated by high-throughput synthesis was received in 200 µL of 50% acetonitrile/ 0.1% trifluoroacetic acid in water while 50 nmol of each peptide synthesized via SPOT synthesis were dissolved in 150 µL of 50% acetonitrile/ 1% formic acid in water. 10 µL of each peptide in a 96 well plate were pooled and mixed. For LC-MS analysis the peptide pools were further diluted to 5% acetonitrile, 0.1% formic acid in water. 25-750 fmol/µL of the crude peptide were subjected to LC-MS/MS and SRM analysis. Peptides were stored long term at -80° C.

Peptide Analysis Using a 6530 Q-TOF LC-MS System

Peptides were analyzed on a G6530A accurate mass Q-TOF LC-MS system equipped with a HPLC-Chip Cube interface and a 1260 Infinity HPLC comprised of a micro autosampler with thermostat set to 4°C, capillary loading pump, and nanoflow pump with microdegasser for gradient delivery (Agilent Technologies Inc., Santa Clara, CA). Peptides were loaded by the capillary pump delivering 0.1% formic acid in 3% acetonitrile / 97% water at a flow rate of 3 µL/min. Peptide separation was performed with a ProtID-Chip-150 (II) (C18, 150 mm, 300 Å, 40 nL enrichment column, Agilent Technologies Inc., Santa Clara, CA) using 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) and a gradient from 3% to 43% B in 80 min and 43%-63% B from 80-85 min at a flow rate of 300 nL/min delivered by the nanoflow pump. Spectra were acquired in a data-directed approach using exclusive lists in an exclusive precursor selection Auto MS/MS mode with the MassHunter Acquisition B.04.00 software (Agilent Technologies Inc., Santa Clara, CA). Exclusive lists were generated based on the expected charge state (z) of each peptide to fragment only the pool of 96 synthesized peptides. For peptides with an expected *z* of 2, a precursor mass to charge (*m/z*) with *z*=2 and 3 was calculated while for peptides with an expected *z* of 3 or higher, a precursor *m/z* with *z*=2, 3 and 4 was considered. Collision energies (CEs) were calculated according to the equations CE = [(2.93 x $m/z_{precursor}$) /100] + 6.72 for doubly and CE = [(3.6 x $m/z_{precursor}$) /100] - 4.8 for triply and higher charged precursor ions. Each *m/z* was fragmented with five CEs, two CEs above and two CEs below the calculated base CE. CE increments are as follows: precursor ions with *z*=2 used ± 5, 10 V, z=3 used ± 3.5, 7 V and z≥4 used ± 2.5, 5V. MS spectra were acquired with a mass range of *m/z* 150-2000 at 4.08 spectra/s. MS/MS spectra were acquired in a mass range of *m/z* 50-3000, scan speeds varied based on the precursor abundance with a maximum acquisition rate of 3.08 spectra/s and a target abundance of 25,000 counts per second (cps). A maximum of 5 precursor ions was selected for each MS/MS cycle with active exclusion after acquiring 3 spectra. Precursor ions selected for MS/MS were based on the expected precursor *m/z* with a 50 ppm mass window at an isolation width of ~4amu. Mass correction was enabled using reference ions *m/z* 321 and *m/z* 1221 (G1969-85003, Agilent Technologies, Inc., Santa Clara, CA). The source gas temperature was set to 350°C and gas flow to 2.4 L/min.

Retention Time Standardization

Chip Cube LC systems utilizing standardized nano RP-HPLC chip formats in combination with a set of 21 retention time peptides (Figure S1) were used to ensure retention time standardization (within 0.2 min) across multiple instruments and several years of data collection, and enabled the direct transfer of the 6530 Q-TOF observed retention times to multiple 6460 QQQs. iRT standard peptides (Biognosys, Schlieren/Zürich, Switzerland) were used to determine iRT values from observed retention times (Escher et al., 2012).

Peptide Analysis Using a QTrap 5500 LC-MS System

Peptides were analyzed on a QTrap 5500 LC-MS system equipped with a Nano Spray Source III and a Tempo nano MDLC system (Sciex, Foster City, CA). Peptides were loaded on a cap trap column (0.5 x 2 mm, Michrom Bioresources, Auburn, CA) in 0.1% formic acid in water for 5 min at a flow rate of 5 µL/min. Chromatographic separation was performed with a C18 Acclaim PepMap 100 analytical column (15 cm, 75 µm, 3 µm, 100 Å, Dionex, Sunnyvale, CA) using 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) and a gradient from 3% to 33% B in 60 min, 33% to 63% B at 60-67.5 min and 63%-83% B at 67.5-70 min at a flow rate of 300 nL/min. The analytical column is connected to a silica tip emitter (New Objective, Woburn, MA). Peptides were analyzed by SRM triggered MS/MS (also MIDAS, MRM Initiated Detection and Sequencing). Peptides were analyzed in SRM mode, triggering the acquisition of a MS/MS spectrum with *m/z* 100-1000 upon the detection of a transition above 1000 cps. Former target ions were excluded after 2 occurrences for 60 s. SRM transitions were acquired with Q1 and

Q3 set to unit resolution, a dwell time of 10 ms per transition and a declustering potential (DP) of 70. MS/MS spectra were acquired in Enhanced Product Ion (EPI) mode with Q1 set to low resolution, dynamic fill time, a scan speed of 10,000 Da/s and a cycle time of 3.1 s. Two transitions per peptide were determined using the doubly and triply charged ion as precursor (Q1) and the first ion in the y-ion series with *m/z* greater than $m/z_{precursor}$ +20 Da as fragment ion (Q3). If the *m/z* of the doubly charged precursor ion exceeds the maximum allowed value of *m/z* 1250 for Q1 and Q3, transitions for the triply and quadruply charged precursor were used instead. If Q3 resulted in ions with *m/z* above the maximum of 1250 Da, Q3 was selected as the first ion in the y-ion serious below $m/z_{precursor}$ -20 Da. For *C*-terminal peptides with an expected charge of 1, transitions were determined by selecting the singly and doubly charged ion as precursor as long as the singly charged ion does not exceed the *m/z* limit of 1250 and by selecting a fragment ion below the precursor mass, otherwise two doubly charged ions were selected as precursors. CEs were calculated according to CE=0.044 x $m/z_{precursor}$ +5.5 for doubly, CE= 0.051 x $m/z_{precursor}$ +0.55 for triply and CE=0.05 x $m/z_{precursor}$ +3 for quadruply charged precursor ions. The minimum allowed CE value was 5.

