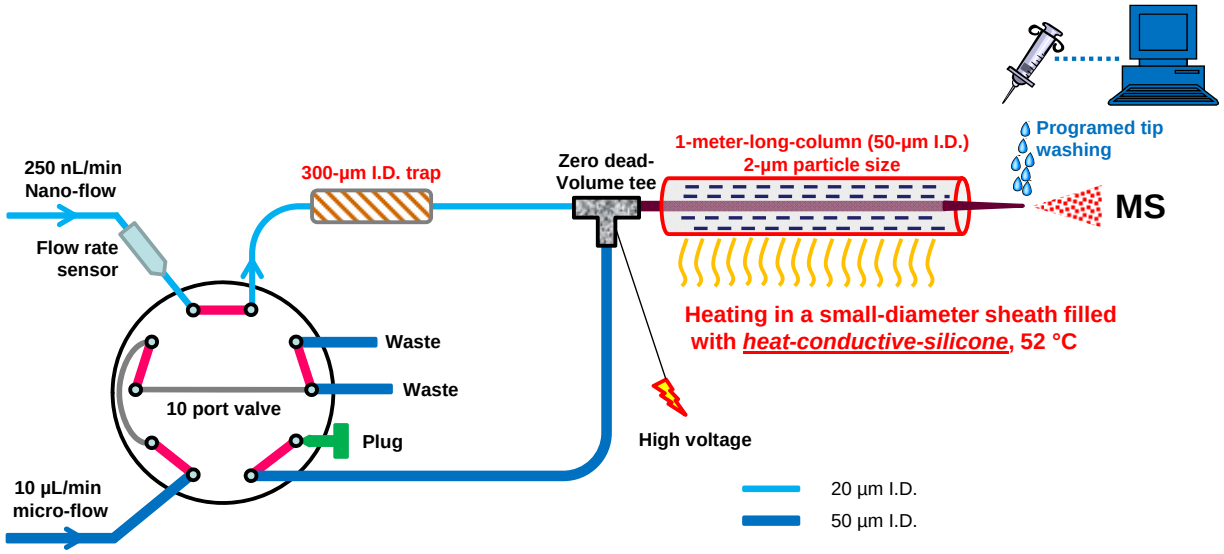
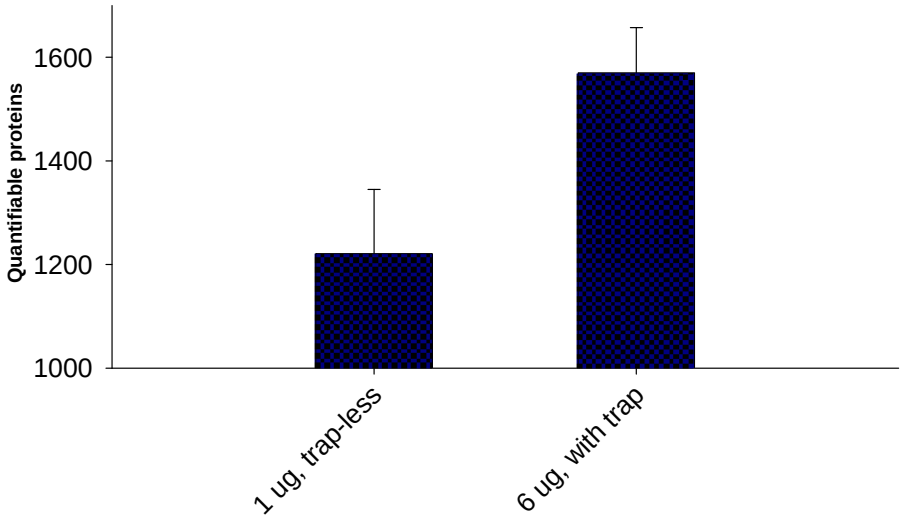
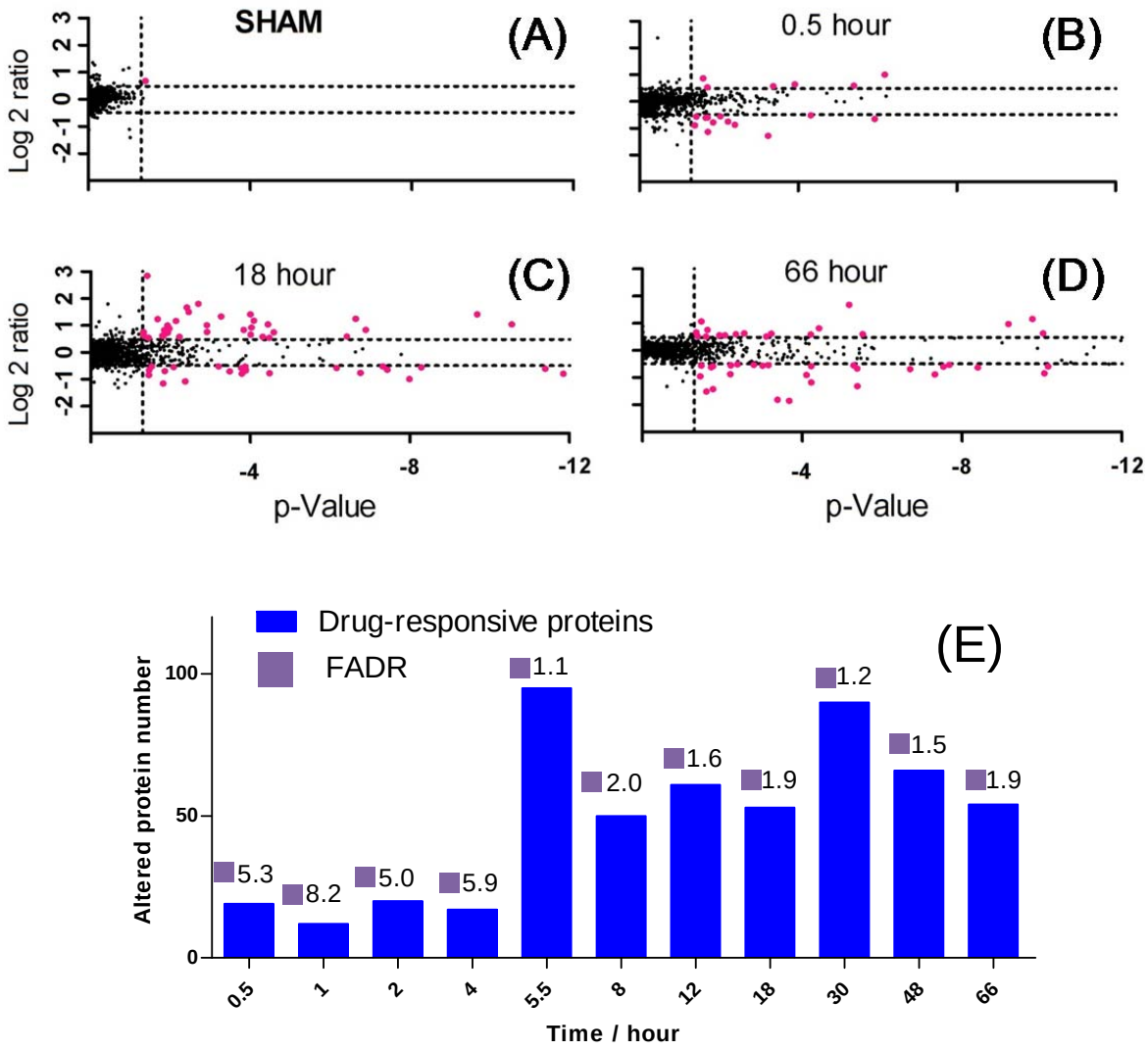
Supplementary Figures



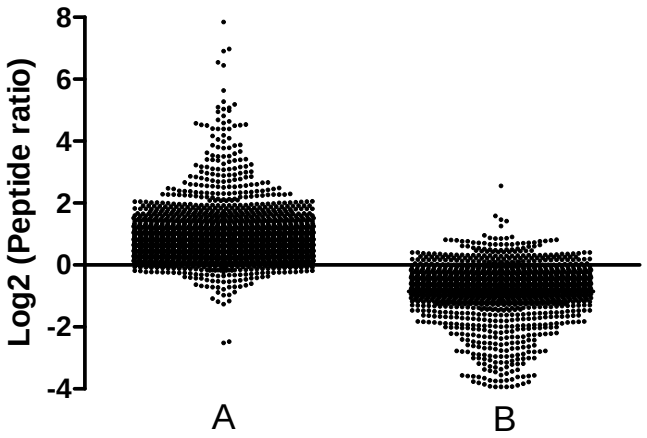
SI Figure S1. The scheme of a high-resolution, reproducible and sensitive nano-LC/MS configuration. The chromatographic approach enables excellent chromatographic resolution and run-to-run reproducibility due to the following features: *i)* separation on a long column (100-cm-long and 50-µm ID) with small particles (Pepmap 2-µm C18, 100 Å) under high pressure (~9,000-11,000 p.s.i. with heating) affords high-resolution separation of tissue samples. *ii)* A unique packing procedure was developed to ensure homogenous and solid packing of the long column. The sorbent flurry was packed to the column from both ends, three cycles per direction; for each cycle, packing pressure was ~12, 000 p.s.i. at 24 °C for 1 hr, followed by controlled gradient venting for 8 hr under constant temperature and humidity. High-pressure, 0.5-mm-long frits (High-Pressure-Frits, patent pending) were placed at both ends to constrain the packed sorbent. Upon completion of packing, a Laser Tip Puller (Sutter Instrument) was used to produce a ~3-5-µm non-coated tip with the fused frit inside. Quality of packing was confirmed by close examination under a microscope, where no void should be found. *iii)* The direct trap-column connection without extra in-valve volume improves peak shapes and reduces tailing. *iv)*The column was homogenously heated to improve the separation of hydrophilic peptides, the reproducibility among consecutive runs and to reduce column pressure. To achieve homogenous heating, the column was folded in a heating sheath, fully filled with heat-conductive silicone. This approach was found to provide far superior run-to-run reproducibility for nano-LC separation than using a column oven. *v)* The use of a large trap-vs.-column ID ratio (6:1) achieves substantially dampened pump delivery variation (i.e. the prevalent “pump noise” on nano-flow pumps) and improved gradient mixing on nanoflow scale (due to the significant volume of the upstream trap), and thus greatly improving reproducibility of mobile phase delivery. The large-ID trap didn’t cause perceivable peak-broadening, because the use of retrograde directions of loading and analysis flows permits minimal peak broadening, and that the C18 material in trap (Agilent SB, 3.5µm) is slightly weaker than that from the column (Dionex Pepmap 2µm), which leads peak compression effect at the inlet of the column. *vi)*Between two runs, the non-coated tip of the fused silica column was subjected to a programmed washing for 10 min (350 µL/min) using 50% methanol with 0.1% formic acid, with a syringe pump triggered by a contact closure signal sent from the Spark autosampler, before the trap is switched in line with the nano-column. This approach drastically improved the stability of ionization over a long period of analysis (e.g. >60 runs over 20 days). Prior to analysis of the large batch of time-course samples, the newly-packed column was “aged” under the gradient conditions (below) by triplicate runs of a pooled liver digest sample for ~21 hr.



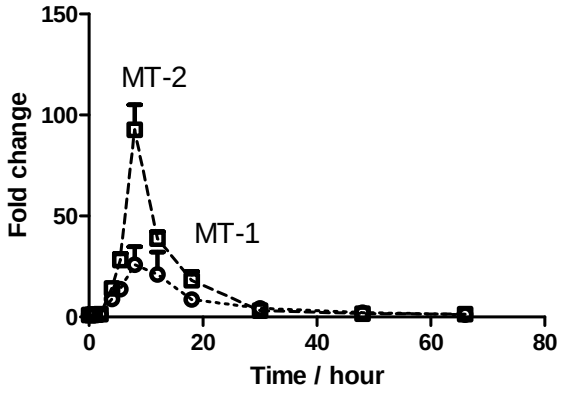
SI Figure S2. Comparison of the number of quantifiable proteins in in rat liver (criteria to define the "quantifiable proteins" can be found the manuscript) with 1-µg sample loadings without trap vs. 6-µg sample loadings with a 300-µm-ID trap (n=3).



