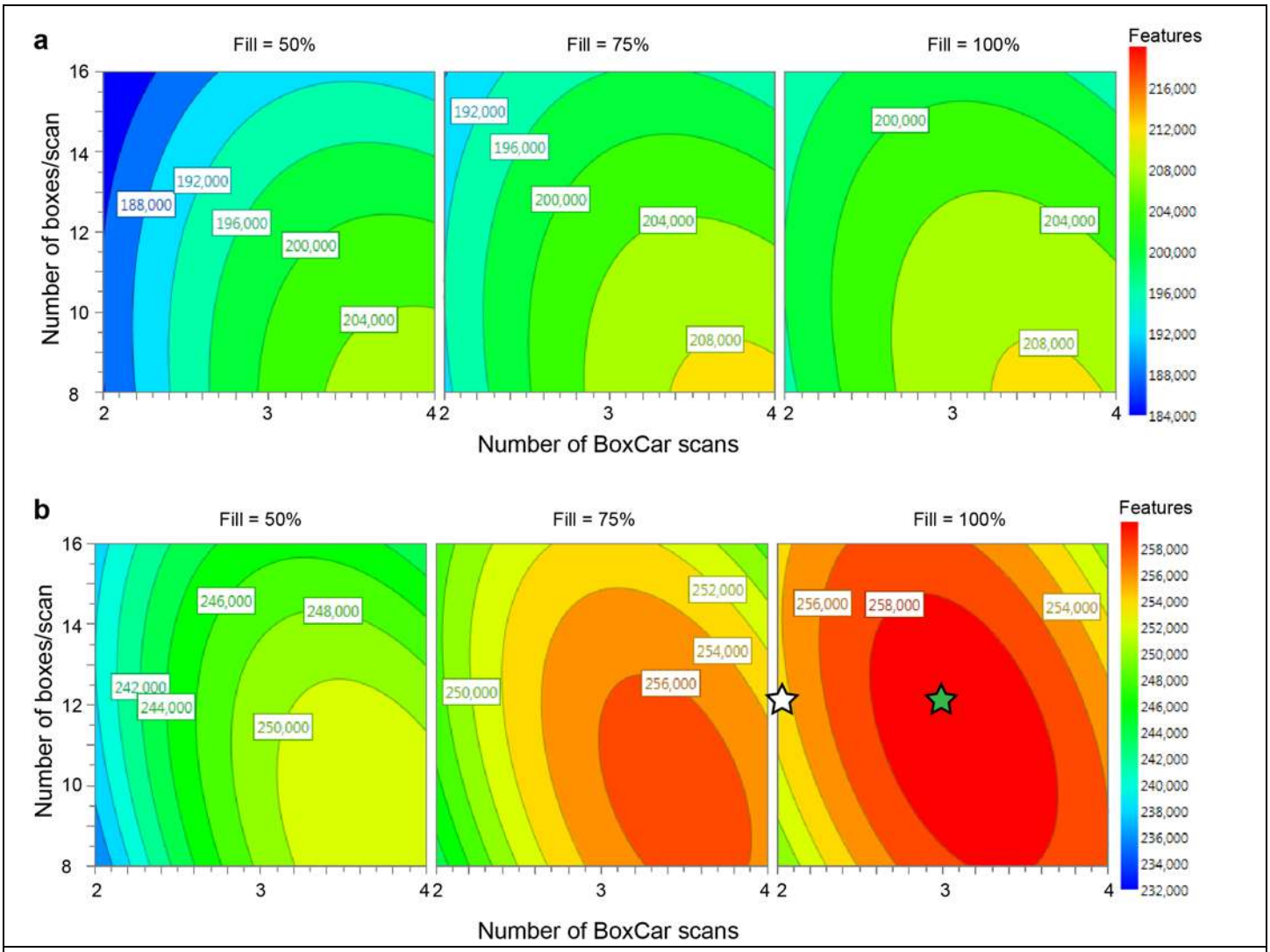
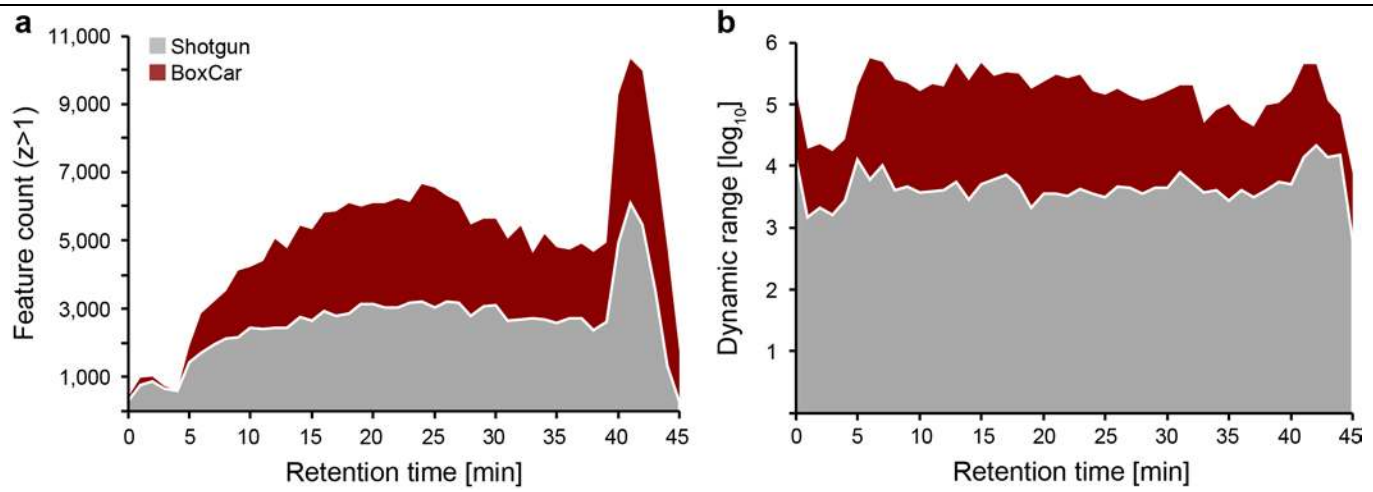


