Supplementary Table 1. Investigation of BoxCar acquisition parameters based on 45 min HeLa single runs using a D-optimal Design of Experiment.

Experiment	Scan parameters				Responses		
	#Scans	#Boxes	Max. IIT [%][a]	Resolution	Isotope patterns	Average IIT [ms]	dpp[b]
N1	2	8	50	60,000	184,698	33.4	11.1
N2	2	16	50	60,000	179,963	36.7	10.9
N3	4	8	100	60,000	212,544	68.4	9.3
N4	4	16	100	60,000	194,923	76.8	8.3
N5	2	8	83	60,000	193,334	48.5	11.1
N6	2	16	67	60,000	186,059	46.3	10.7
N7	2	11	100	60,000	194,912	59.3	11.1
N8	2	13	100	60,000	199,018	64.3	10.7
N9	4	8	67	60,000	208,152	47.1	9.4
N10	4	16	83	60,000	193,196	65.5	8.4
N11	4	11	50	60,000	205,202	43.2	9.3
N12	4	13	50	60,000	194,523	42.5	9.2
N13	3	8	50	60,000	199,799	36.6	10.3
N14	3	8	100	60,000	201,316	66.0	10.2
N15	3	16	50	60,000	190,807	44.0	9.7
N16	3	16	100	60,000	195,100	71.1	9.1
N17	3	12	75	60,000	202,094	53.0	10.1
N18	4	8	50	120,000	252,366	71.9	7.4
N19	4	16	50	120,000	241,590	85.6	7.7
N20	2	8	100	120,000	246,348	99.8	8.8
N21	2	16	100	120,000	254,941	133.1	8.9
N22	2	8	67	120,000	257,910*	81.4	8.8
N23	2	16	83	120,000	247,667	102.8	8.8
N24	2	11	50	120,000	236,583	61.9	8.8
N25	2	13	50	120,000	235,090	63.8	8.8
N26	4	8	83	120,000	250,987	113.1	7.7
N27	4	16	67	120,000	247,663	104.3	7.7
N28	4	11	100	120,000	252,243	143.4	7.5
N29	4	13	100	120,000	255,037	140.8	7.6
N30	3	8	50	120,000	249,782	65.2	8.2
N31	3	8	100	120,000	262,647	130.1	8.2
N32	3	16	50	120,000	245,879	72.2	7.9
N33	3	16	100	120,000	254,449	135.1	8.1
N34	3	12	75	120,000	253,899	100.6	8
N35	3	12	75	120,000	256,839	102.6	8.3
N36	3	12	75	120,000	257,902	99.7	8
N37	3	12	75	120,000	256,281	98.2	7.9
N38	3	12	75	120,000	259,599	101.5	8

* Not included in fitting of the model after residual analysis (abs. studentized residual > 4 s.d.).

[a] Ion injection time (IIT) as a percentage of the Orbitrap transient time.

[b] Average number of data points per peak (dpp).

Supplementary Table 2. Adapting the box width to the *m/z* distribution of tryptic peptides.

Scan	Quadrupole isolation windows (*m/z* low, *m/z* high)
2 BoxCar scans, 24 boxes	
BoxCar scan #1	(400,423.2), (441.2,459.9), (476.3,494.3), (510.3,528.8), (545,563.8), (580.8,600.3), (618.4,639.8), (660.3,684.3), (708.3,735.4), (764.4,799.9), (837.9,885.4), (945,1032)
BoxCar scan #2	(422.2,442.2), (458.9,477.3), (493.3,511.3), (527.8,546), (562.8,581.8), (599.3,619.4), (638.8,661.3), (683.3,709.3), (734.4,765.4), (798.9,838.9), (884.4,946), (1031,1201)
3 BoxCar scans, 36 boxes	
BoxCar scan #1	(400,416.3), (441.2,454.2), (476.3,488.8), (510.3,523.3), (545,557.8), (580.8,594), (618.4,633), (660.3,676.4), (708.3,726.3), (764.4,788.4), (837.9,868.8), (945,999)
BoxCar scan #2	(415.3,429.7), (453.2,465.9), (487.8,499.9), (522.3,534.8), (556.8,569.6), (593,606.6), (632,646.8), (675.4,692.3), (725.3,745), (787.4,812.4), (867.8,903.5), (998,1071.1)
BoxCar scan #3	(428.7,442.2), (464.9,477.3), (498.9,511.3), (533.8,546), (568.6,581.8), (605.6,619.4), (645.8,661.3), (691.3,709.3), (744,765.4), (811.4,838.9), (902.5,946), (1070.1,1201)

Supplementary Table 3. Deep proteome coverage of a HeLa digest in 45 min single runs.