MS/MS and SRM-MS/MS Data Analysis

Instrument-native data files were converted to mzML (Martens et al., 2011) using msConvert from ProteoWizard (Kessner et al., 2008), with centroiding into peak lists performed using the vendor-supplied routines in the DLL. MS/MS spectra were associated with peptide sequences using X!Tandem (Craig and Beavis, 2004) with the K-score plugin (MacLean et al., 2006) as well as Mascot (version 2.3.02, Matrix Science, London). The X!Tandem search algorithm was modified to allow the search for unassigned ions in charge state *z*=4 in addition to charge states of z=2 and z=3, an attribute needed for the analysis of QTrap 5500 data. A monoisotopic mass error tolerance of ± 0.1 Da with the isotope error setting activated was used for 6530 Q-TOF data while a monoisotopic mass tolerance of 1.0 Da was used for QTrap 5500 data. The search parameters included a fixed modification of +57.021464 to account for carbamidomethylated cysteines, a variable modification of +15.9949 for oxidized methionine's and semi-tryptic peptides allowed in refinement mode. The Mascot required mgf format was generated from mzML files using the MzXML2Search program as part of the Trans-Proteomic Pipeline (TPP) (Deutsch et al., 2010; Keller et al., 2005) (Deutsch et al., 2015a) with the option -c1-4 to search charge states +2, +3, and +4. Parameters were set to use trypsin, ESI-QUAD-TOF as instrument, monoisotopic mass with a fragment ion mass tolerance of 0.8 Da, peptide mass tolerance of 0.1 Da, peptide isotope error of 2, carbamidomethyl as fixed modification, and auto reporting of hits. The mzML and mgf files were searched against an artificial protein database consisting of the 166,174 peptide sequences targeted in this study. In this database, peptides from each 96 well plate were concatenated into one protein entry and sequence-shuffled decoys were appended. The search results were processed with the TPP including PeptideProphet (Keller et al., 2002) and iProphet (Shteynberg et al., 2011). Peptide spectrum matches (PSM) generated by the search engine were analyzed with PeptideProphet to assign each PSM a probability of being correct. The accurate mass binning model was enabled in the PeptideProphet analysis. PeptideProphet results were further processed with iProphet to combine results from both search engines. iProphet was run with the TopCat model, which applied a mixture model to the attribute of whether a PSM is observed in the expected pooled plate sample or not. Separate consensus spectral libraries from the synthetic peptide fragmentation spectra of each MS instrument were generated with SpectraST (Lam et al., 2008) using iProphet results with a minimum probability threshold of 0.9.

Generation of Transition Lists

For each peptide precursor ion, the ten most intense y or b fragment ions from the 6530 Q-TOF consensus spectrum were extracted together with the retention time to generate transition lists. Fragments were extracted above *m/z* of 136 Da. For multiply charged precursors, ions above the precursor *m/z* were rewarded by multiplying the intensity by two prior to selection. If peptide fragmentation resulted in less than ten y or b ions, the maximum available number of y and b fragments was chosen. Extracted ions from each pool of 96 peptides were compiled into two or more transition lists depending on the number of transitions per peptide pool and their retention times to reduce concurrency. Transition lists were divided into two or more MS runs if the number of transitions exceeded 200 per time window.

Acquisition of SRM Chromatographic Traces and Verification of Transitions

Peptide pools were analyzed with the selected transitions on a G6460A QQQ LC-MS system equipped with a HPLC Chip Cube interface and a 1260 Infinity HPLC (Agilent Technologies Inc., Santa Clara, CA) to obtain the chromatographic traces of each peptide ion. Data were acquired in dynamic MRM mode with the base CE and chromatography conditions as described for the 6530 Q-TOF LC-MS/MS analysis. A fixed cycle time of 2500 ms, a minimum dwell time of 10 ms and a retention time window of 5-7 minutes was applied (depending on the number of

concurrent transitions). The following parameters were used: MS1 resolution wide, MS2 resolution unit, fragmentor 125V, cell accelerator voltage 5, source gas temperature 350°C and gas flow 5 L/min. To validate each set of SRM traces we assessed several criteria and assigned a quality score to each SRM chromatogram, specifically to each transition set consisting of a Q1 ion and its associated Q3 ions. The SRM chromatogram quality score considered if trace intensities are above background intensity within an expected retention time window, the correlation of the rank order of transition intensities with the 6530 Q-TOF derived consensus spectrum (also taking potential interference traces into account), and if retention times at the apex of each trace are aligned. 6460 QQQ native data files were converted to mzML using msConvert and processed with the corresponding transition list to extract the intensity at the apex of each chromatographic trace and to generate MS/MS like spectra. In addition, we implemented a statistical model utilizing a decoy transition set to estimate positive and negative SRM chromatogram peptide score distributions using a kernel-density estimation. Score values were transformed to a probability and an estimated false detection rate was computed. All QQQ runs were collectively processed and only SRM chromatograms with a probability corresponding to ≤1% false detection rate were accepted.