Supplementary Figure 1

Investigation of key BoxCar acquisition parameters using a D-optimal Design of Experiment to model linear and quadratic effects.

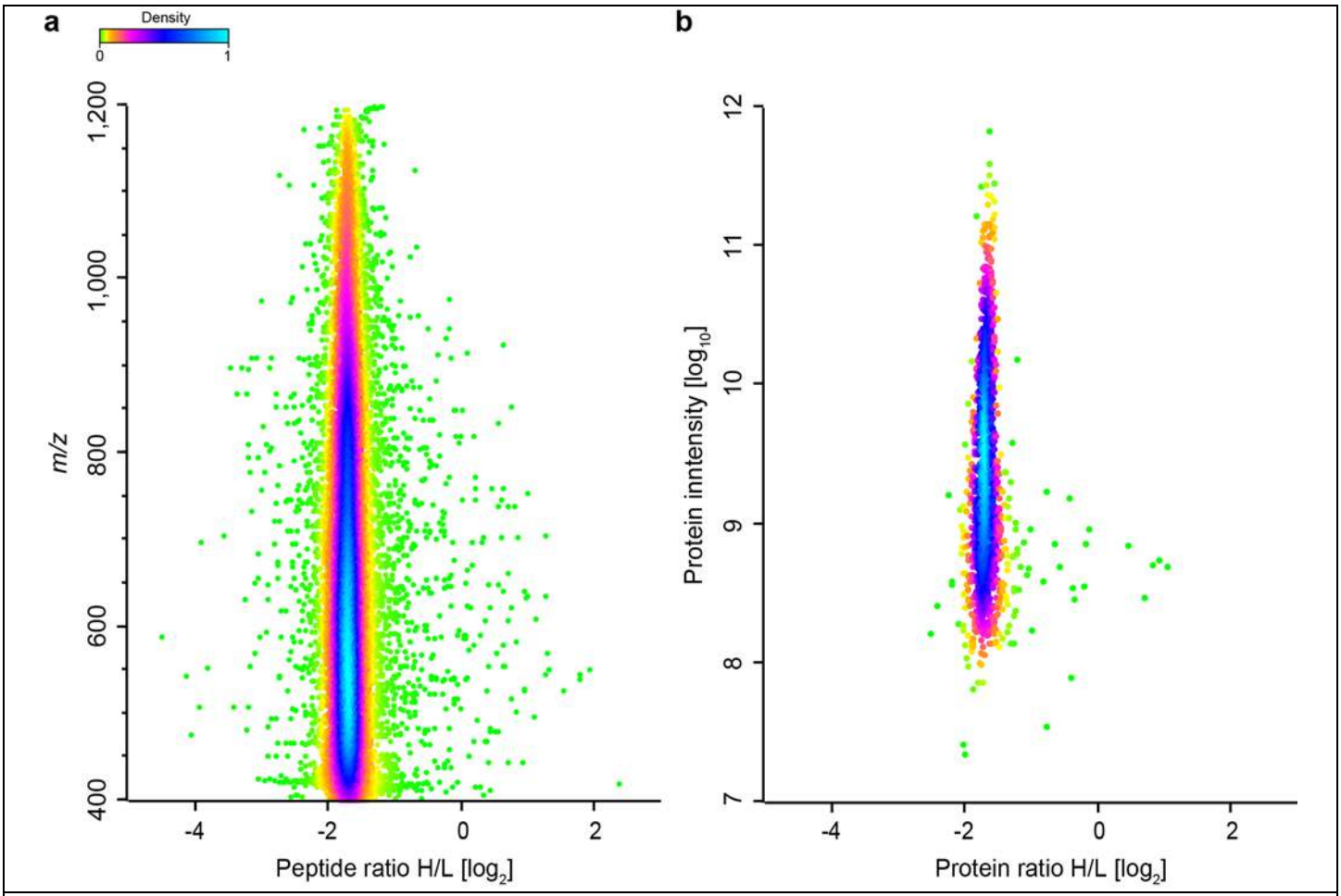
The objective of the DoE was to maximize the number of detected peptide features (*m/z* 400-1200, z>1) in 45 min runs of 1 µg HeLa digest. The 4D response contour plot illustrates the effect of the following factors: the number of BoxCar scans per cycle (x axis), the number of boxes per scan (y axis) as well as the effect of the maximum ion injection time in percent of the transient time (Fill) for a resolution of (a) 60,000 and (b) 120,000 at *m/z* 200. The results indicate that the number of features increases with the maximum fill time, which is in accordance with the expected increase in dynamic range