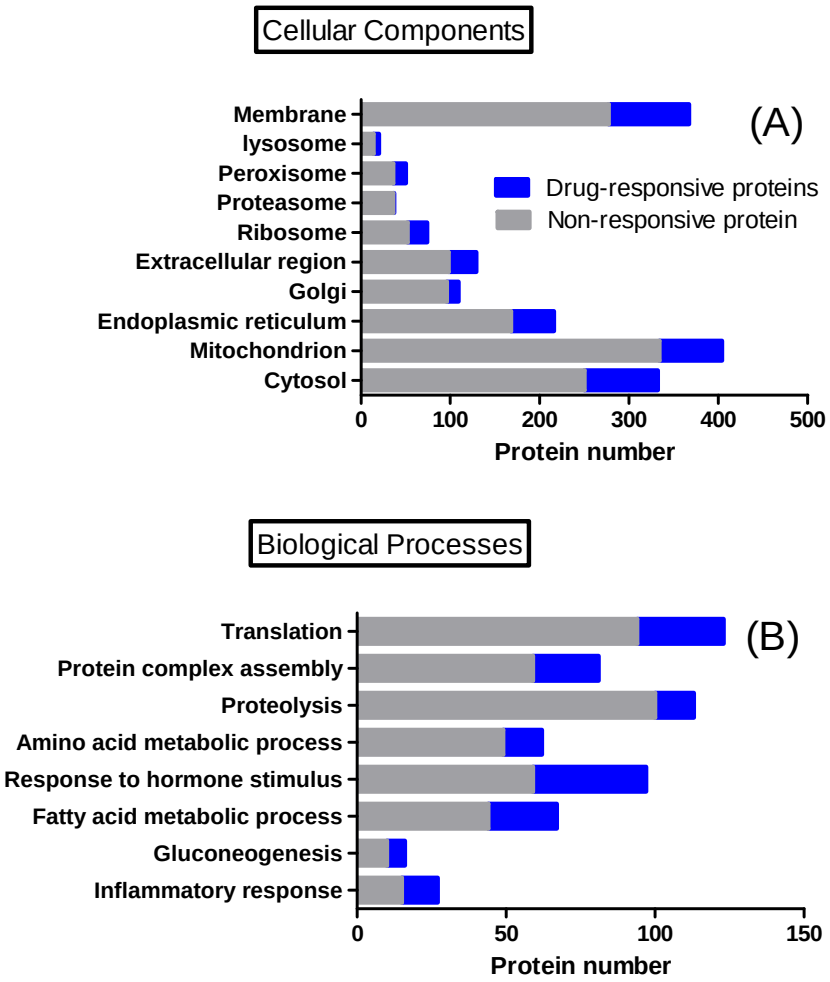
SI Figure S3. Measurement of false-altered protein discovery rates (FADR). Representative Volcano Plots (fold-of-changes vs p values): (A) Sham sample set (5 control vs 5 control animals, the biological and technical null sample set), (B) 0.5 h, (C) 18 h and (D) 66 h after dosing with MPL (*n*=5 per group). Red dots denote the significantly-altered proteins under the optimal cutoff thresholds (1.5-fold change and $p<0.05$). Under these criteria, the number of altered proteins and the corresponding FADR in each time point is shown in (E).



SI Figure S5. Constant protein and peptide ratios in the 323 significantly-altered proteins. The ratios of all peptides in the elevated proteins (A) and decreased proteins (B) are shown.



SI Figure S6. Time profiles for the metallothionein proteins, which are involved in the metal ion homeostasis and strongly up-regulated by MPL.



SI Figure S7. Cellular components (A) and biological processes (B) for the quantified proteins. Drug-responsive proteins are designated with blue color, while proteins not found as drug-responsive are in grey.