Assessment of Peptide Selection Success

Proteotypic peptides chosen for SRM assay development were selected for synthesis based on a scoring system that considered both empirical and predicted observability. Since the time we specified the peptide set underlying this study, new data have become available and the state of the human proteome as viewed through PeptideAtlas in 2015 (Deutsch et al. 2015b), which incorporates data from other draft human proteomes (Farrah et al., 2013; Farrah et al., 2014; Kim et al., 2014; Wilhelm et al., 2014), reports ~113 million high-quality PSMs identifying more than 1 million distinct peptides which represent 14, 070 (70%) confidently identified human proteins, 5% ambiguous and 9% redundant detections, leaving 16% (3166) undetected using 20,061 neXtProt primary protein entries as reference. Given a large number of peptides discovered since the peptide selection for the SRMAtlas was performed, we retrospectively investigated the success of selecting suitable peptides that were observed in the recent PeptideAtlas and were not available in the initial selection database. The empirical component for peptide selection is based on the human PeptideAtlas build 2010-05, supplemented with peptides from the human PeptideAtlas build 2014-08 for new protein entries derived from the human reference proteome 2014 (20,193 proteins) and 2015 (20,203 proteins). The selected peptides were compared to the human PeptideAtlas build 2014-08 containing more than 1 million distinct peptides. In order to make a fair comparison between these two database selections, only fully tryptic peptides, with no missed cleavages, that map to the reference database in each build (SwissProt core 2010-05 and 2014-06) were evaluated since these defined the basic selection pool. From this pool, we excluded peptides for which the predictor score was not materially altered, specifically those whose score was not revised downwards based on synthesis suitability criteria such as hydrophobicity, length, or undesirably amino acid content; those which were less desirable in the context of the proteome, such as non-proteotypic peptides, or peptides containing commonly occurring SNPs; and we only counted up to a maximum of five peptides per protein, the initial coverage level. While we selected additional peptides for higher molecular weight proteins (>50 kDa), they were constrained to distribute them equally along the entire protein sequence or selected for specific sequence motifs, which limits their utility in measuring the predictor success. From the newly observed peptides in the 2014-08 build that fulfill the criteria above we successfully selected 84.2% for peptide synthesis.

While the analysis above determined the percentage of newly observed peptides that were selected, it did not address whether the most abundant peptides observed in the majority of mass spectrometry analyses of various experiments of the human proteome (all derived from ~133 million peptide-spectrum matches identifying more than 1 million distinct peptides) were selected. To address this question, we ranked the peptides based on spectral count, and determined, after applying the same criteria as above, what percentage of the top observed candidate peptides for each protein were selected. We considered the five peptides with the most spectral counts for each protein, and allowed subsequent peptides if their count was at least 50% of the fifth peptide and at least 20% of the first most abundant peptide in cases where several peptides have similar spectral counts. We determined a success rate of 85.4% in selecting the most abundant peptides based on the predictors to utilize in creating synthetic peptides and subsequent SRM assays for each of these deposited in the human SRMAtlas. This approach provides confidence that the peptides chosen for the human SRMAtlas are the best possible peptides commonly observed in mass spectrometry analyses of tryptic digests of human proteins and are able to be readily synthesized for use in SRM assays.

Human SRMAtlas Access and Query Options

The human SRMAtlas database is hosted under www.srmatlas.org. Customized queries for SRM transitions can be executed via *Search SRM Assays* under DATA ACCESS in the sidebar on the left. Default settings are provided for

novice users for the immediate return of transitions. The selection of the SRMAtlas Build 'Complete Human SRMAtlas' and the entry of at least one protein accession or peptide sequence is required to successfully return a query result (www.srmatlas.org → Data Access → Search SRM Assays → Select 'Complete Human SRMAtlas' as SRMAtlas Build). Further details on how to set up a query are displayed by clicking on or moving the mouse cursor over the question mark icons. A question mark icon is provided for each entry field in the query form to provide immediate help, if needed.
The user can select the target instrument (the MS the experiment will be performed on) and transition sources (the MS the assays were developed on). The majority of proteins in the human SRMAtlas are represented by several, independent SRM assays per protein which were developed in a consistent manner on a 6530 Q-TOF, 6460 QQQ and QTrap 5500 mass spectrometer and we recommend selecting these three transition sources for best results. For proteins where only one or two assays per protein could be developed or for studies where an extraordinary large number of assays per protein should be of interest, the query result will be supplemented with assays derived from ion trap data or with predicted peptides and transitions which have not been verified. The rank order of these transitions may not be in agreement with observations on quadrupole type mass spectrometers due to the different fragmentation behavior of ion trap instruments and some uncertainty using prediction tools. The human SRMAtlas is built on UniProt/SwissProt accessions, but search options allowing for Ensembl and neXtProt accessions (and IPI for legacy) are likewise implemented. If heavy labeled standard peptides for quantification purposes should be measured, select heavy label (e.g., Lysine +8 and Arginine +10) and L&H under Labeled Transitions to return transitions for both, the endogenous peptide as well as the heavy labeled analogue accounting for the selected heavy label, either as synthetic internal standard or as SILAC (Ong et al., 2002) approach. Since different MS instruments cover different *m/z* ranges, the query form allows users to set a minimum and maximum *m/z* value to report only transitions within this range. This will avoid error messages when importing a transition list into the MS method. The provided retention time values may need to be adjusted to a users' specific liquid chromatography system. This can be approached by selecting a wider RT window in a first analysis, the use of retention time peptides or by performing unscheduled experiments. Several peptide modifications can be selected to be included in the query: C[160], carbamidomethylated cysteine; K[136] and R[166], heavy labeled *C*-terminus; N[115], deglycosylated peptide (D → N); M[147], oxidized, methionine; C[143], *N*-terminal S-carbamoylmethylcysteine cyclization; Q[111] and E[111], *N*-terminal cyclization of glutamine and glutamic acid. K[136] and [R166] checked will not conflict with 'Light only' selected.
The result page in the human SRMAtlas not only reports verified assay coordinates but also integrates with external knowledge bases offering comprehensive information on a protein of interest. Detailed information about neXtProt (www.nextprot.org) (Lane et al., 2012), PeptideAtlas (www.peptideatlas.org) (Desiere et al., 2004; Deutsch et al., 2015b), the Human Protein Atlas (www.proteinatlas.org) (Uhlen et al., 2005) (Uhlén et al., 2015; Uhlen et al., 2010), Pathway Commons (www.pathwaycommons.org) (Cerami et al., 2011) and SRMCollider (www.srmcollider.org) (Röst et al., 2012) is available in the respective publication.

