Supplemental data:

A systematic comparison of label-free, SILAC and TMT techniques to study early adaption towards inhibition of EGFR signaling in the colorectal cancer cell line Difi

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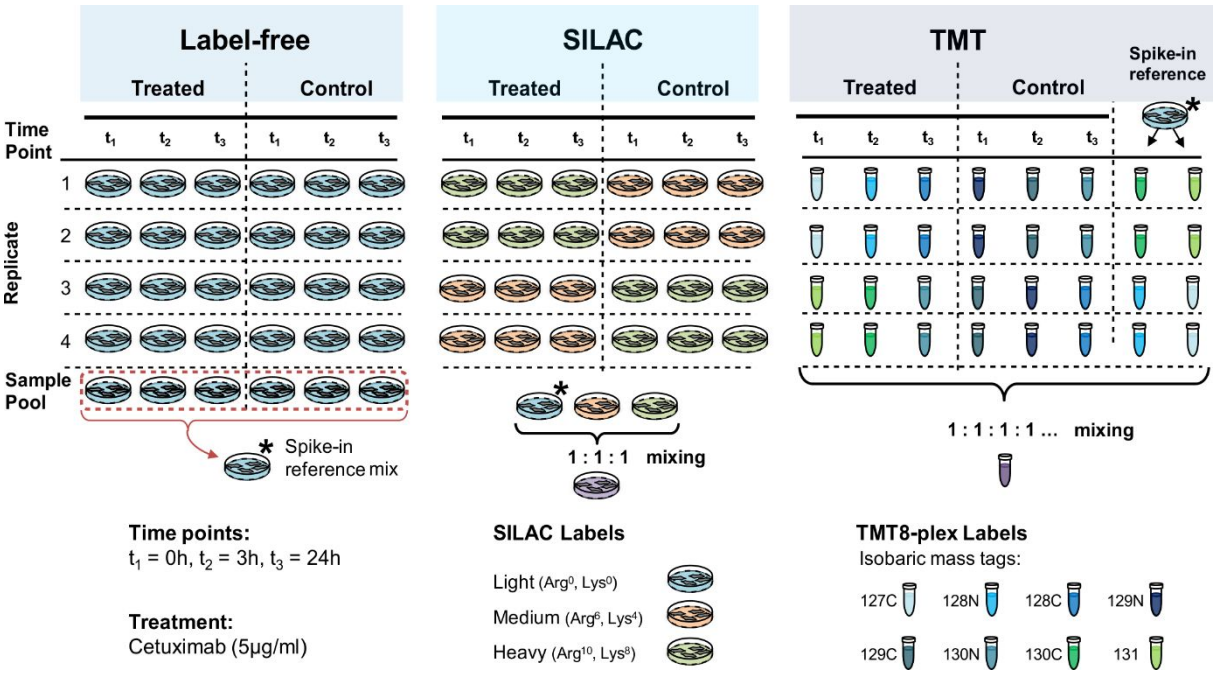
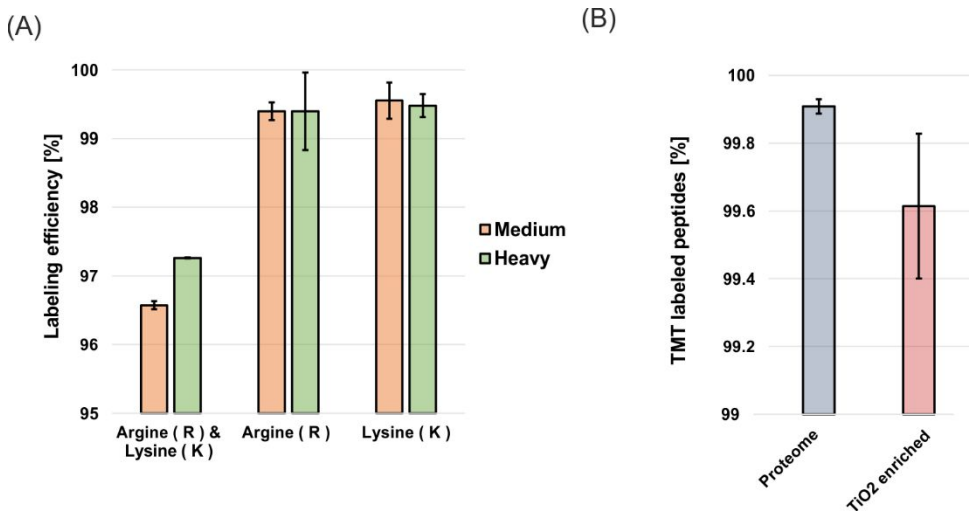
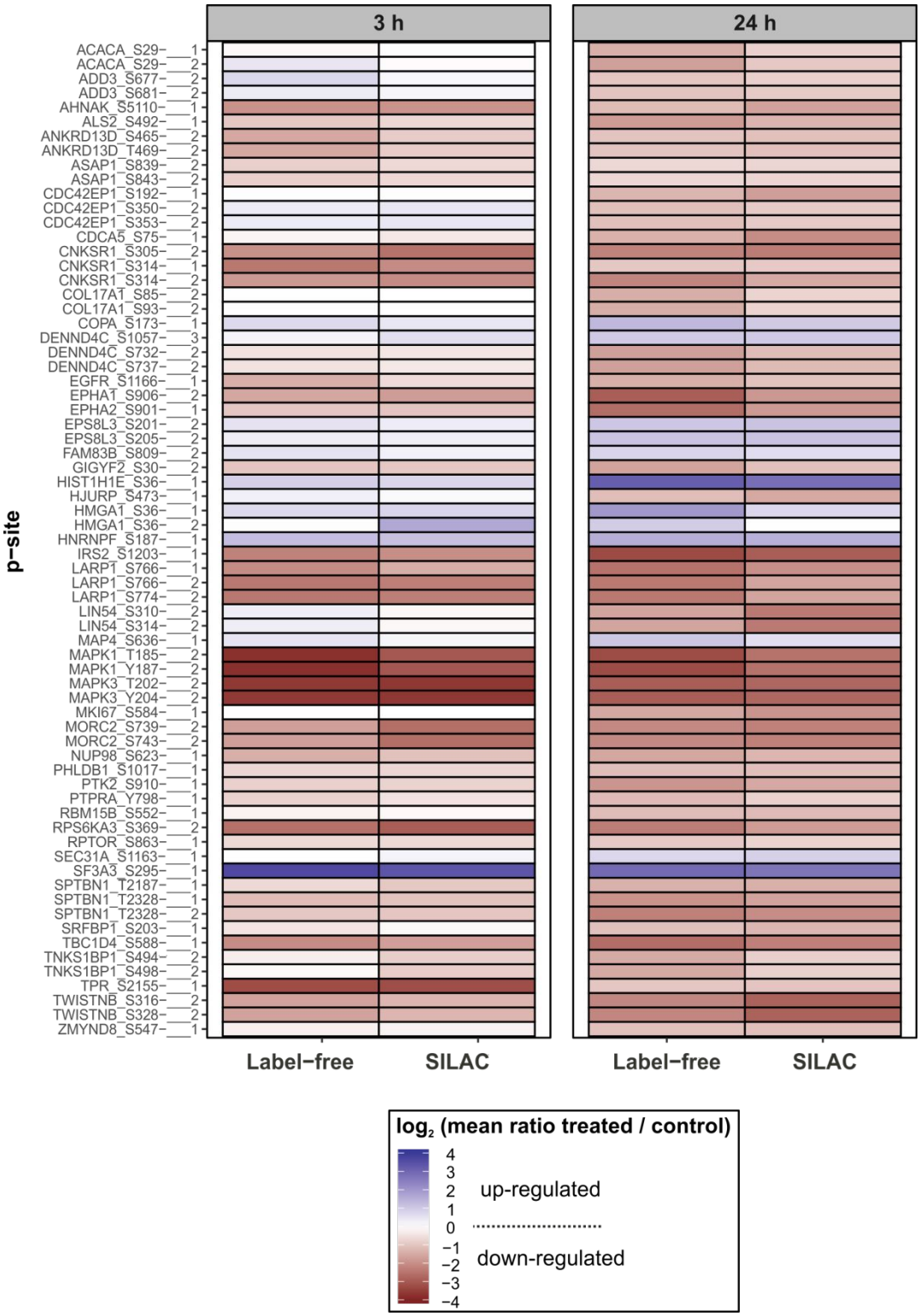
The Supplemental Tables are available as Excel-files:



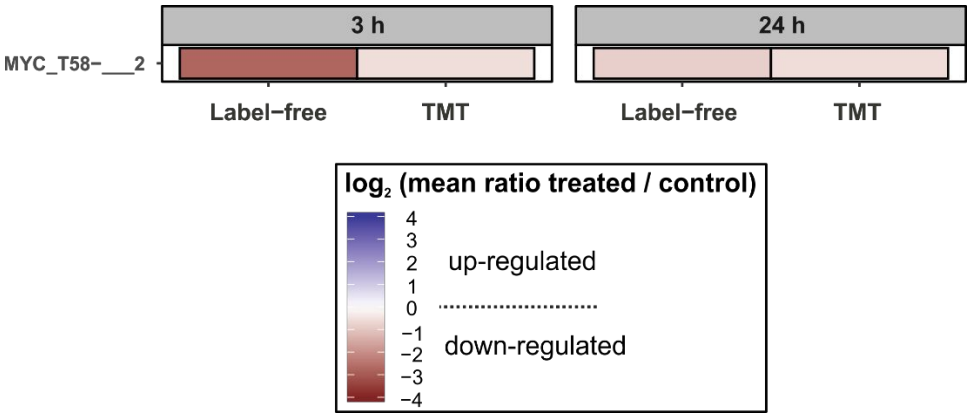
Supplemental Figure S1: Schematic representation of the applied labelling strategy. For label-free quantification (LF) all experimental replicates were processed and analyzed individually. SILAC samples were metabolically labeled using three different states. The experimental replicates were mixed directly after sampling and subsequently processed in combination. For TMT, eight different channels were used. The samples were digested using trypsin and labelled with TMT reagents individually, before they were mixed and processed together.



