SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. (A) Comparison of peptide intensities from the different labeling workflows. A recurring pattern between forward and reverse experiments can be seen in the boxplots before normalization. This pattern was affiliated with the labeling reagent (CH_2O or C^2H_2O), but not the workflow, as the labeling reagent was the only variable in these four replicate experiments. (B) Peptide ratio boxplots after normalization. The experiment is described in Figure 1A. Median ratios are listed at the bottom of each boxplot. Boxplots were normalized to a factor (0.0239) due to a consistent H/L ratio offset associated with the heavy label. The factor was derived from all of the 24 runs in this data set. (C) Comparison of peptides properties between different dimethyl labeling workflows. Differences in peptides properties observed between different workflow included length and hydrophobicity. Median peptide lengths are listed at the bottom of each boxplot.

Supplementary Figure 2. Boxplot of $\log_2$ ratio of SILAC and dimethyl labeling samples analyzed in separated MS runs. Results are combined from four separated experiments. Ratios are normalized so that the median of H1:L1s are centered at $\log_2 1$. Dotted lines indicate the expected log ratios from $\log_2 10$ to $\log_2 \frac{1}{10}$.

Supplementary Figure 3. Histogram of H/L peptide ratios including N-terminal acetylated peptides. Data is from one of the replicates in Figure 2, with N-acetylated peptides as a variable modification in MaxQuant search. (Left) A sub-population of peptides appear to the left (dotted circle) of the main cluster, which led to lower H/L ratios in SILAC samples when compared to dimethyl labeling (right). These N-acetylated peptides are not identified in dimethyl labeling

samples because these blocked N-termini would not be labeled by the chemical labeling reaction and the variable modification of N-terminal acetylation is not allowed in place of N-terminal dimethyl labeled N-termini at search time. In fact, these N-acetylated peptides that could not be dimethyl labeled were only detected when peptide search and quantification was performed in the SILAC mode. Black lines indicate the expected log ratios from $\log_2 10$ to $\log_2 \frac{1}{10}$.

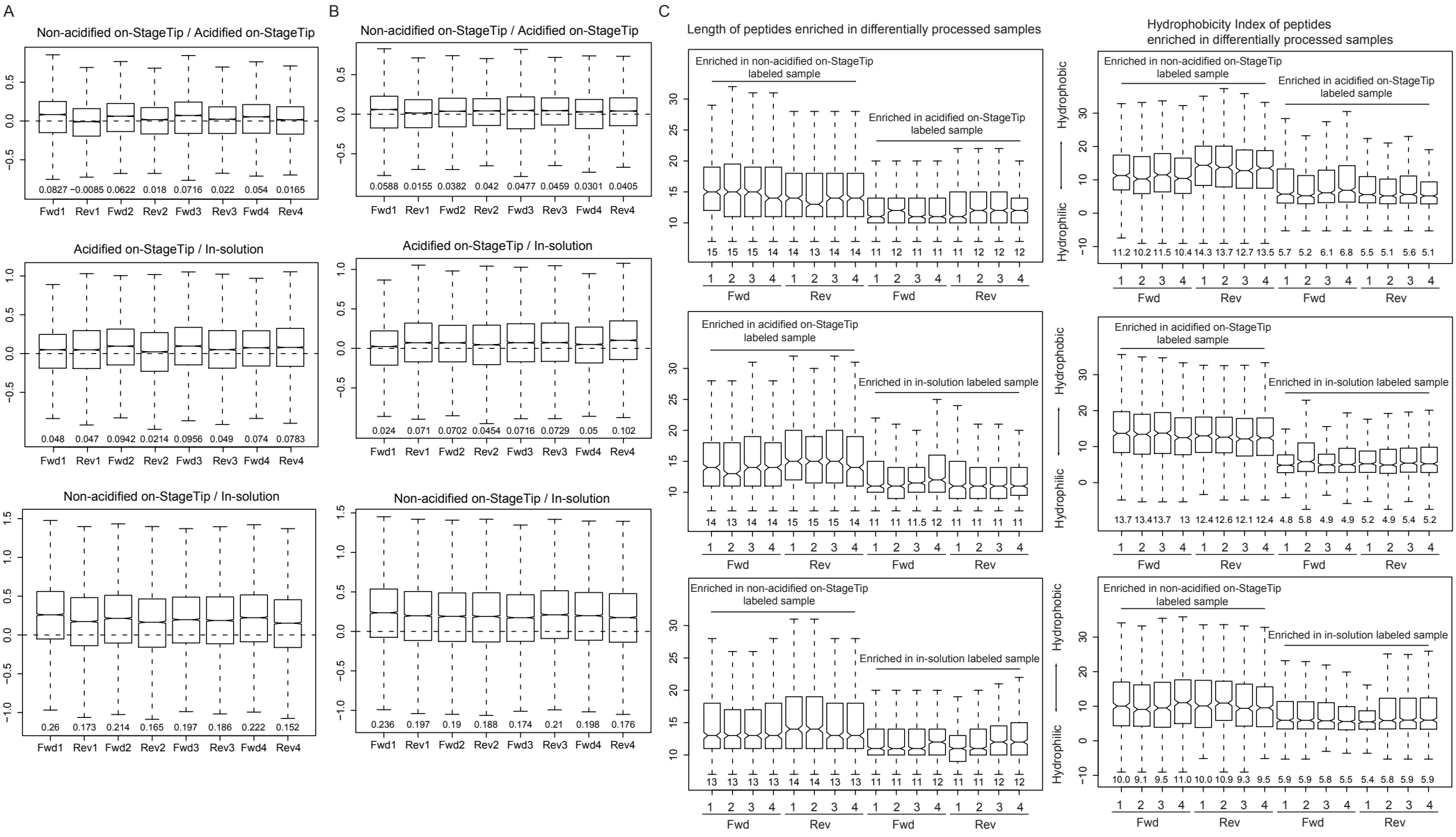
Supplementary Figure 4. Boxplot of $\log_2$ protein ratios for 1:1:1:1 experiment. Experiments were performed as shown in Figure 3A. The three panels show the three inter-day replicate groups in Figure 3A, each panel contains four intra-day replicates. The 12 sets of data were combined, normalized and illustrated in Figure 3B. DiMe and cDiMe are used to indicate DiMe labeled samples run either with SILAC samples or cSILAC samples, respectively.