Sample Preparation and SRM Analysis of Huh7 and HepG2 cells
Huh7 and HepG2 cells were grown in DMEM and MEM medium respectively that was supplemented with 10% FBS. Both cell lines have been authenticated using highly-polymorphic short tandem repeat loci (STRs) (PowerPlex® 16 HS System, Promega) and tested for mycoplasma contamination. To perturb the cells, drugs were added for 48h before the cells were harvested. Cells were lysed and proteins denatured in 8M Urea in NH_4HCO_3. Proteins were reduced with 2.5 mM Tris(2-carboxyethyl)phosphine (TCEP), alkylated using 40 mM Iodoacetamide (IAM) and digested using 1:100 Lys-C (Wako) and 1:75 Trypsin (enzyme/protein, Promega) before desalting on C18 columns (NEST group). For the treated condition, cells were incubated in medium containing 10% LPDS (Lipoprotein deficient serum) and 1 µM or 5µM atorvastatin (Sigma I1149). The control conditions consisted of untreated and 0.1% DMSO treated cells. The SRMAtlas resource was queried to obtain SRM assays for 64 proteins selecting Agilent QQQ as target instrument, RT_Catalog_ChipCube for retention times and 6 transitions per peptide. The maximum m/z for transitions was set to 1250, y2 and b2 ions were excluded and assays downloaded as dynamic SRM method. First we determined which peptides can be detected in a pooled sample of treated and untreated cells considering the expected retention time and fragment rank order, undetected peptides and interfering transitions were removed or replaced. The final method used to measure the samples included 74 peptides for 33 proteins in addition to 24 transitions added for 8 peptides of "housekeeping" proteins and 33 transitions for iRT peptides resulting in a total of 512 transitions analyzed in a single dynamic SRM analysis. Experiments were performed for all conditions in three biological replicates and measurements were performed in triplicates on the nanoChipCube 6490 QQQ (Agilent Technologies), the gradient is described under 6530 Q-TOF LC-MS. Analysis of the data was performed with Skyline (MacLean et al., 2010) and the R/Bioconductor package MSstats (www.msstats.org), data

were normalized to the measured housekeeping proteins (ACTB/G, HIST2B, and GAPDH - GAPDH was only included in HepG2 as it was regulated in Huh7 cells). The transition list is provided in Table S5. SRM mass spectrometry data was deposited in PASSEL and can be accessed at the webpage http://www.peptideatlas.org/PASS/PASS00867.

Sample Preparation and SRM Analysis of DU 145, LNCaP and PC-3 cells

DU145 (ATCC HTB-81), LNCaP clone FGC (ATCC CRl-1740) and PC-3 (ATCC CRL-1435) cells were cultured at 37° C with 5% $C0_2$ in EMEM, RPMI-1640 and F-12K medium, respectively. Cell culture medium was supplemented with 10% FBS. Cells were tested for mycoplasma contamination. Cells were allowed to incubate 48h prior to treatment with docetaxel (Biotang Inc.). Docetaxel was dissolved in ethanol and diluted in media. For the treated condition, DU145 was incubated with media containing 9 nM docetaxel, PC-3 with 10 nM and LNCaP with 3.5 nM (previously determined IC50 concentration for each cell line; LNCaP cells treated with 10 nM docetaxel showed visual damage by 8h and all cells died within 48h). For time point controls, cells were cultured in media containing an equal amount of ethanol (0.005%). Cells were harvested at 0h and treated and control cells at 8, 24, 48 and 72h post treatment. Cells were lysed and proteins denatured in 8 M urea, 0.1% RapiGest, and 100 mM NH_4HCO_3. Protein amounts were determined by BCA. Proteins were reduced with 5 mM TCEP (60 min, 37°C), alkylated with 10 mM IAM (30 min, room temperature, darkness), digested using 1:50 trypsin (enzyme/protein, Promega) and samples desalted with tC18 SepPak cartridges (Waters). Similar as described above, the SRMAtlas resource was queried to obtain SRM assays for 36 protein selecting QTrap 5500 as target instrument and for retention times, 6 transitions per peptide, a maximum m/z of 1250, and y2 and b2 ions being excluded (P42166 and P42167 are both associated with *TMPO*, P42166/*TMPO* is displayed in Figure 6). Interfering transitions were removed or replaced with a different fragment ion, two proteins, P49462 and Q96T88, were not detected in each of the three cell lines and therefore transitions excluded in the final method, peptides for Q16777 (Histone H2A type 2-C) map to multiple proteins, unique peptides are not available for this protein. The final method included 87 peptides for 34 target proteins in addition to transitions of peptides for "housekeeping" proteins, in total 530 transitions were measured in a single scheduled SRM analysis. Samples were measured in triplicate on a QTrap 5500, the gradient is described above under QTrap 5500, Q1 and Q3 were set to unit resolution. Analysis of the data was performed with Skyline and the R/Bioconductor package MSstats (www.msstats.org) using equalize medians to normalize data. The transition list is provided in Table S5. SRM mass spectrometry data was deposited in PASSEL and can be accessed at the webpage http://www.peptideatlas.org/PASS/PASS00868.