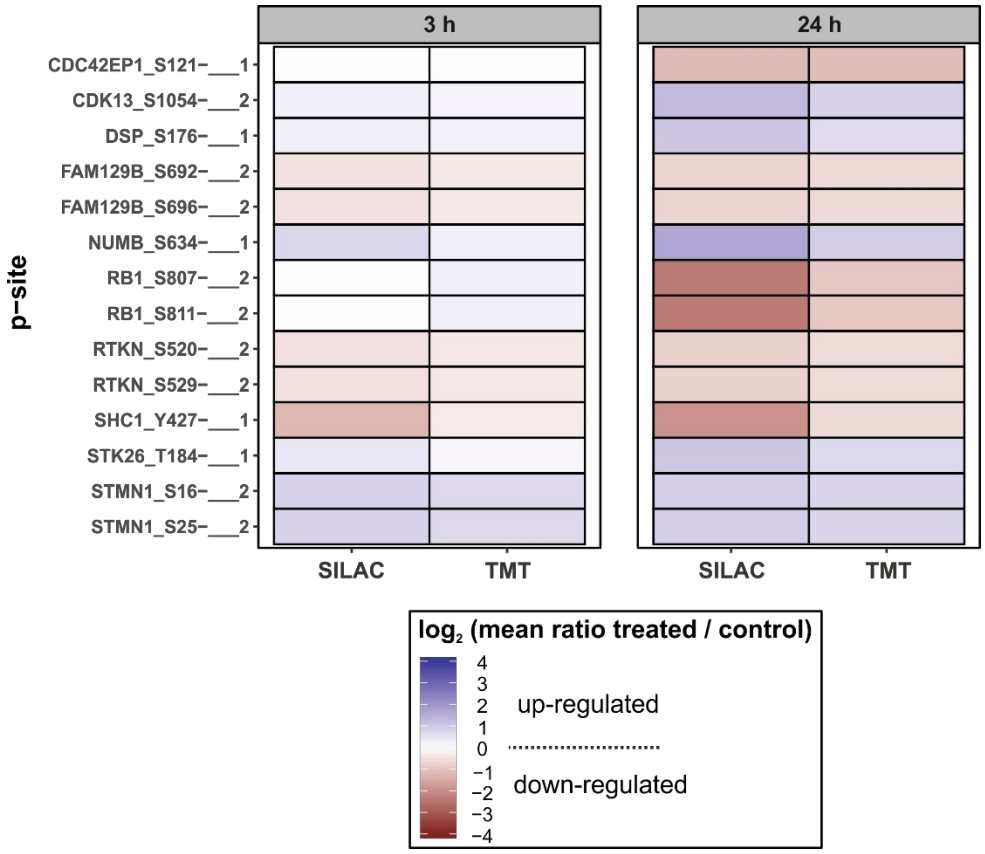
Supplemental Figure S2: Labelling efficiencies for SILAC and TMT labelled samples. (A) SILAC labelling efficiencies are shown as mean values with standard deviations (n = 2). Efficiencies are represented as fraction of labelled peptides (%)with respect to the respective peptide species. Samples were generated after 12 days of SILAC labelling (5 -6 passages in cell culture). **(B)** TMT labelling efficiencies are shown as mean values with standard deviation (n = 4) for proteome samples and TiO2 enriched peptide samples. Efficiencies (%) were calculated based on peptide numbers measured in the performed experiment.



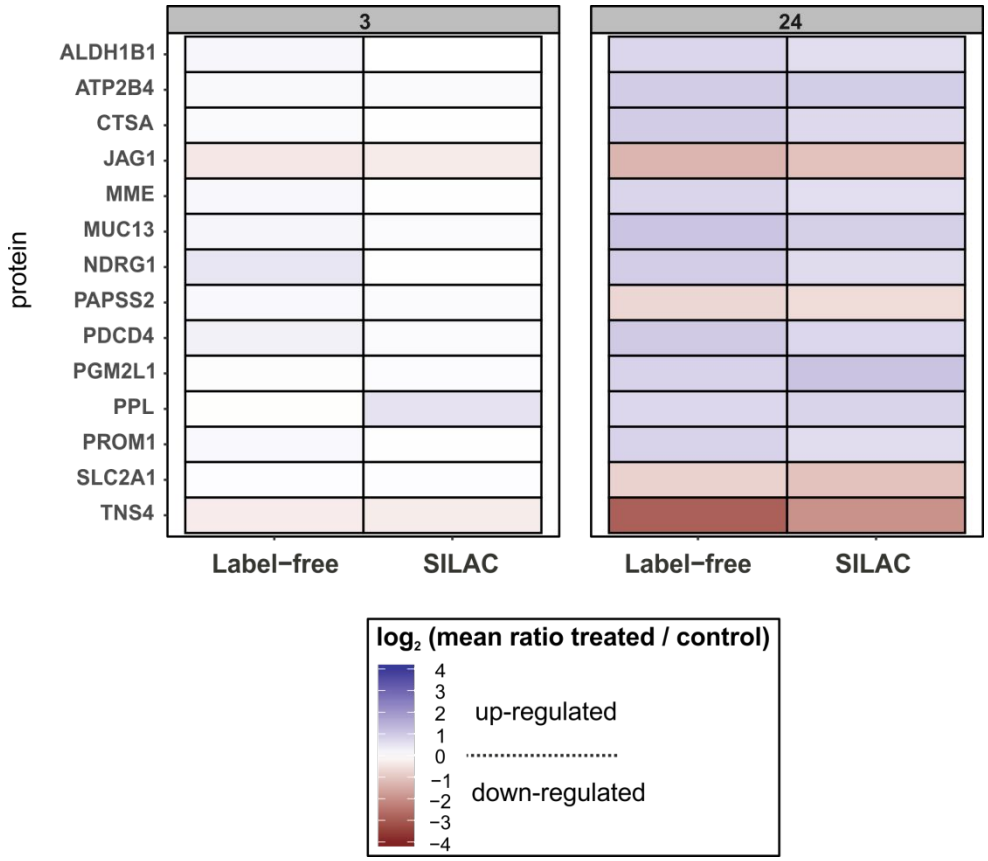
Supplemental Figure S3: Ratios of p-sites regulated in label-free and SILAC quantification. Ratios between treated and control samples at 3 and 24 hours for p-sites found to be regulated in label-free and SILAC approaches. P-sites displayed with gene name, amino acid, position, and multiplicity information.