Supplementary Figure 5. (A) Boxplot of $\log_2$ ratio of (dimethyl labeled peptides intensity / SILAC labeled peptides intensity) of common peptides in two samples. Median of the $\log_2$ ratios is -0.0797, which translates to 5% less signal intensity in dimethyl labeled samples (DiMe). (B) Boxplot of different peptide properties for unique and common peptides in SILAC and dimethyl labeled samples. Median values are listed at the bottom of boxplots. (C) Boxplot of different hydrophobicity scales.

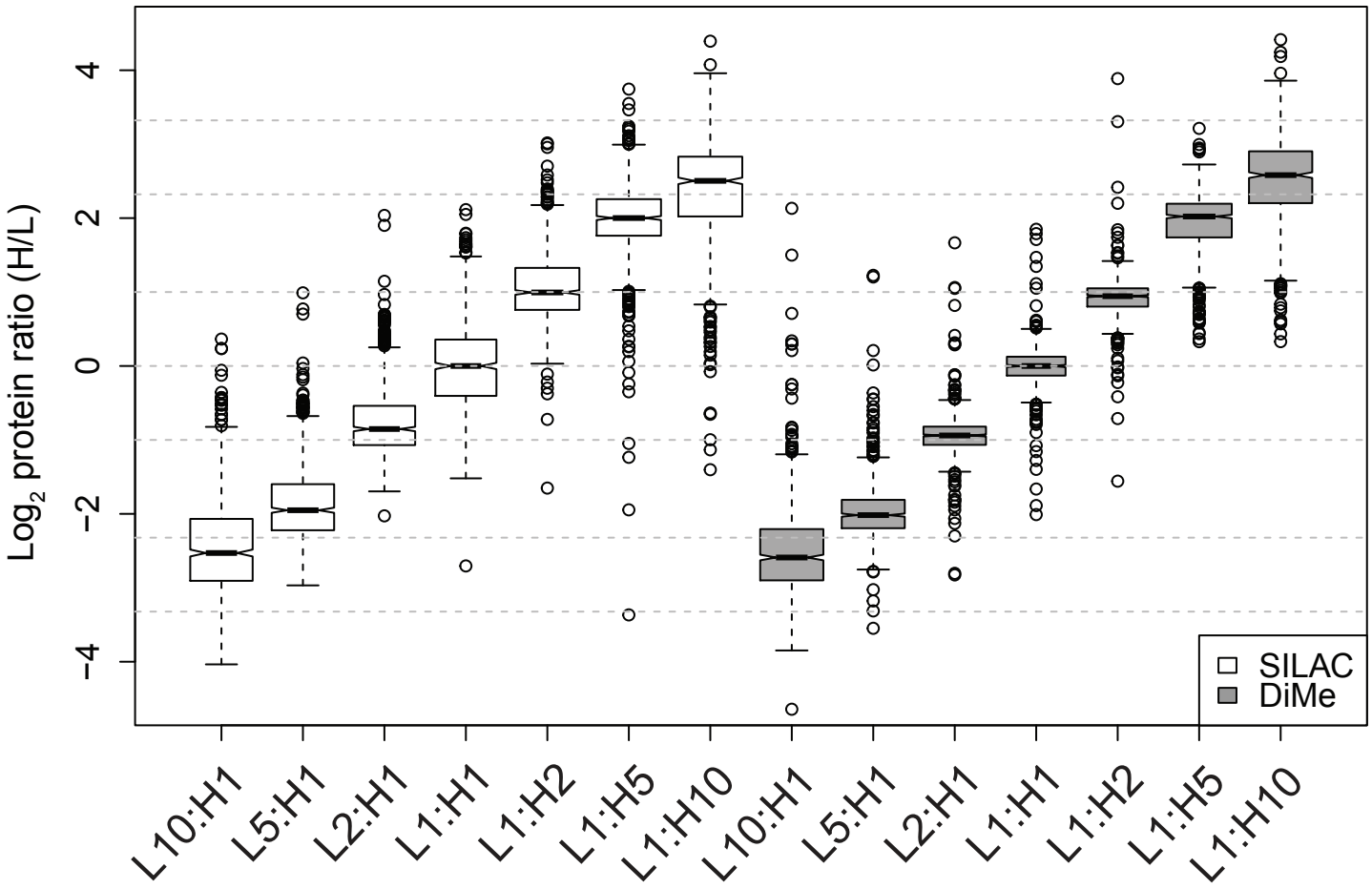
Supplementary Figure 6. FLAG-tagged GRB2-SH2 domain pull-down. (A) Experimental design of 'forward' experiment. Four populations of HeLa cells were grown in SILAC medium, serum starved and treated with EGF or PBS (vehicle). Bacterially expressed FLAG-GRB2-SH2 protein was immobilized on Sigma M2 anti-FLAG beads and used as affinity reagent. Captured proteins were eluted from the beads and resolved with SDS-PAGE gel, in-gel digested with trypsin. Trypsin digested peptides were desalted on StageTips. Dimethyl labeled samples were labeled with either light or heavy formaldehyde. SILAC and dimethyl labeled samples were

mixed and analyzed by MS. (B) Scatterplot of protein ratios from label swap replicates. EGFR and SHC1 are known GRB2-SH2 domain-binding proteins.[46, 51, 52] UBC is a cleavage product from ubiquitinylated EGFR after EGF treatment.[58] (C, left) Percent sequence coverage of SH2 domain interacting proteins. (C, right) Number of identified peptides from UBC, SHC1 and EGFR.