Additional details study II

We measured the effect of docetaxel treatment to three differentially responsive prostate cancer cell lines, LNCaP, DU145 and PC-3, based on a transcriptional time course response by microarray analysis. These cell lines represent both androgen independent (DU145 and PC-3) and androgen sensitive (LNCaP) cells and are derived originally from patient's metastatic tumors (LNCaP - supraclavicular lymph node adenocarcinoma metastasis; DU145 - adenocarcinoma metastatic to the brain; PC-3 - poorly-differentiated adenocarcinoma of the bone). Docetaxel binds to tubulin inhibiting microtubule disassembly and results in cell cycle arrest at G/M phase and thus cell death. As a cell-cycle specific antineoplastic agent, we investigate the effect of docetaxel on gene regulatory networks of the cell cycle. Based on a comprehensive database of drug sensitivity in cancer cells (Welcome Trust Sanger Institute, http://www.cancerrxgene.org) (Garnett et al., 2012), LNCaP is the most sensitive cell line to docetaxel followed by 50 fold more resistance cell-lines, PC3 and DU145 cells. An earlier study reported LNCaP, DU145, and PC3 as prostate cancer cell lines with low, moderate, and high metastatic potential, respectively (Pulukuri et al., 2005). The transcriptional time course response by microarray indicated decreasing abundance of genes associated in the network over time (0-72h). Overall, concordance of mRNA and protein abundance was observed with docetaxel time course treatments of the cell-cycle network, showing larger abundance changes at 48 and 72h compared to 8 and 24h, but also highlighted the differences of the three cell lines such as a stronger changes in abundance in PC3 compared to DU145 and LNCaP. Two clusters of differential protein abundance were defined in PC3 cells and to a lesser extent in DU145 cells and of DNA repair, synthesis and cell cycle proteins. The DNA repair, synthesis and cell cycle proteins in latter time scales of PC3 cell lines show good concordance in abundance with mRNA highlighting the effect on these cell cycle specific proteins by docetaxel.

Microarray Analysis of DU 145, LNCaP and PC-3 Prostate Cancer Cell Lines

Total RNA was extracted from docetaxel treated cells and untreated controls at each time point and cell line using the RNeasy mini kit (Qiagen), cells for microarray analysis were from an equivalent preparation than for SRM analysis. Total RNA was treated on-column with RNase-free DNase (Qiagen). RNA quality was assessed with an

Agilent 2100 Bioanalyzer to ensure RNA integrity. Microarray experiments were carried out using whole human genome oligo arrays with 8x60k 60-mer probes (Agilent Technologies) with 25 ng total RNA starting material according to the manufacturer's protocol. Hybridized arrays were scanned with Agilent's dual laser-based scanner. Feature Extraction software version 10.5 (Agilent Technologies) was used to link a feature to a design file and to determine the relative fluorescence intensity between two samples. Dye swap strategy with alternate cy3 and cy5 labeling on docetaxel treated and control groups over four time points was used to have technical replicates and decrease dye bias. Array raw data were imported into BRB Array Tools version 4.3.0 for further analysis. Lowess normalization was applied to each array. The ratios of the average Lowess normalized gene expression values of treated versus control samples for each time point of each cancer cell line were used to determine differential gene expression over time. Genes with absolute fold-change ≥ 2 at least in one time point of one cancer cell line were hierarchically clustered using *clustergram* MATLAB function with Euclidean distance metric and average linkage. A functional network formed by 35 genes from the downregulated PC-3 gene cluster was visualized in IPA (Ingenuity System Inc., USA; http://www.ingenuity.com/) Path Designer. The structure of the network is based on the IPA Core Analysis, STRING (Szklarczyk et al., 2015) and Pathway Commons (Cerami et al., 2011) derived direct interactions and indirect relationships of the selected components.

Supplemental Tables

Table S1. UniProtKB/Swiss-Prot and PeptideAtlas Proteins by Evidence Level, Related to Figure 2

Protein Evidence Level	UniProtKB/ Swiss-Prot 2010	PeptideAtlas 2010	UniProtKB/ Swiss-Prot 2015	PeptideAtlas 2015
1 Evidence at Protein Level	13,171 (65.0%)	8,721 (43.0%)	14,610 (72.3%)	13,501 (66.8%)
2 Evidence at Transcript Level	6,253 (30.8%)	1,106 (5.5%)	4,224 (20.9%)	2,639 (13.1%)
3 Inferred from Homology	209 (1.0%)	17 (0.1 %)	652 (3.2%)	209 (1.0%)
4 Predicted	96 (0.5%)	17 (0.1%)	126 (0.6%)	31 (0.2%)
5 Uncertain	548 (2.7%)	85 (0.4%)	591 (2.9%)	194 (1.0%)

Number of human proteins by their protein evidence level as defined in the UniProtKB/Swiss-Prot database. Absolute numbers and percent for the 20,277 proteins in UniProtKB/Swiss-Prot and PeptideAtlas 2010 and for 20,203 proteins in UniProtKB/Swiss-Prot and PeptideAtlas 2015.

Table S2. PABST Weight Adjustments, Related to Figure 1 and Experimental Procedures.

Parameter	Weight	Description
4H	0.5	4 consecutive hydrophobic residues: C,F,I,L,V,W,Y
5H	0.5	5 straight hydrophobic residues: F,I,L,V,W,M
Hper	0.5	More than 75% hydrophobic residues: F,I,L,V,W,M
ssr_p	0.5	Peptides with SSR hydrophobicity <10 or >46
C	0.95	Cysteine-containing peptides
D	1	Asparagine-containing peptides
M	0.95	Methionine-containing peptides
P	0.95	Proline-containing peptides
R	1	Arginine-containing peptides
S	1	Serine-containing peptides
W	1	Tryptophan-containing peptides
nQ	0.2	*N*-terminal Glutamine
nE	1	*N*-terminal Glutamic Acid
nM	1	*N*-terminal Methionine
Xc[a]	0.2	Any *C*-terminal peptide
nX	1	Any *N*-terminal peptide
NxST	1	Peptides with NxST motif
BA	1	More than 4 basic (protonatable) sites: H,K,R,n-ter
obs	2	Peptides observed in Peptide Atlas
PATR	5	Peptide exists in PA transition resource
min_l[b]	7	Minimum length for peptide
min_p	0.1	Peptides under min length
max_l[b]	20[c]	Maximum length for peptide
max_p	0.2	Peptides over max length

Peptides were evaluated with the PABST algorithm by applying a multiplicative weight factor to each parameter. A value <1 penalizes an attribute and reduces the overall score while a value >1 results in a reward and increases the score.

