Supplementary Figures

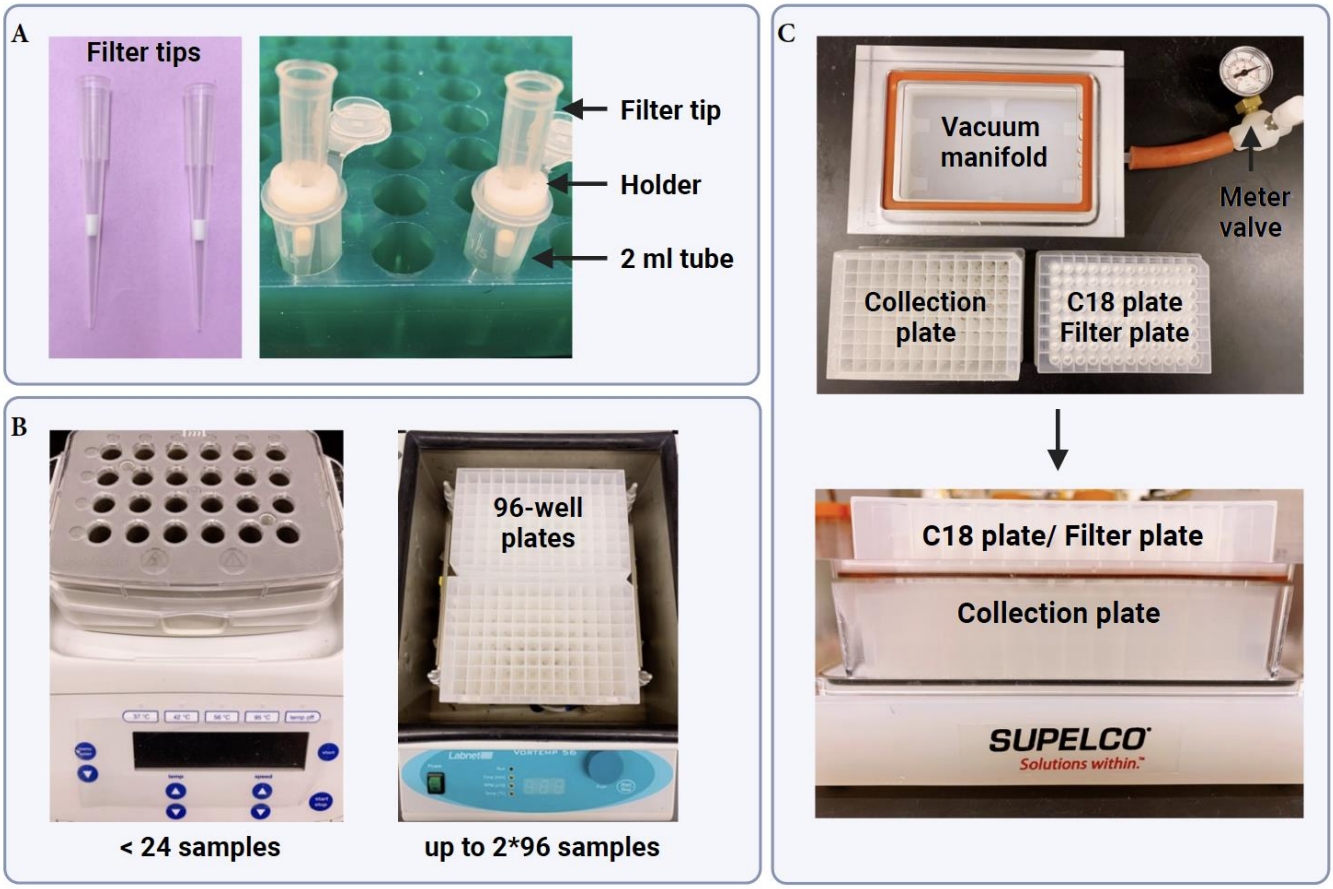

Fig. S1 Tricks from low-throughput to high-throughput workflows in peptide purification and phosphopeptide enrichment. **A** The filter tips assembled for single phosphopeptide enrichment, individual filter tips (left panel) and assembled filter tips (right panel). **B** The 24-well Eppendorf thermo mixture instrument (left); 96-well plate based protein digestion instrument (right). **C** The vacuum manifold system for the large-scale peptide purification and phosphopeptides enrichment