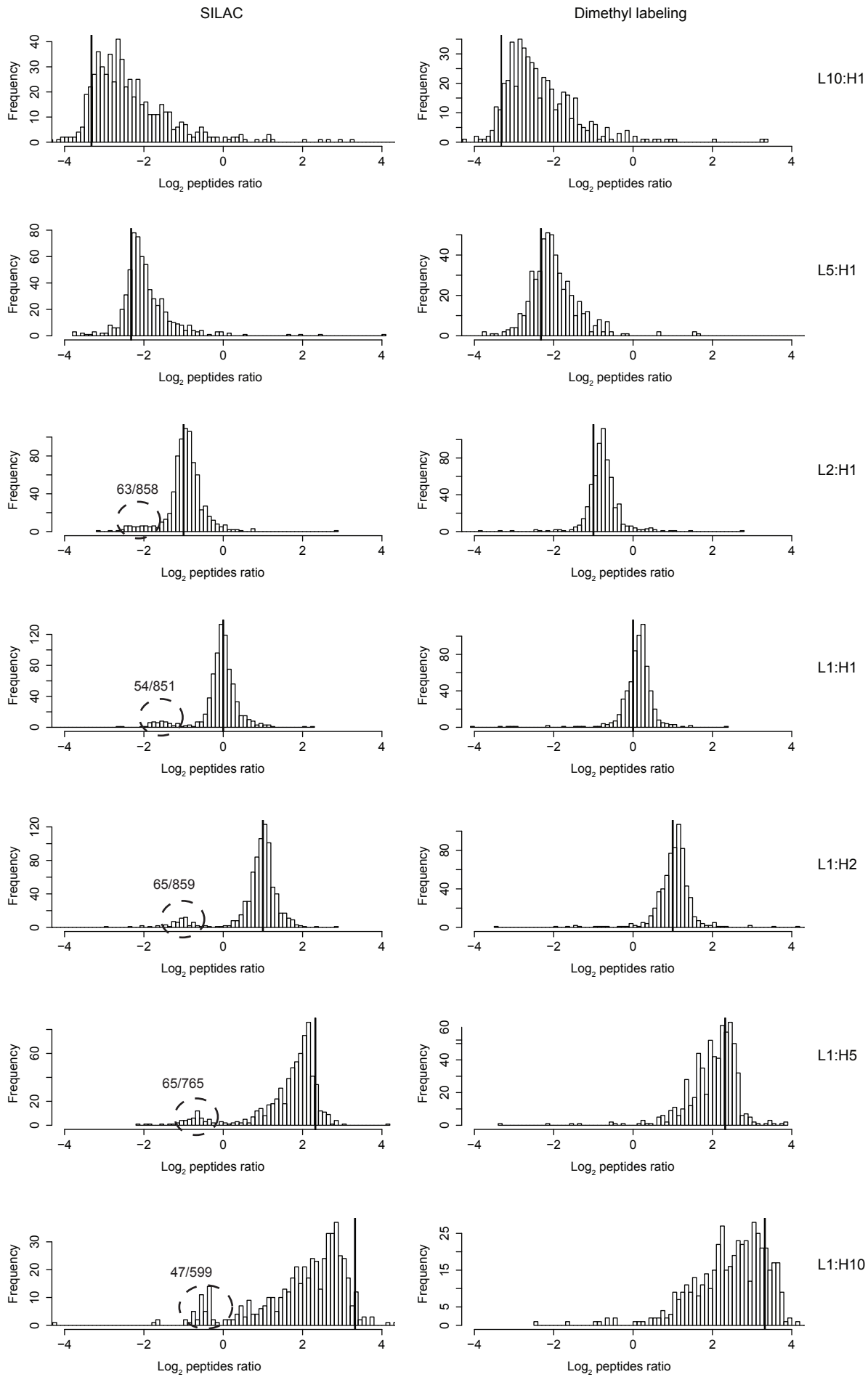
Supplementary Figure 1_Lau et. al.