and improved signal-to-noise ratios. Furthermore, the benefits of increasing the resolving power overcompensate the downside of lengthening the cycle time. The effects of varying the number of BoxCar scans and boxes were less prominent, however, the results imply that a combination of three scans with about 12 boxes each yields best performance. Stars indicate the settings used for data acquisition in the present study for 45 min (white) and 100 min gradients (green).



Supplementary Figure 2

Feature detection in BoxCar and standard full scans.

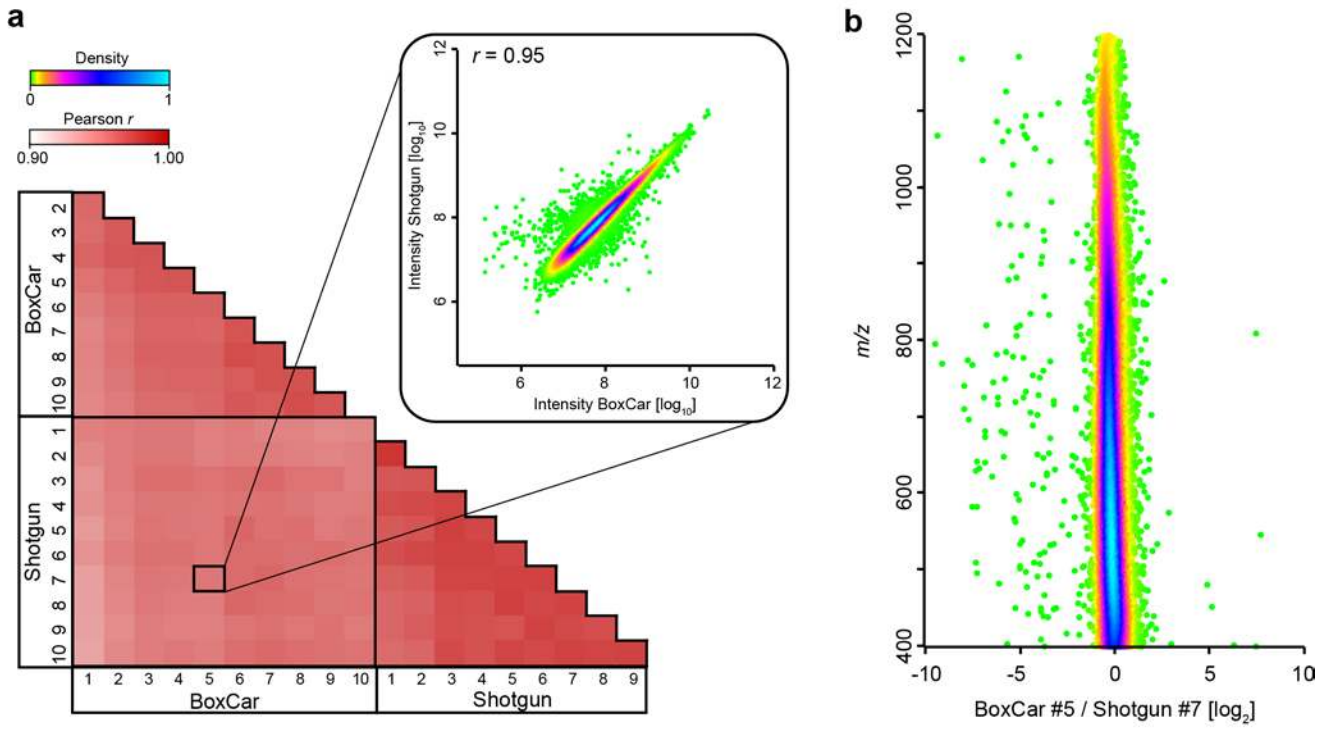
(a) Number of detected multiply charged features from 1 µg HeLa digest over a 45 min gradient with the standard and BoxCar method. (b) Dynamic range of the detected features as a function of retention time.