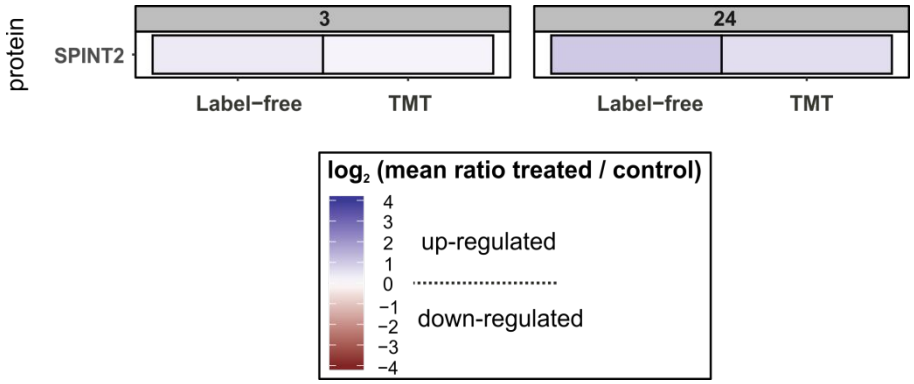
Supplemental Figure S4: Ratios of p-sites regulated in label-free and TMT quantification. Ratios between treated and control samples at 3 and 24 hours for p-sites found to be regulated in label-free and TMT approaches. P-sites displayed with gene name, amino acid, position, and multiplicity information.



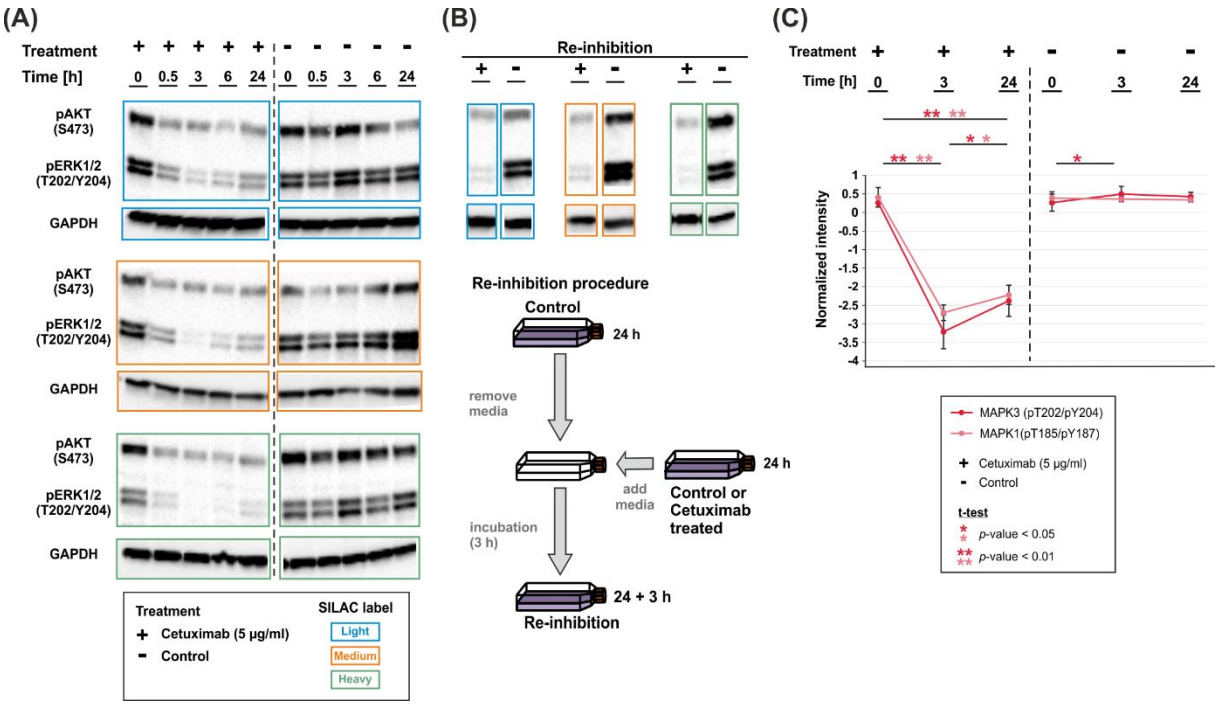
Supplemental Figure S5: Ratios of p-sites regulated in SILAC and TMT quantification. Ratios between treated and control samples at 3 and 24 hours for p-sites found to be regulated in SILAC and TMT approaches. P-sites displayed with gene name, amino acid, position, and multiplicity information.