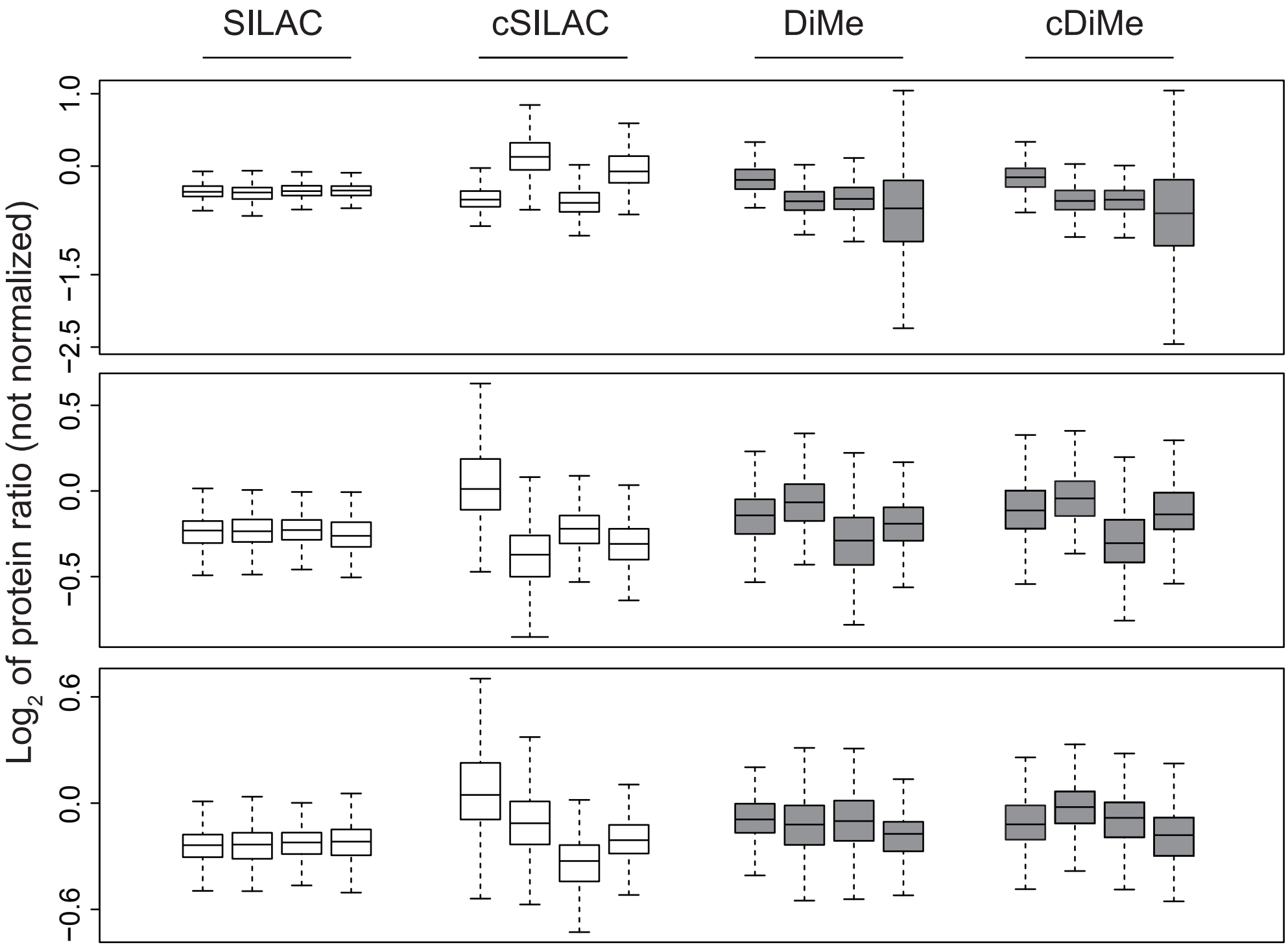
Supplementary Figure 2_Lau et. al.