Supplementary Figure 3

Stable-isotope label based quantification with BoxCar.

Quantification of (a) peptide (N=24,487) and (b) protein (N=1,647) ratios from a human cancer cell line in a two-channel SILAC experiment, acquired in triplicate single runs with the BoxCar method and applying the intensity correction as described in the main text. The heavy and light channels were mixed in a 1:3 ratio, which is accurately reflected in the density plots.

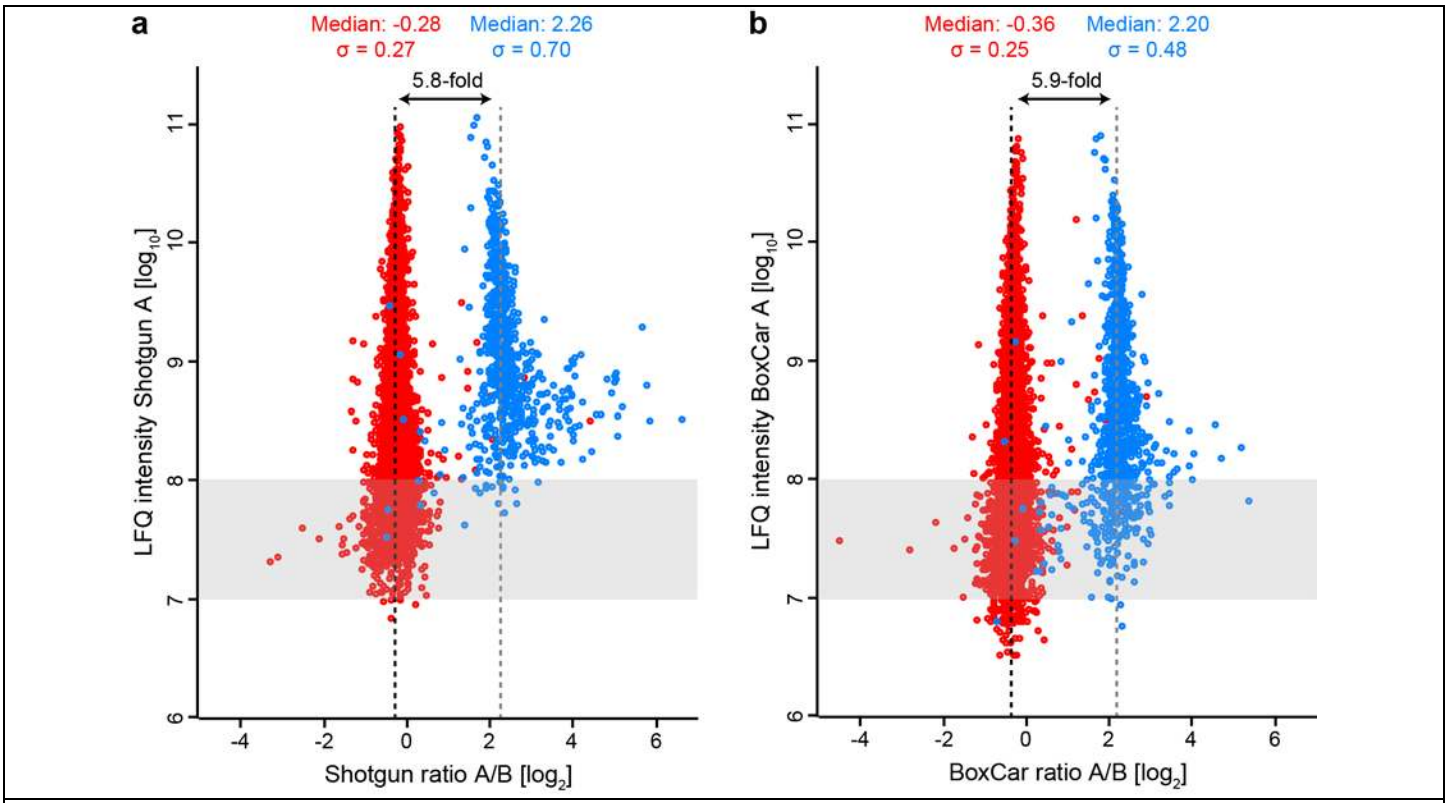


Supplementary Figure 4

Peptide label-free quantification in ten replicate 45 min runs with the BoxCar method and our standard shotgun method.

(a) Pearson correlation analysis of the non-normalized peptide intensities. The median pairwise correlation coefficients were 0.96 and 0.97 for BoxCar and shotgun replicates, and 0.95 for cross-correlated pairs (total N=36,736 peptides).

