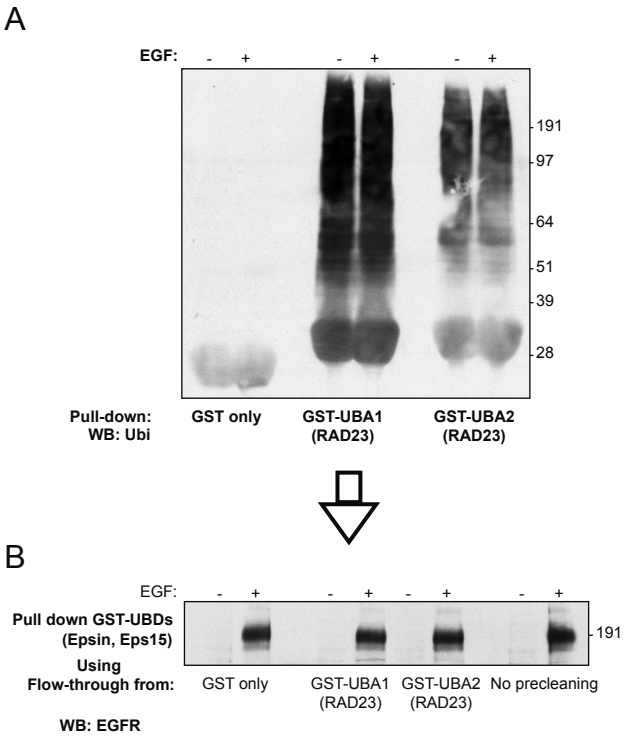
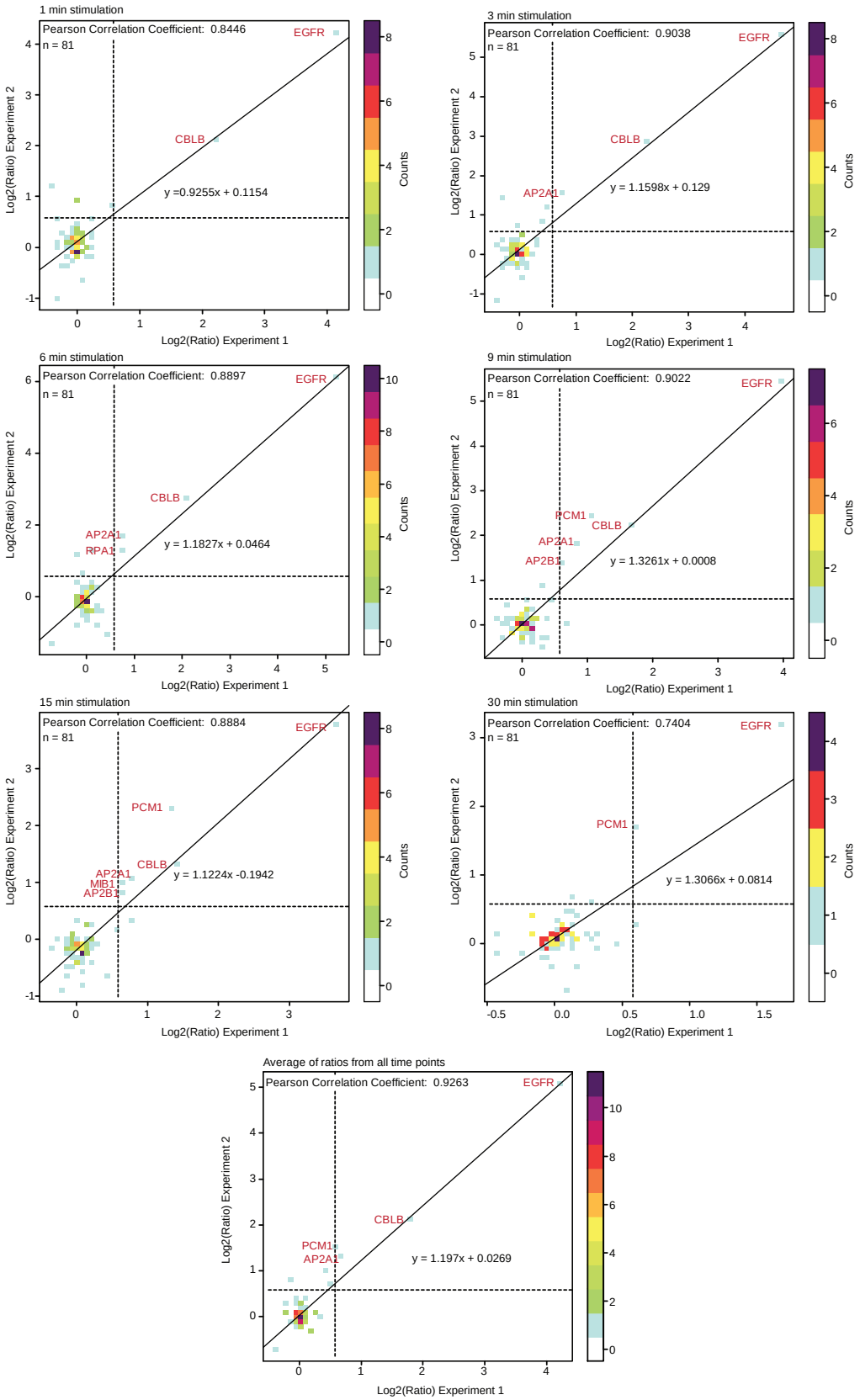
Supplemental Figure 1