[a]Xc was not penalized for the intended selection of *C*-terminal peptides.

[b]For a small number of peptides length was relaxed to 7 to 30 amino acids.

Table S3. 22 Unrepresented Proteins in the Human SRMAtlas, Related to Figure 2.

Protein	Sequence	Names and Attributes	Reason
O15225	MSGPLSPVCSCPQLPFMLSPCHMH HHPGHVALSQTVSPASLLTQGLGLP QH	INE1_HUMAN Putative inactivation escape 1 protein OS=Homo sapiens GN=INE1 PE=2 SV=5	No tryptic peptides between 6-50 AA
O95424	MLGARVAAHLDALGPLVPYVPPPLL PSMFYVGLFFVNVLILYYAFLMEYIV LNVGLVFLPEDMDQALVDLGVLSDP GSGLYDADSELDVFDAYLE	DEXI_HUMAN Dexamethasone-induced protein OS=Homo sapiens GN=DEXI PE=2 SV=2	No tryptic peptides between 6-50 AA
P01358	LAAGKVEDSD	GAJU_HUMAN Gastric juice peptide 1 OS=Homo sapiens PE=1 SV=1	No tryptic peptides between 6-50 AA
P01858	TKPR	TUFT_HUMAN Phagocytosis-stimulating peptide OS=Homo sapiens PE=1 SV=1	No tryptic peptides between 6-50 AA
P04553	MARYRCCRSQSRSRYYRQRQRSR RRRRRSCQTRRRAMRCCRPRYRP RCRRH	HSP1_HUMAN Sperm protamine P1 OS=Homo sapiens GN=PRM1 PE=1 SV=2	No tryptic peptides between 6-50 AA
P0C5Y4	MASCSTSGTCGSSCCQPSCCETSC CQPSCCQTSSCGTGCGIGGGIGYG QEGSGGSVSTRIRWCHPDCHVEGT CLPPCYLVSCTPPSCCQLHHAEASC CRPSYCGQSCCRPACCCHCCEPTC	KRA14_HUMAN Keratin-associated protein 1-4 OS=Homo sapiens GN=KRTAP1-4 PE=2 SV=1	No tryptic peptides between 6-50 AA
P22103	AGEPKLDAGV	PNEU_HUMAN Pneumadin OS=Homo sapiens PE=1 SV=1	No tryptic peptides between 6-50 AA
P31358	MKRFLFLLLTISLLVMVQIQTGLSGQ NDTSQTSSPSASSNISGGIFLFFVAN AIIHLFCFS	CD52_HUMAN CAMPATH-1 antigen OS=Homo sapiens GN=CD52 PE=1 SV=1	No tryptic peptides between 6-50 AA
P59991	MCHTSCSSGCQPACCAPSPCQPAC CVPSSCQASCCVPVGCQSSVCVPV SFKPAVCLPVSCQSSVCVPMSFKSA VCVPVSCQSSVCVPVSCRPIVCAAP SCQSSLCVPVSCRPVVYAAPSCQS SGCCQPSCTSVLCRPISYSISSCC	KR122_HUMAN Keratin-associated protein 12-2 OS=Homo sapiens GN=KRTAP12-2 PE=1 SV=1	No tryptic peptides between 6-50 AA
P60329	MCHTSHSSGCPMACPGSPCCVPST CYPPEGYGTSCCCSAPCVALLCRPL CGVSTCCQPACCVPSPCQVACCVP VSCKPVLCVASFCPTSGCCQPFCPT LVYRPVTWSTPTGC	KR124_HUMAN Keratin-associated protein 12-4 OS=Homo sapiens GN=KRTAP12-4 PE=2 SV=1	No tryptic peptides between 6-50 AA
P60896	MSEKKQPVDLGLLEEDDEFEEFPAE DWAGLDEDEDAHVWEDNWDDDNV EDDFSNQLRAELEKHGYKMETS	DSS1_HUMAN 26S proteasome complex subunit DSS1 OS=Homo sapiens GN=SHFM1 PE=1 SV=1	No tryptic peptides between 6-50 AA
P62945	MRAKWRKKRMRRLKRKRRKMRQR SK	RL41_HUMAN 60S ribosomal protein L41 OS=Homo sapiens GN=RPL41 PE=2 SV=1	No tryptic peptides between 6-50 AA
Q156A1	MQQQQQQQQQQQQQQQQQQQQ QQQQQQQQQQQQQQQQQQQQQ QQQQQQQQQQQQQQQQQQQQQ QQQQQQQQQQQQQQQQQ	ATX8_HUMAN Ataxin-8 OS=Homo sapiens GN=ATXN8 PE=1 SV=1	No tryptic peptides between 6-50 AA