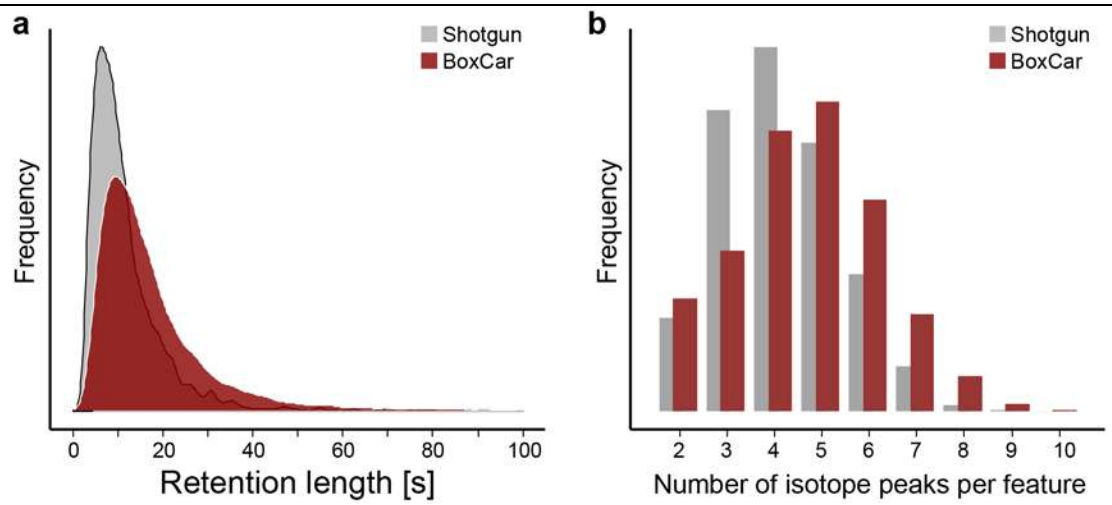
(b) Pairwise peptide feature intensity ratios of a representative BoxCar/shotgun pair as a function of *m/z* (N=30,924).



Supplementary Figure 5

Label-free quantification benchmark.

E.coli lysate was mixed with a human cancer cell line (HeLa) lysate in 1:2 and 1:12 ratios (peptide w/w, E.coli : HeLa). The scatter plot indicates median MaxLFQ ratios of human (red) and E.coli (blue) proteins that were fully quantified in triplicate single runs (N=3) of each sample with the (a) shotgun (N=5,214 proteins) and (b) BoxCar (N=5,699 proteins) acquisition method. One-sided student's t-test returns in total 962 significantly changing E.coli proteins at a permutation-based FDR below 0.05 for BoxCar, which is 35% more than with the standard method.