Precleaning of cell lysates for subsequent purification of ubiquitinated targets by GST-UBDs. (*A*) Reducing the amount of the abundant Lys-48 poly-ubiquitin protein conjugates from the lysates with recombinant GST-UBA domains derived from human Rad23A. (*B*) Recombinant GST-UBDs (derived from Epsin and Eps15) were used for enrichment of ubiquitinated EGFR from such precleaned lysetes as well from cellular lysate not subjected to the precleaning step for comparison.

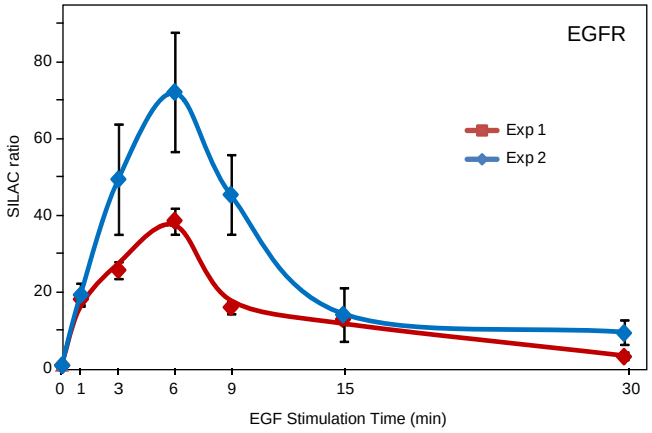
Supplemental Figure 2



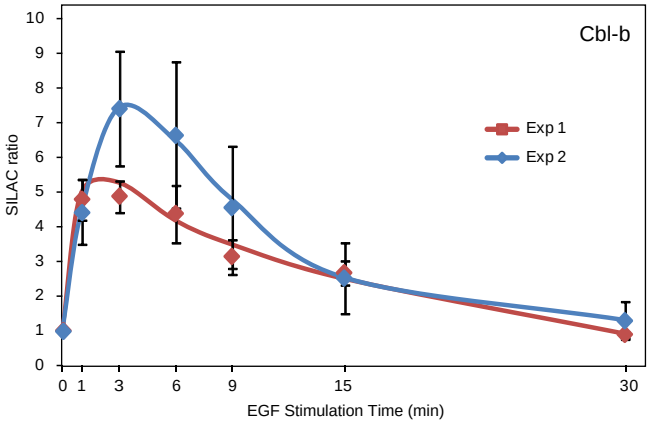
Correlation of all protein ratios between the two biological experiments. Density scatter plots represent correlations between two independent experiments at each individual time-point. Dashed lines show regulation threshold and full line displays best linear correlation between two experiments. The bottom scatter plot is an overall summary representing the average protein ratios from all time points.