Q16617	MELCRSLALLGGSLGLMFCLIALSTD FWFEAVGPTHSAHSGLWPTGHGDII SGYIHVTQTFSIMAVLWALVSVSFLV LSCFPSLFPPGHGPLVSTTAAFAAAI SMVVAMAVYTSERWDQPPHPQIQT FFSWSFYLGWVSAILLLCTGALSLG AHCGGPRPGYETL	NKG7_HUMAN Protein NKG7 OS=Homo sapiens GN=NKG7 PE=2 SV=1	hydrophobicity
Q3LI58	MCCNYYGNSCGYGSGCGCGYGSG SGCGCGYGTGYGCGYGCGFGSHY GCGYGTGYGCGYGSGSGYCGYRP FCFRRCYSSC	KR211_HUMAN Keratin-associated protein 21-1 OS=Homo sapiens GN=KRTAP21-1 PE=2 SV=1	No tryptic peptides between 6-50 AA
Q3MUY2	MFLSLPTLTVLIPLVSLAGLFYSASVE ENFPQGCTSTASLCFYSLLLPITIPVY VFFHLWTWMGIKLFRHN	PIGY_HUMAN Phosphatidylinositol N-acetylglucosaminyltransferase subunit Y OS=Homo sapiens GN=PIGY PE=1 SV=1	No tryptic peptides between 6-50 AA
Q6UWW 9	MSRSRLFSVTSAISTIGILCLPLFQLV LSDLPCEEDEMCVNYNDQHPNGW YIWILLLLVLVAALLCGAVVLCLQCW LRRPRIDSHRRTMAVFAVGDLDSIY GTEAAVSPTVGIHLQTQTPDLYPVP APCFGPLGSPPPYEEIVKTT	TM207_HUMAN Transmembrane protein 207 OS=Homo sapiens GN=TMEM207 PE=2 SV=1	No tryptic peptides between 6-50 AA
Q9BY19	MNSMTSAVPVANSVLVVAPHNGYP VTPGIMSHVPLYPNSQPQVHLVPGN PPSLVSNVNGQPVQKALKEGKTLG AIQIIIGLAHIGLGSIMATVLVGEYLSIS FYGGFPFWGGLWFIISGSLSVAAEN QPYSYCLLSGSLGLNIVSAICSAVGV ILFITDLSIPHPYAYPDYYPYAWGVN PGMAISGVLLVFCLLEFGIACASSHF GCQLVCCQSSNVSVIYPNIYAANPVI TPEPVTSPPSYSSEIQANK	M4A8B_HUMAN Membrane-spanning 4-domains subfamily A member 8B OS=Homo sapiens GN=MS4A8B PE=2 SV=1	No tryptic peptides between 6-50 AA
Q9BYP8	MGCCPGDCFTCCTQEQNCCEECC CQPGCCGCCGSCCGCGGSGCGG SGCGGSCCGSSCCGSGCGGCGGC GGCGGGCCGSSCCGSSCCGSGCC GPVCCQPTPICDTK	KR171_HUMAN Keratin-associated protein 17-1 OS=Homo sapiens GN=KRTAP17-1 PE=2 SV=1	No tryptic peptides between 6-50 AA
Q9BZ97	MKTQDDGVLPPYDVNQLLGWDLNL SLFLGLCLMLLLAGSCLPSPGITGLS HGSNREDR	TTY13_HUMAN Putative transcript Y 13 protein OS=Homo sapiens GN=TTTY13 PE=5 SV=1	No tryptic peptides between 6-50 AA
Q9HC47	MFVIISLHNCVVISFVLFLFGGNNFIQ NFYLPQNYIDQFLLTSFPTFTSVGVLI VLVLCSAFLLLWQGEGVNLR	CTGE1_HUMAN Cutaneous T-cell lymphoma-associated antigen 1 OS=Homo sapiens GN=CTAGE1 PE=1 SV=1	No tryptic peptides between 6-50 AA
Q9P1J0	MLIPLQQYLVSLLPIPVSFLQLQWAL FLNNFPTLYFVYDMPFCAYSKTLSK SN	YN007_HUMAN Putative uncharacterized protein PRO1617 OS=Homo sapiens GN=PRO1617 PE=2 SV=1	Intact protein is 47 amino acids long, rejected due to hydrophobicity

SRM assays were not developed for 22 proteins of the human proteome as the sequences did not allow the selection of tryptic peptides between 6-50 amino acids (AA) and suitable hydrophobicity. The protein accession, sequence and attributes including protein name, organism name (OS), gene name (GN), protein existence (PE) and sequence version (SV) as well as the rejection reason are shown.

Supplemental References

Braisted, J., Kuntumalla, S., Vogel, C., Marcotte, E., Rodrigues, A., Wang, R., Huang, S.-T., Ferlanti, E., Saeed, A., Fleischmann, R., *et al.* (2008). The APEX Quantitative Proteomics Tool: Generating protein quantitation estimates from LC-MS/MS proteomics results. BMC Bioinformatics *9*, 529.

Cerami, E.G., Gross, B.E., Demir, E., Rodchenkov, I., Babur, Ö., Anwar, N., Schultz, N., Bader, G.D., and Sander, C. (2011). Pathway Commons, a web resource for biological pathway data. Nucleic Acids Research *39*, D685-D690.

Craig, R., and Beavis, R.C. (2004). TANDEM: matching proteins with tandem mass spectra. Bioinformatics *20*, 1466-1467.

Deutsch, E.W., Mendoza, L., Shteynberg, D., Farrah, T., Lam, H., Tasman, N., Sun, Z., Nilsson, E., Pratt, B., Prazen, B., *et al.* (2010). A guided tour of the Trans-Proteomic Pipeline. Proteomics *10*, 1150-1159.

Escher, C., Reiter, L., MacLean, B., Ossola, R., Herzog, F., Chilton, J., MacCoss, M.J., and Rinner, O. (2012). Using iRT, a normalized retention time for more targeted measurement of peptides. Proteomics *12*, 1111-1121.

Farrah, T., Deutsch, E.W., Hoopmann, M.R., Hallows, J.L., Sun, Z., Huang, C.-Y., and Moritz, R.L. (2013). The State of the Human Proteome in 2012 as Viewed through PeptideAtlas. Journal of Proteome Research *12*, 162-171.

Farrah, T., Deutsch, E.W., Omenn, G.S., Sun, Z., Watts, J.D., Yamamoto, T., Shteynberg, D., Harris, M.M., and Moritz, R.L. (2014). State of the Human Proteome in 2013 as Viewed through PeptideAtlas: Comparing the Kidney, Urine, and Plasma Proteomes for the Biology- and Disease-Driven Human Proteome Project. Journal of Proteome Research *13*, 60-75.

Fusaro, V.A., Mani, D.R., Mesirov, J.P., and Carr, S.A. (2009). Prediction of high-responding peptides for targeted protein assays by mass spectrometry. Nat Biotechnol *27*, 190-198.