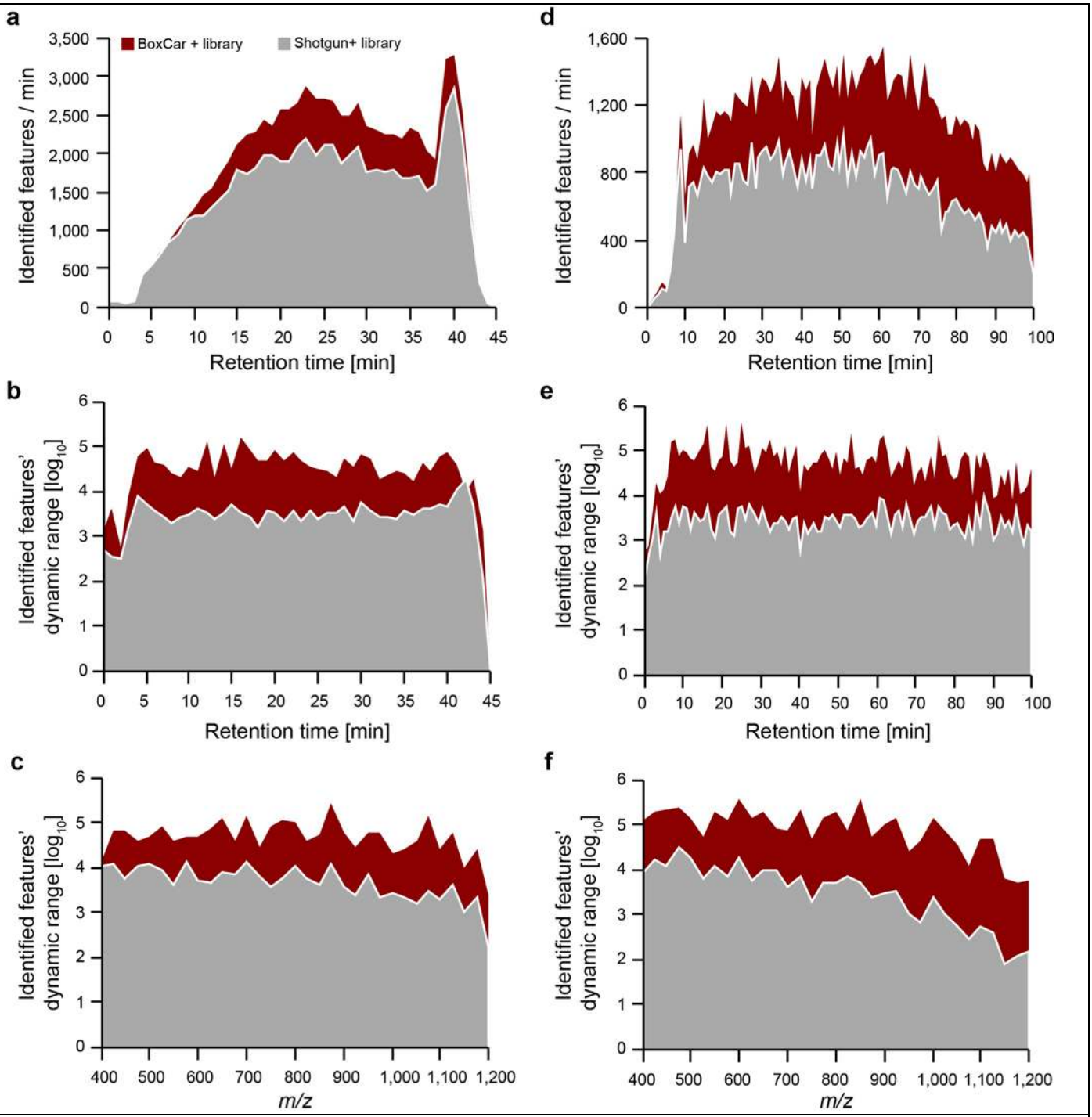
Supplementary Figure 6

Dynamic range in clinical samples.

Comparison of isotope patterns (features) from human plasma samples that were commonly identified and quantified in triplicate 45 min shotgun and BoxCar runs (*m/z* 400-1200, N=11,918 for each).

(a) Detection time per feature as a measure of sensitivity.