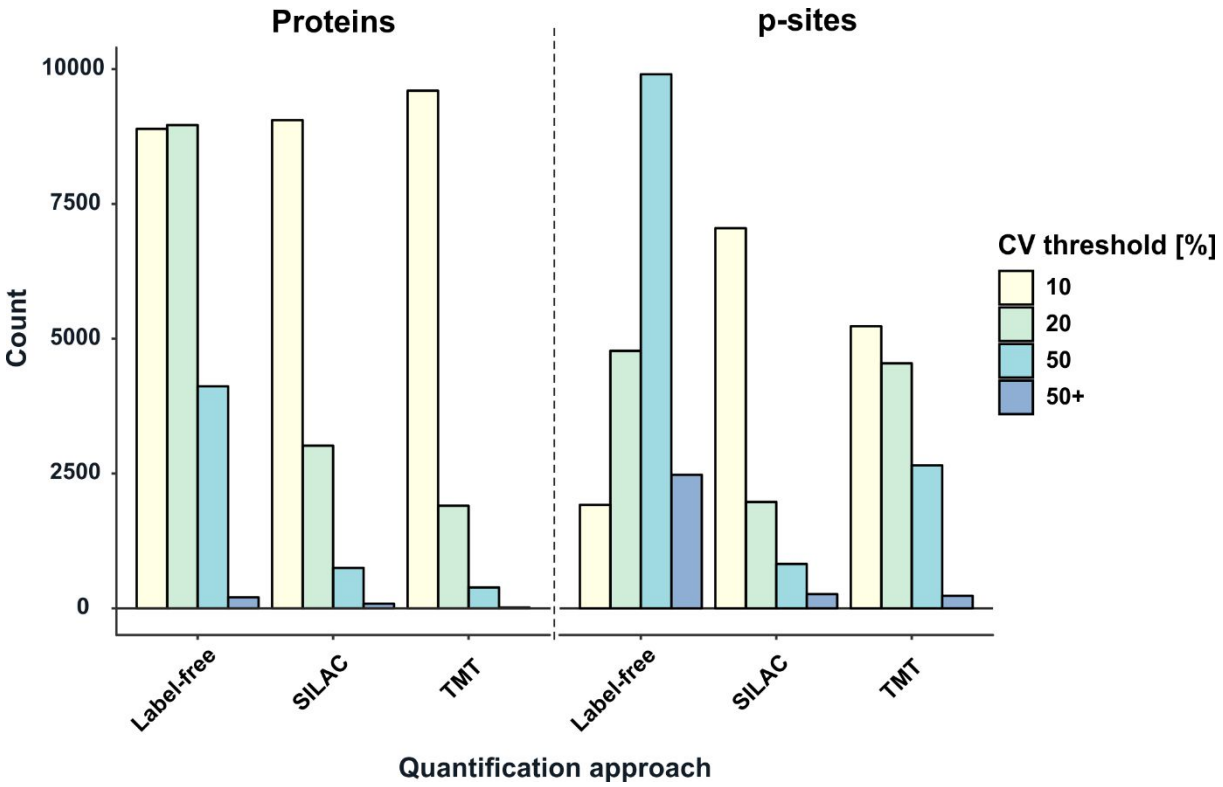
Supplemental Figure S6: Ratios of proteins regulated in label-free and SILAC quantification. Ratios between treated and control samples at 3 and 24 hours for proteins found to be regulated in label-free and SILAC approaches. Proteins indicated by gene names.



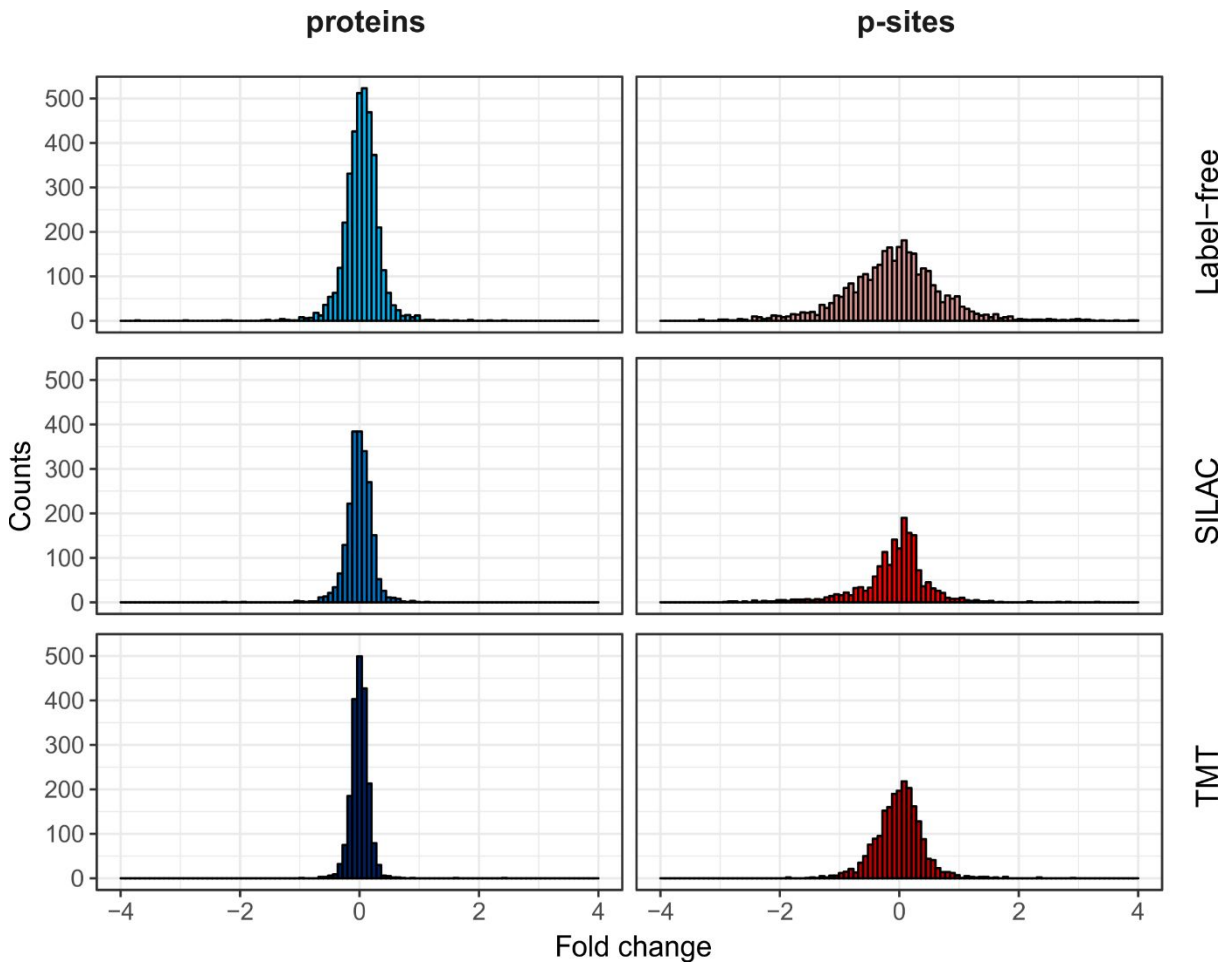
Supplemental Figure S7: Ratios of proteins regulated in label-free and TMT quantification. Ratios between treated and control samples at 3 and 24 hours for proteins found to be regulated in label-free and TMT approaches Proteins indicated by gene names.