Garnett, M.J., Edelman, E.J., Heidorn, S.J., Greenman, C.D., Dastur, A., Lau, K.W., Greninger, P., Thompson, I.R., Luo, X., Soares, J., *et al.* (2012). Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature *483*, 570-575.

Grant, G.A.E. (2002). Synthetic Peptides, A User's Guide. New York: Oxford University Press, 2nd edition.

Keller, A., Eng, J., Zhang, N., Li, X., and Aebersold, R. (2005). A uniform proteomics MS/MS analysis platform utilizing open XML file formats. Mol Syst Biol, 1 - 17.

Keller, A., Nesvizhskii, A., Kolker, E., and Aebersold, R. (2002). Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. Anal Chem *74*, 5383-5392.

Kessner, D., Chambers, M., Burke, R., Agus, D., and Mallick, P. (2008). ProteoWizard: open source software for rapid proteomics tools development. Bioinformatics *24*, 2534-2536.

Kim, M.-S., Pinto, S.M., Getnet, D., Nirujogi, R.S., Manda, S.S., Chaerkady, R., Madugundu, A.K., Kelkar, D.S., Isserlin, R., Jain, S., *et al.* (2014). A draft map of the human proteome. Nature *509*, 575-581.

Krokhin, O.V. (2006). Sequence-Specific Retention Calculator. Algorithm for Peptide Retention Prediction in Ion-Pair RP-HPLC: Application to 300- and 100-Å Pore Size C18 Sorbents. Analytical Chemistry *78*, 7785-7795.

Lam, H., Deutsch, E.W., Eddes, J.S., Eng, J.K., Stein, S.E., and Aebersold, R. (2008). Building consensus spectral libraries for peptide identification in proteomics. Nat Meth *5*, 873-875.

Lane, L., Argoud-Puy, G., Britan, A., Cusin, I., Duek, P.D., Evalet, O., Gateau, A., Gaudet, P., Gleizes, A., Masselot, A., *et al.* (2012). neXtProt: a knowledge platform for human proteins. Nucleic Acids Research *40*, D76-D83.

MacLean, B., Eng, J.K., Beavis, R.C., and McIntosh, M. (2006). General framework for developing and evaluating database scoring algorithms using the TANDEM search engine. Bioinformatics *22*, 2830-2832.

MacLean, B., Tomazela, D.M., Shulman, N., Chambers, M., Finney, G.L., Frewen, B., Kern, R., Tabb, D.L., Liebler, D.C., and MacCoss, M.J. (2010). Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics *26*, 966-968.

Martens, L., Chambers, M., Sturm, M., Kessner, D., Levander, F., Shofstahl, J., Tang, W.H., Roempp, A., Neumann, S., Pizarro, A.D., *et al.* (2011). mzML - a Community Standard for Mass Spectrometry Data. Molecular & Cellular Proteomics *10*, R110.000133.

Olsen, J.V., Ong, S.-E., and Mann, M. (2004). Trypsin Cleaves Exclusively C-terminal to Arginine and Lysine Residues. Molecular & Cellular Proteomics *3*, 608-614.

Ong, S., Blagoev, B., Kratchmarova, I., Kristensen, D., Steen, H., Pandey, A., and Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Molecular & cellular proteomics : MCP *1*, 376-386.

Pulukuri, S.M., Gondi, C.S., Lakka, S.S., Jutla, A., Estes, N., Gujrati, M., and Rao, J.S. (2005). RNA Interference-directed Knockdown of Urokinase Plasminogen Activator and Urokinase Plasminogen Activator Receptor Inhibits Prostate Cancer Cell Invasion, Survival, and Tumorigenicity in Vivo. Journal of Biological Chemistry *280*, 36529-36540.

Rodriguez, J., Gupta, N., Smith, R.D., and Pevzner, P.A. (2008). Does Trypsin Cut Before Proline? Journal of Proteome Research *7*, 300-305.

Röst, H., Malmström, L., and Aebersold, R. (2012). A Computational Tool to Detect and Avoid Redundancy in Selected Reaction Monitoring. Molecular & Cellular Proteomics *11*, 540-549.

Shteynberg, D., Deutsch, E.W., Lam, H., Eng, J.K., Sun, Z., Tasman, N., Mendoza, L., Moritz, R.L., Aebersold, R., and Nesvizhskii, A.I. (2011). iProphet: Multi-level Integrative Analysis of Shotgun Proteomic Data Improves Peptide and Protein Identification Rates and Error Estimates. Molecular & Cellular Proteomics *10*, M111.007690.

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K.P., *et al.* (2015). STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Research *43*, D447-D452.

Tang, H., Arnold, R., Alves, P., Xun, Z., Clemmer, D., Novotny, M., Reilly, J., and Radivojac, P. (2006). A computational approach toward label-free protein quantification using predicted peptide detectability. Bioinformatics *22*, e481 - e488.

Uhlén, M., Fagerberg, L., Hallström, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., *et al.* (2015). Tissue-based map of the human proteome. Science *347*.

Uhlen, M., Oksvold, P., Fagerberg, L., Lundberg, E., Jonasson, K., Forsberg, M., Zwahlen, M., Kampf, C., Wester, K., Hober, S., *et al.* (2010). Towards a knowledge-based Human Protein Atlas. Nature Biotechnology *28*, 1248-1250.

Webb-Robertson, B.-J.M., Cannon, W.R., Oehmen, C.S., Shah, A.R., Gurumoorthi, V., Lipton, M.S., and Waters, K.M. (2010). A support vector machine model for the prediction of proteotypic peptides for accurate mass and time proteomics. Bioinformatics *26*, 1677-1683.

Wilhelm, M., Schlegl, J., Hahne, H., Gholami, A.M., Lieberenz, M., Savitski, M.M., Ziegler, E., Butzmann, L., Gessulat, S., Marx, H., *et al.* (2014). Mass-spectrometry-based draft of the human proteome. Nature *509*, 582-587.