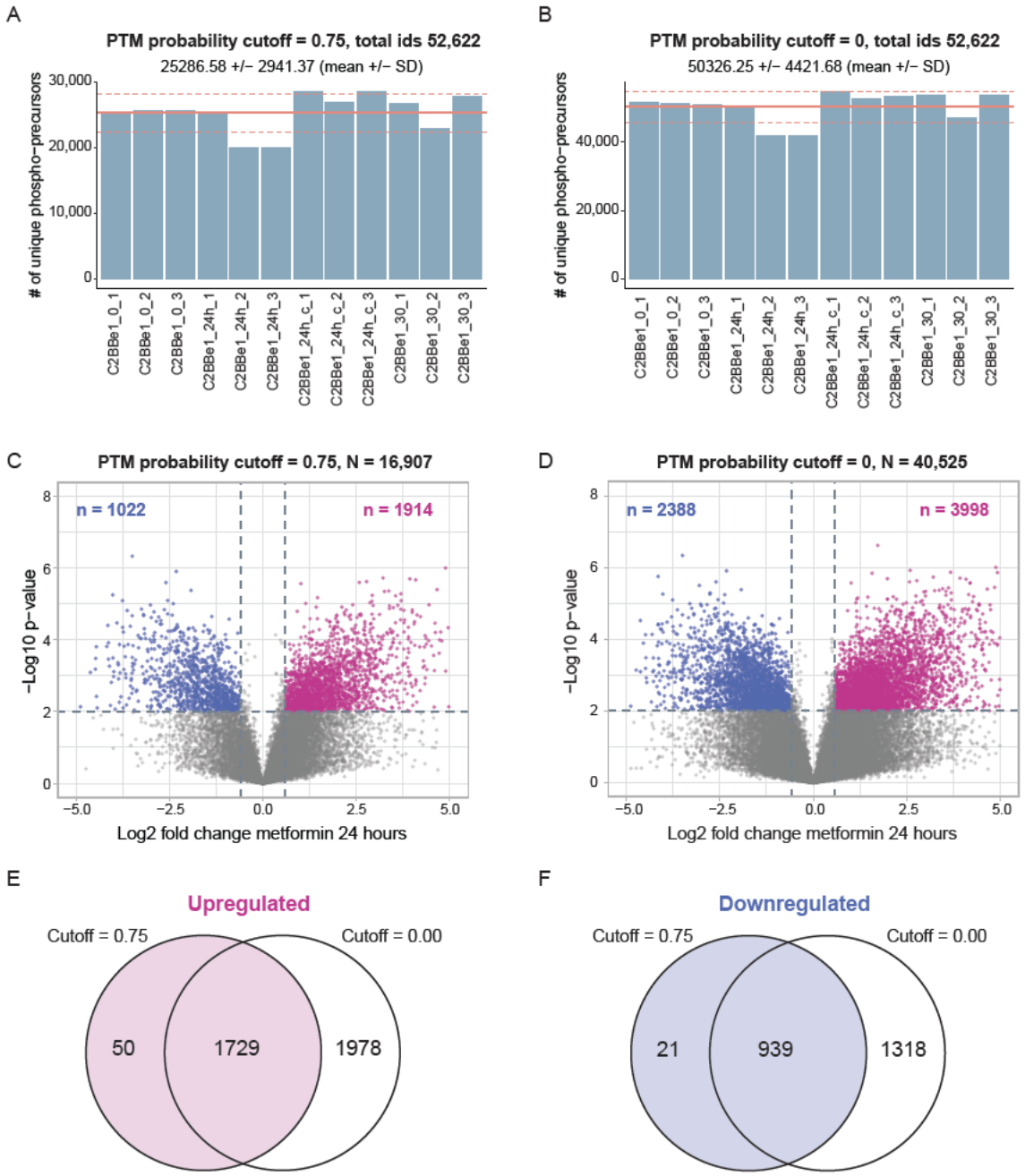

Fig. S2 Improvement in phosphoproteomics data completeness using our workflow employing two different PTM cutoffs. The presented data were extracted from our dataset published previously (Salovska *et al.* 2023). **A,B** Number of quantified phospho-precursors in each MS run after application of the PTM probability cutoff of 0.75 **(A)** and 0.00 **(B)** in Spectronaut. Panels A and B show the same unique phospho-precursor ids (PTM probability cutoff < 0.75 for identification, N = 52,622). **C,D** Numbers of phospho-precursors with significant change in abundance after 24 h of metformin treatment compared to an untreated control sample in the C2BBe1 cell line (two-sample t-test $p < 0.01$, absolute fold change > 1.5). N = 16,907 and N = 40,525 indicate the number of phospho-precursors that passed a filtering based on valid values (at least 2 replicate values in both compared groups) and were used for the statistical analysis. **E,F** Overlap between significantly up- and down-regulated phopsho-precursors corresponding to the volcano plots in Panels C and D