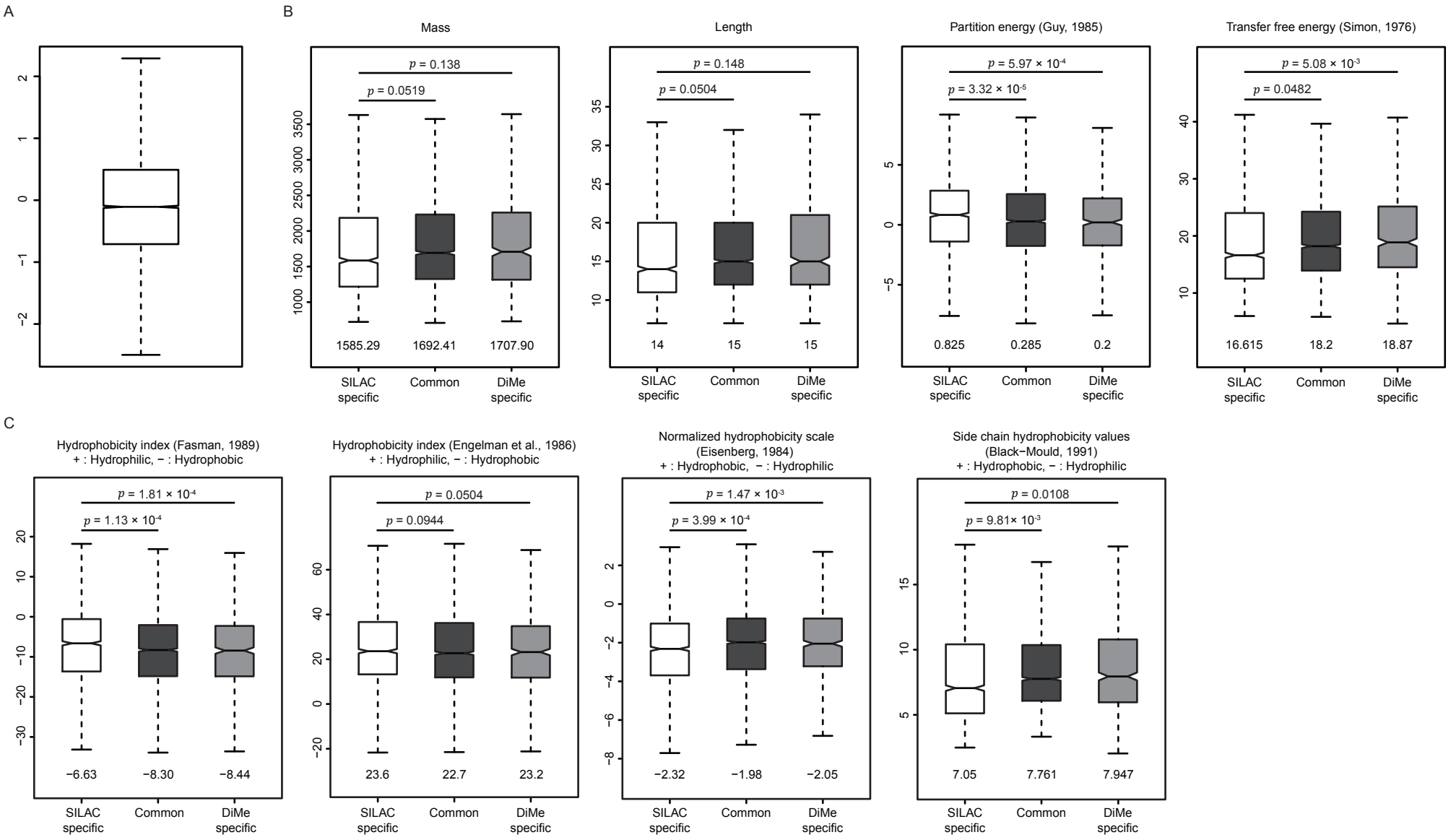
Supplementary Figure 3_Lau et. al.