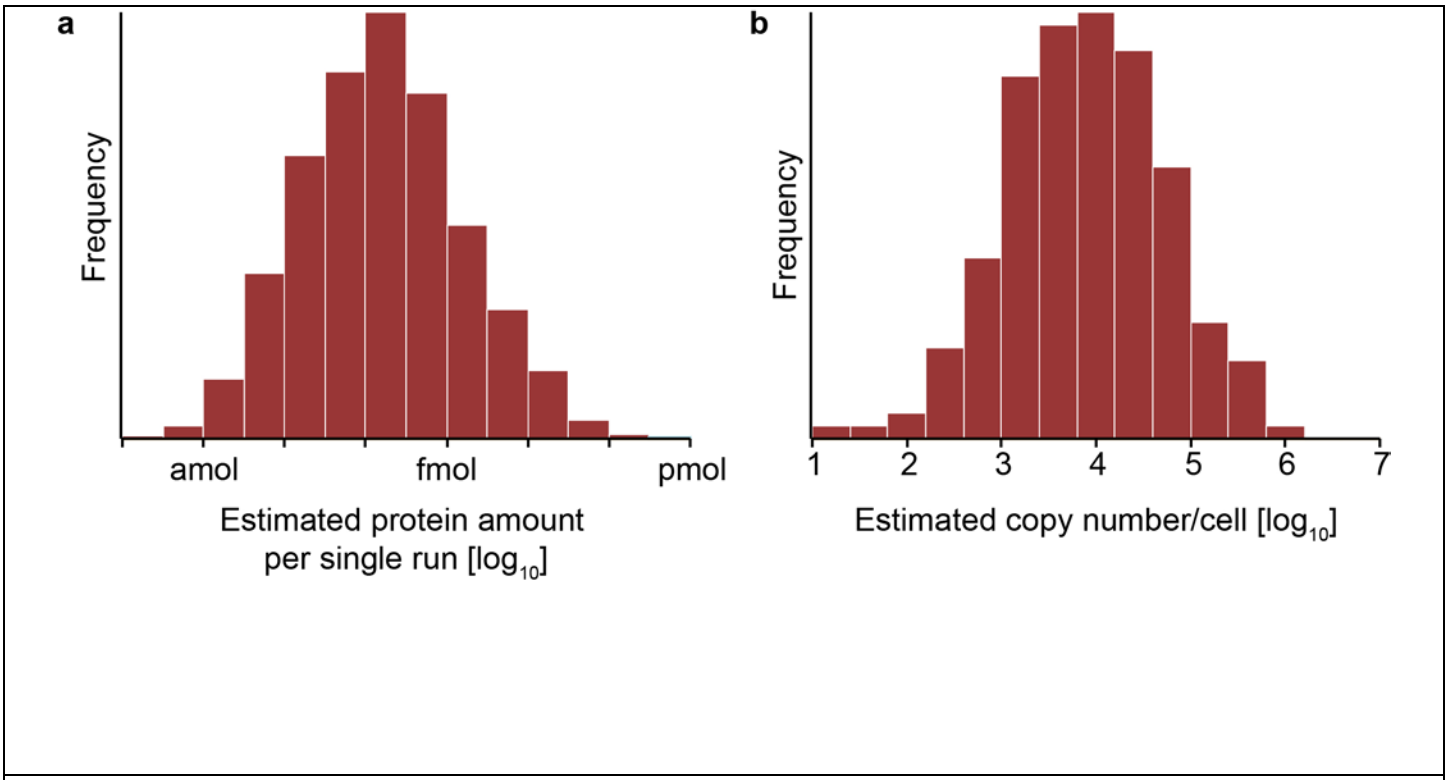
(b) Number of detected isotope peaks as a measure of intra-scan dynamic range.



Supplementary Figure 7

Single run protein quantification with peptide libraries.

Comparison of the number and dynamic range of identified features by matching from a deep library into single shotgun (grey) and BoxCar (red) runs. (a-c) Analysis of a human cancer cell line digest in a 45 min gradient. (d-f) Analysis of a mouse cerebellum digest in a 100 min single run.



Supplementary Figure 8

Absolute abundance range of mouse cerebellum proteins quantified in 100 min BoxCar single runs.

(a) Estimated protein amount on the analytical column per 1 µg peptide injection (N=10,569).

(b) Estimated copy numbers per cell for proteins annotated with the GOCC term ‘transcription factor complexes’ (N=185).