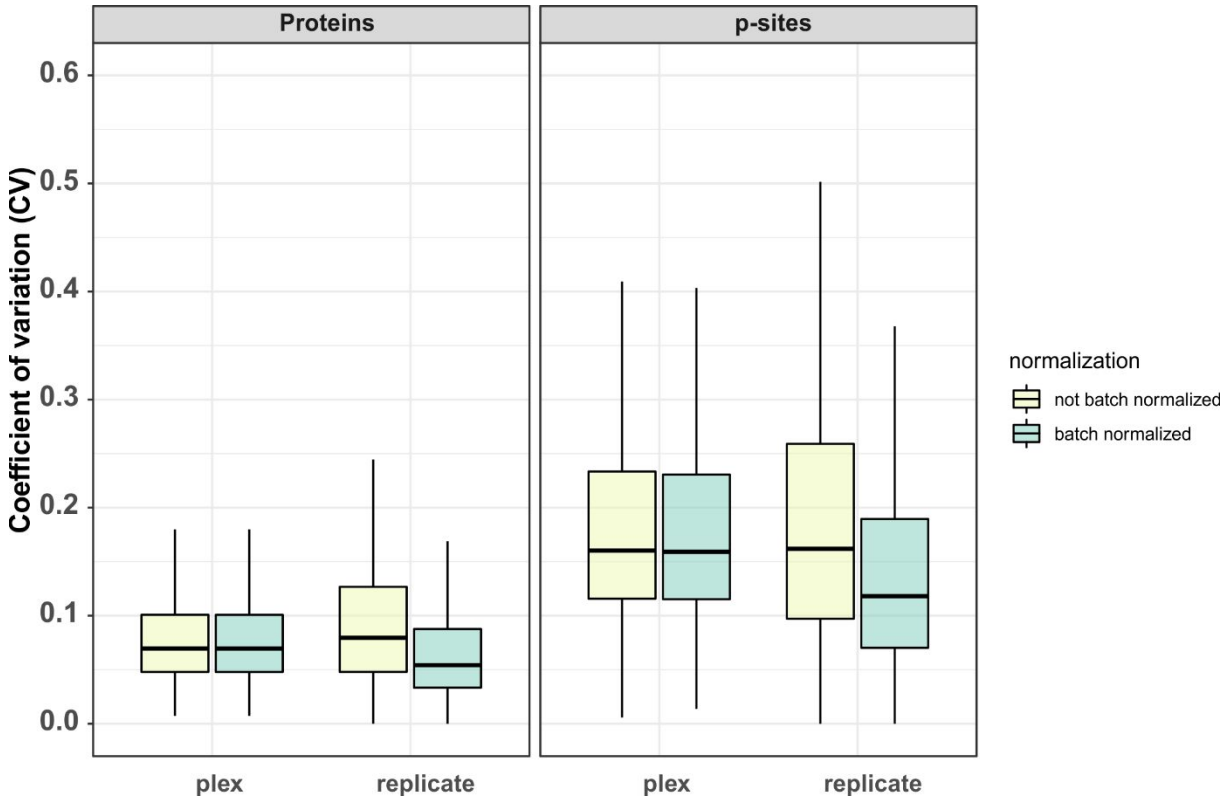
Supplemental Figure S8: Dynamics of MAPK phosphorylation upon cetuximab treatment. (A) Immunoblots showing the phosphorylation of EGFR signalling targets pAkt (Ser-473) and pErk1/2 (Thr202/Tyr204) upon treatment with cetuximab. SILAC-labelled cells (light, medium and heavy) were probed to verify similar phosphorylation patterns. The initial downregulation of pAkt and pErk was found to recover towards the 24h time point (without reaching the baseline level). GAPDH was used as loading control **(B)** Re-inhibition experiment: Cells were cultured in media with or without cetuximab. After 24 h media from control cells were replaced with media from cetuximab-treated cells and incubated for another 3 h. Cells were harvested and western blot analysis of pAkt and pErk was performed to assess the bioactivity of the cetuximab antibody after 24h incubation time. **(C)** Phosphorylation dynamics of MAPK1/3 as quantified using SILAC. The LC-MS/MS data was found to be consistent with observations made by immunoblotting. Data displayed as mean values (n=4) and standard deviation.