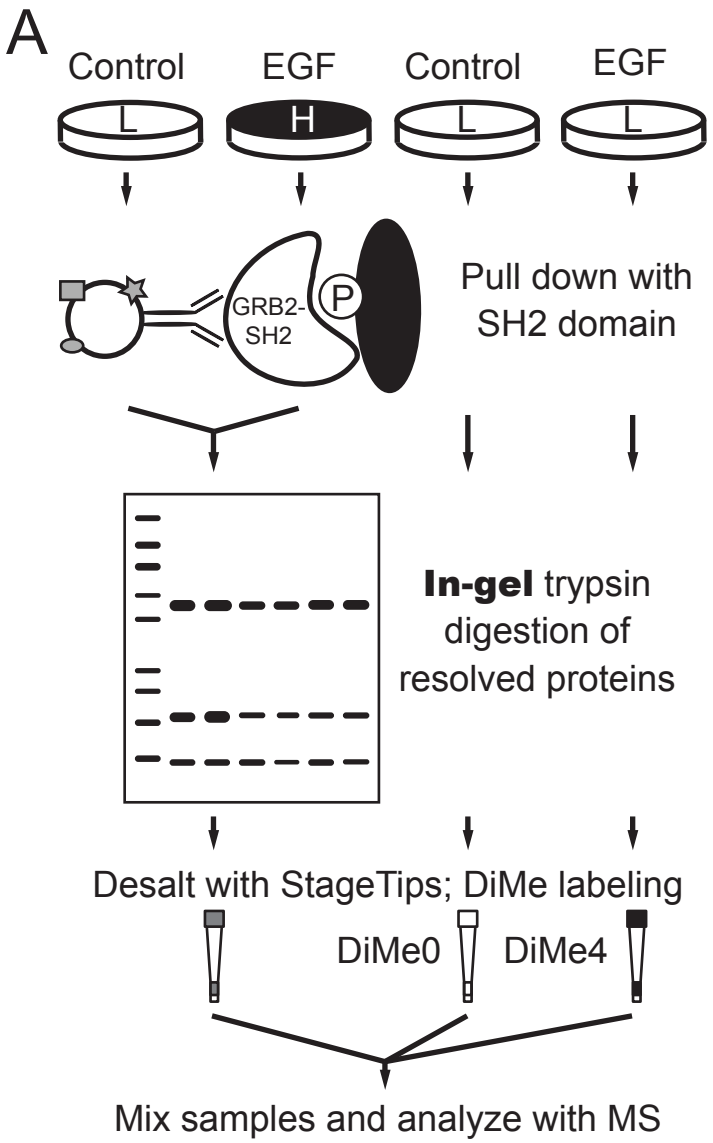
Supplementary Figure 4_Lau et. al.