Type	Experiment	Isotope patterns	Isotope patterns (z>1)	Evidences	Protein groups
Shotgun	Standard_01	130,085	118,857	28,414	4,536
Shotgun	Standard_02	133,008	121,281	28,804	4,536
Shotgun	Standard_03	126,892	115,241	27,444	4,341
Shotgun	Standard_04	129,605	118,000	27,886	4,446
Shotgun	Standard_05	127,810	116,156	27,273	4,299
Shotgun	Standard_06	131,465	119,682	28,175	4,447
Shotgun	Standard_07	127,289	115,645	27,239	4,354
Shotgun	Standard_08	128,616	116,928	27,203	4,316
Shotgun	Standard_09	132,983	120,755	28,002	4,407
Shotgun	Standard_10	128,503	116,547	27,238	4,370
Shotgun + MBR	Standard_01	130,085	118,857	41,543	5,357
Shotgun + MBR	Standard_02	133,008	121,281	41,951	5,340
Shotgun + MBR	Standard_03	126,892	115,241	41,313	5,322
Shotgun + MBR	Standard_04	129,605	118,000	41,606	5,355
Shotgun + MBR	Standard_05	127,810	116,156	41,387	5,294
Shotgun + MBR	Standard_06	131,465	119,682	41,734	5,358
Shotgun + MBR	Standard_07	127,289	115,645	41,184	5,318
Shotgun + MBR	Standard_08	128,616	116,928	41,221	5,289
Shotgun + MBR	Standard_09	132,983	120,755	41,666	5,352
Shotgun + MBR	Standard_10	128,503	116,547	41,149	5,308
Boxcar	Boxcar_01	288,298	226,941	81,782	7,799
Boxcar	Boxcar_02	289,469	227,710	81,153	7,768
Boxcar	Boxcar_03	289,645	227,802	80,900	7,761
Boxcar	Boxcar_04	283,870	224,444	80,446	7,759
Boxcar	Boxcar_05	286,971	226,753	80,788	7,776
Boxcar	Boxcar_06	280,940	223,284	80,609	7,753
Boxcar	Boxcar_07	288,181	228,859	81,398	7,843
Boxcar	Boxcar_08	282,526	224,169	80,079	7,768
Boxcar	Boxcar_09	289,030	227,901	80,249	7,785
Boxcar	Boxcar_10	281,747	222,619	79,734	7,738

Supplementary Note 1.

To reduce the potential for false library matches, the following points should be considered.

An important factor is the size and origin of the matching library itself. Very large libraries, for example, derived from various tissue types, organisms or community data, contain a large proportion of proteins that may not be present in the sample of interest. Depending on the relative size of this truly absent population, more stringent statistical tests and strategies to control the accumulation of false positives on peptide and protein levels are required, a situation that also occurs in data independent acquisition schemes (Rosenberger *et al.*, Statistical control of peptide and protein error rates in large-scale targeted data-independent acquisition analyses, *Nat. Methods* **14**, 921-927 (2017)). In the present study, peptide libraries have been generated from the very same cell culture batch or specimen and, if applicable, processed in parallel with the samples for single run experiments. Identifications in the library were stringently filtered for an FDR <1% on the peptide spectrum match and protein group level. Only peptides that passed these filtering criteria were considered for matching between runs. Thus, the proteins in the library are also present in the sample, minimizing the potential for false protein identifications.

Despite the low error rate at the protein level, the error rate of individual feature matches still needs to be assessed. To estimate the rate of potentially false matches in the current study, we devised a decoy model on the level of MS-features for the entire HeLa dataset. In particular, we asked if the increased number of detected isotope patterns in BoxCar scans as compared with standard full scans inflates the rate of random matches to the peptide library. We hypothesize that true matches are sampled from a zero-centered normal distribution in both mass error and retention time difference, while false matches are derived from a uniform background population. To generate such a decoy population from the measured data in silico, we first altered all detected isotope patterns in both dimensions by first shifting all masses by + 40 ppm - outside the tolerance window for matching between runs. Second, we permutated all retention times with a minimal shift of 180 s. Adding these decoy features to the original mass spectra did not alter the properties of the original data set, such as charge distribution, retention length and mass uncertainty. To distinguish both populations, we multiplied all intensities of the original features (target population) by an arbitrary factor of 10^9. This resulted on average in 144,000 decoy and 227,000 target features in ten 45 min single BoxCar runs of 1 µg HeLa digest.

Next, we processed the in silico data in MaxQuant together with the entire peptide library using 'matching between runs'. Importantly, mass re-calibration and retention time alignment, which are both crucial for the matching process, were not affected by the insertion of decoy features. Target and decoy identifications ('evidences') formed distinct intensity populations and, in accordance with our starting hypothesis, the distribution of mass errors and match time differences was significantly wider for the decoy population. In particular, we observed that the target population showed a narrow Gaussian distribution of retention time differences, while decoy matches were nearly uniformly distributed throughout the entire tolerance window. Note that this procedure also allows modeling the rate of false matches as a function of the size of the matching time windows.

Using a 0.3 min matching tolerance window (**Online Methods**), we estimated the FDR as follows, with *N* being the number of quantified evidences:

$$FDR = \frac{N_{Decoy}}{N_{Target}}$$

In this way, we determined the average FDR for the ten 45 min BoxCar single runs described in the manuscript, to be 3.6 ± 0.1%. Based on these results and the considerations above, we estimate that the FDR in the BoxCar library matching approach is below 5% for the features.