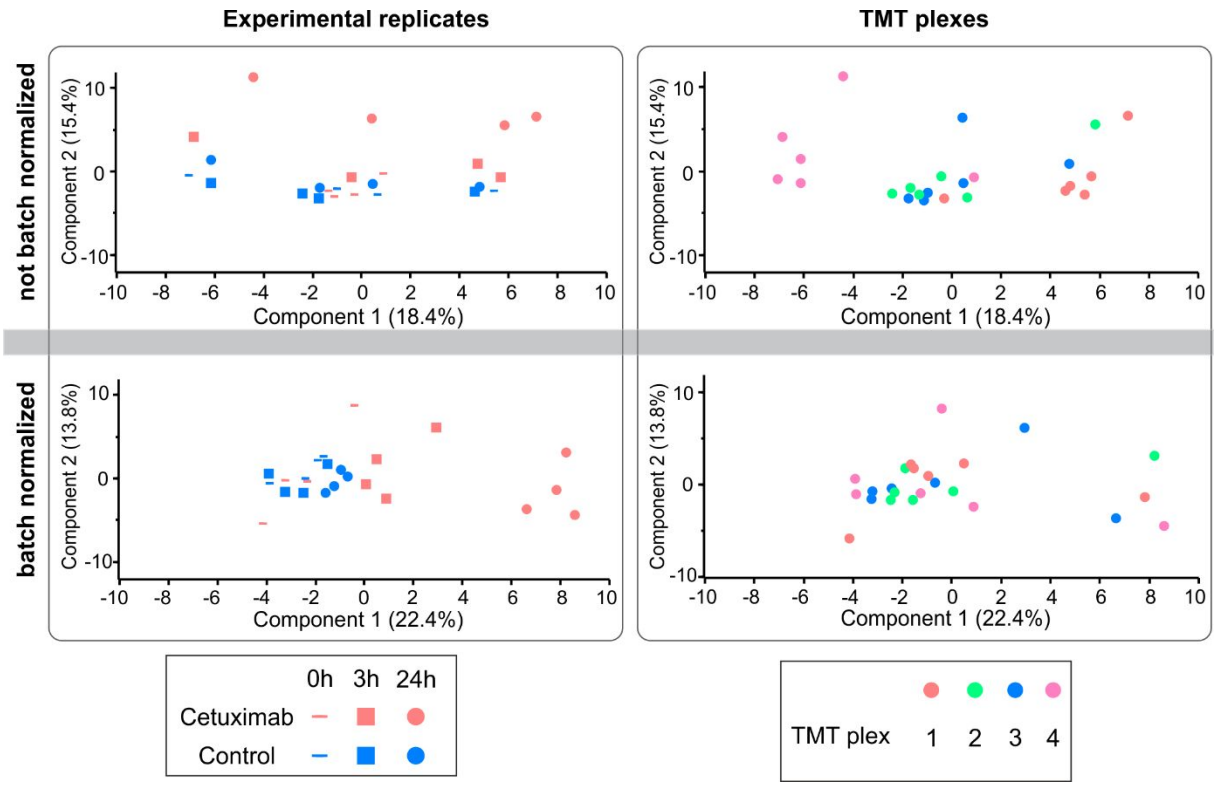
Supplemental Figure S9: Analysis of coefficients of variation. Coefficients of variation were grouped according to the CV thresholds (≤ 10%, ≤ 20%, ≤ 50%, and > 50%) and displayed for each quantification strategy and proteins and p-sites, respectively. The displayed numbers add up from three analyzed time points and do not represent the actual numbers of quantified proteins and p-sites.



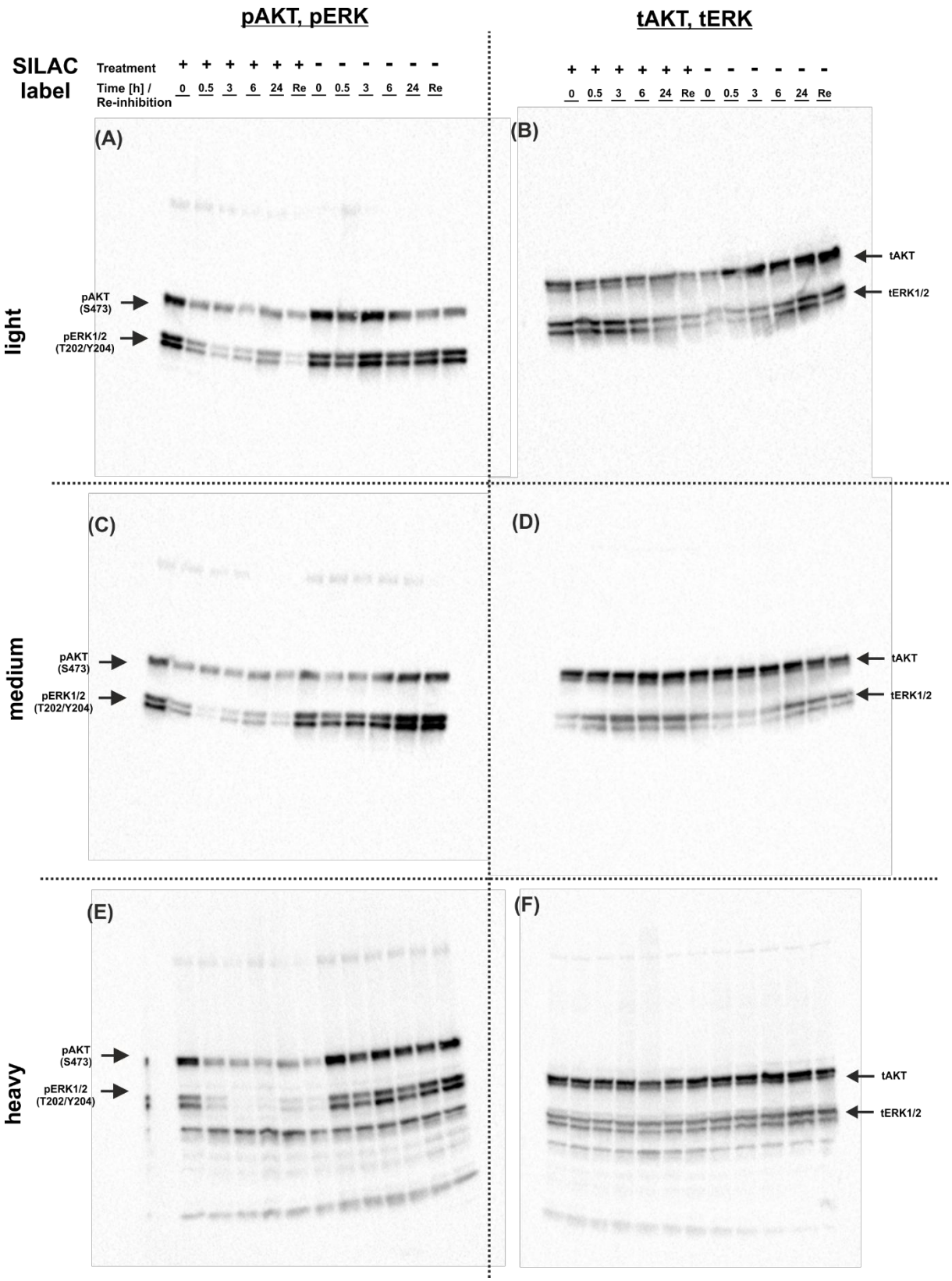
Supplemental Figure S10: Fold change distributions for LF, SILAC and TMT data. Distribution of the $\log_2$ fold changes of proteins and p-sites. Displayed for the comparison between treated and control group at t = 24 h. Histogram representation (bins = 100) for each quantification approach. Scales of x-axes are limited to -4 to 4.