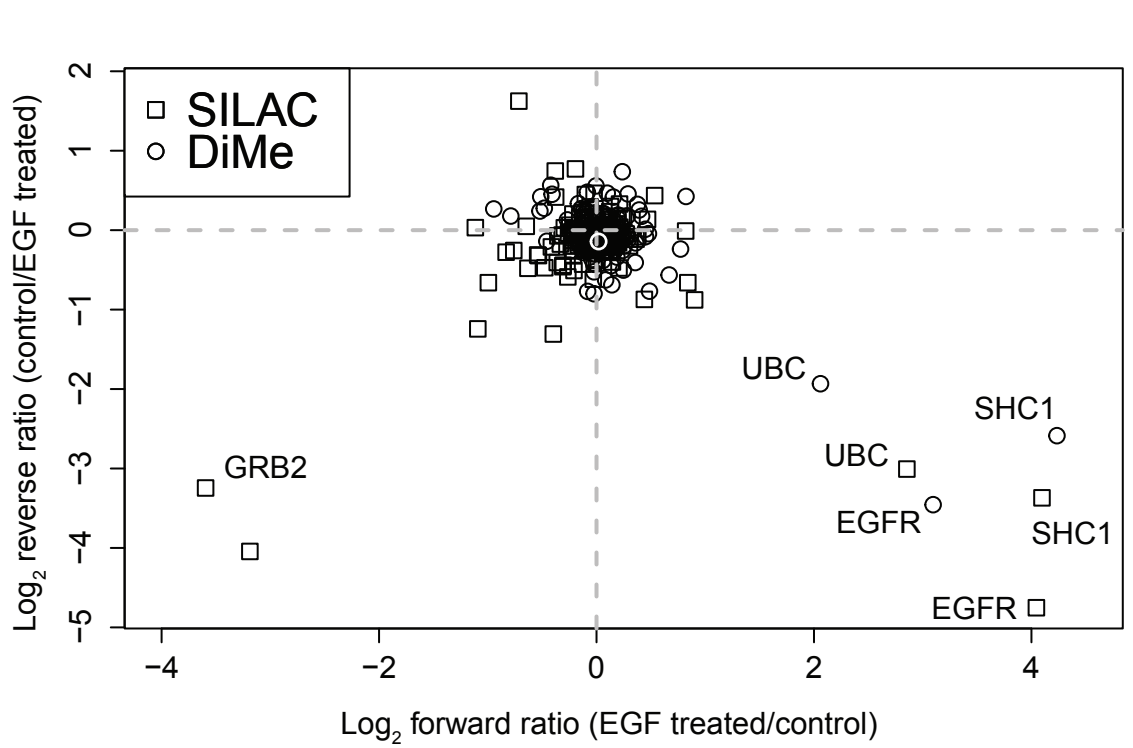


Supplementary Figure 5_Lau et. al.



Supplementary Figure 6_Lau et. al.





C

	SILAC		DiMe	
	Fwd	Rev	Fwd	Rev
UBC	61.8	49.9	61.8	61.8
SHC1	46.7	52.2	36.8	33.2
EGFR	29.8	33.5	27.7	26.1

% coverage 0-20 21-40 41-60 60-80