Supplemental Figure 3

A

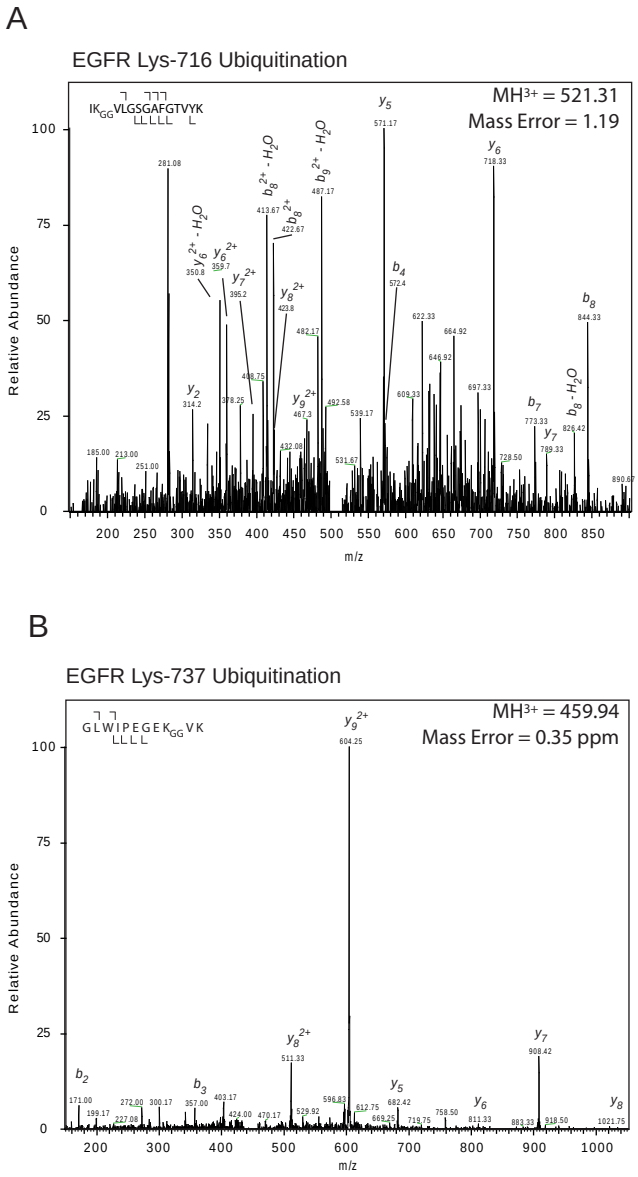


B



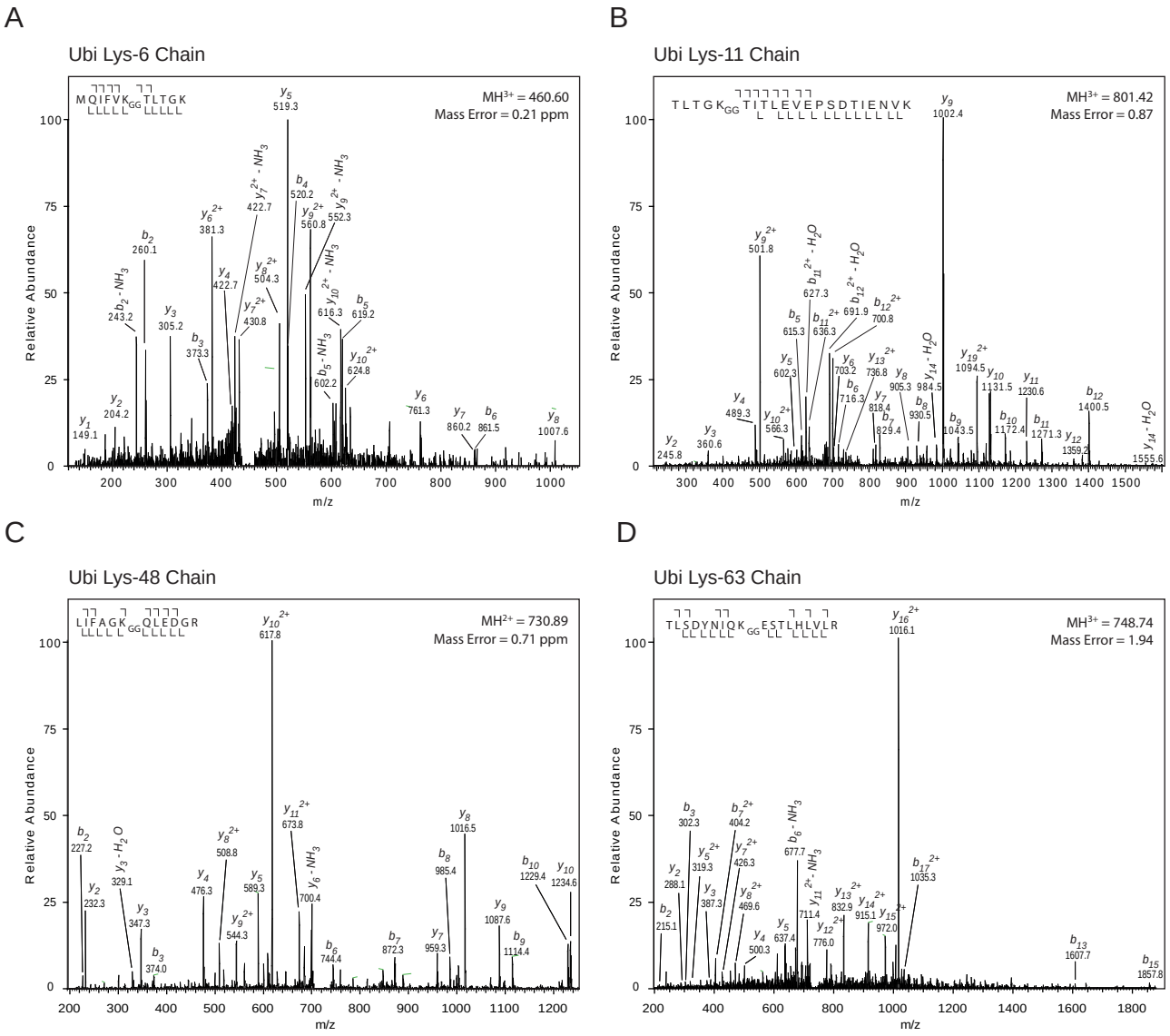
Dynamic profiles of EGFR and Cbl-b originating from two independent biological experiments. (*A*) Profiles of the ubiquitinated EGFR over 30 min of ligand stimulation as determined by quantitative mass spectrometry. (*B*) Ubiquaitination dependant dynamics of Cbl-b in response to EGF stimulation.

Supplemental Figure 4



Identification of two EGFR ubiquitination sites. Digestion of ubiquitylated proteins with trypsin results in two glycine residues remnant from the C-terminus of ubiquitin attached to the targeted lysine. This