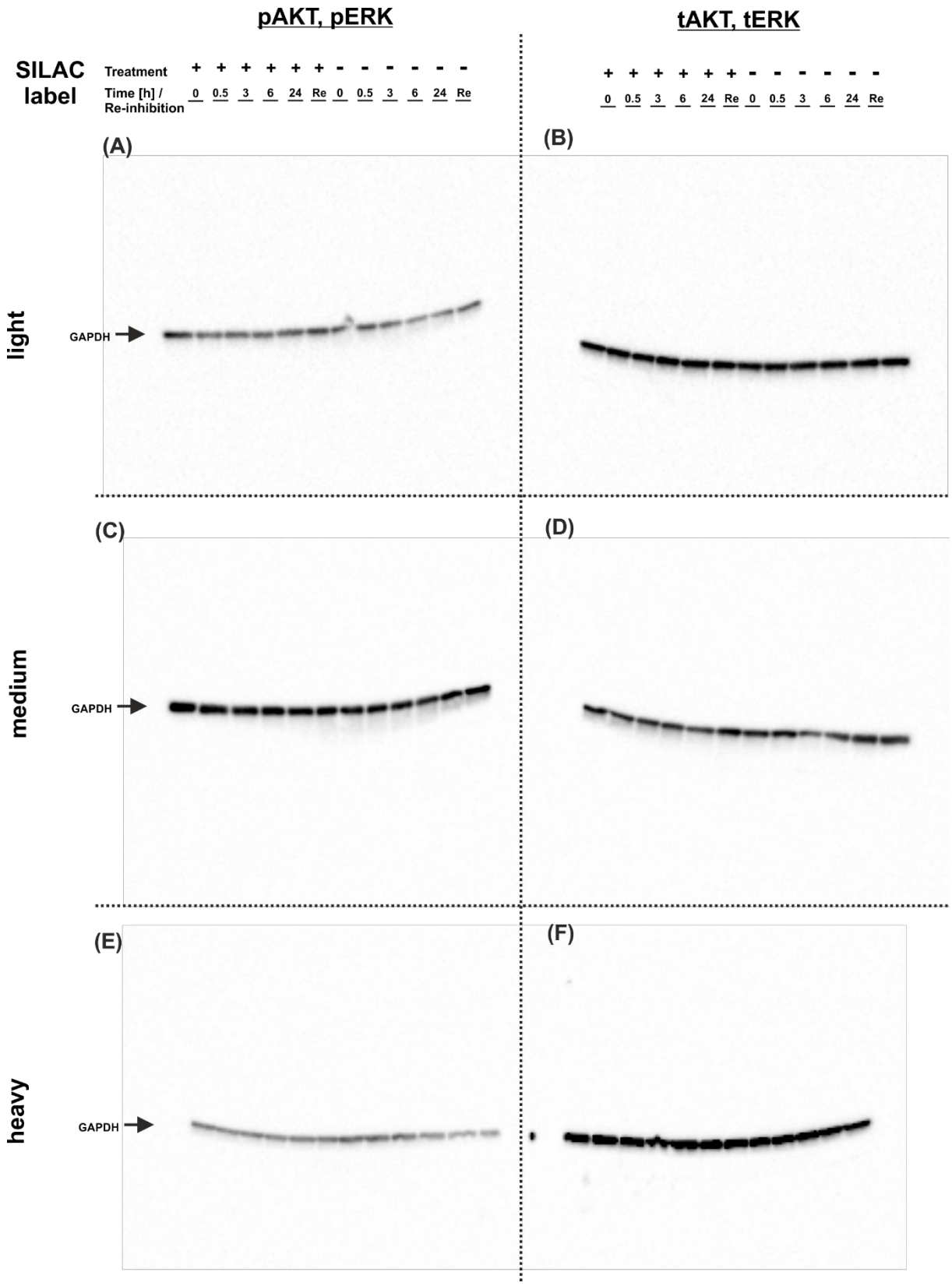
Supplemental Figure S11: Technical variability before and after batch normalization of TMT data. Boxplots representing CVs of quantified proteins and p-sites displayed before and after batch normalization by mean subtraction as described in Section 2.11. CVs were grouped either according to the TMT plex (i.e. MS measurement, n = 4) or according to the experimental groups denoted as experimental replicates (n = 6). Boxes represent 25th and 75th percentiles; whiskers extend to the most extreme data points within 1.5 range of interquartile range; median shown as a horizontal line.



Supplemental Figure S12: Principal component analysis of TMT data. Principal component analysis was performed using quantified p-sites. The upper panel represents data before batch effects were normalized by mean subtraction as as described in Section 2.11; the lower panel shows the respective normalized data. Replicates in the left panel are labeled according to the experimental groups (time point and treatment condition); in the right panel replicates are labeled according to the TMT plexes (MS measurements).



Supplemental Figure S13: Western blot analyses of AKT and ERK. Full membranes corresponding to the analyses presented in Supplemental Figure 8.



Supplemental Figure S14: Western blot analysis of GAPDH. Complete membranes corresponding to the analyses presented in Supplemental Figure S8.