leads to a mass shift of 114.0429 Da and a missed cleavage at the modified lysine. (*A*) MS/MS spectrum of the EGFR peptide that contain the ubiquitinated Lys-716. (*B*) MS/MS spectrum of the EGFR peptide containing the ubiquitinated Lys-737. Both peptides originated from the Lys4/Arg6 SILAC-labeled cells. K_{GG} indicates the remnant diglycine signature from the ubiquitin on the corresponding lysine residue.

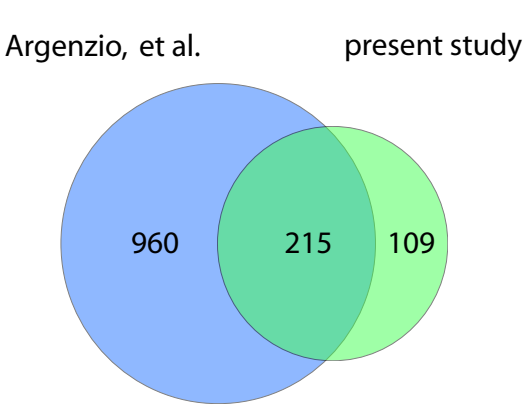
Supplemental Figure 5



Identification of branched polyubiquitin peptides derived from the same gel band as the EGFR. MS/MS spectra of the ubiquitin peptides corresponding to (*A*) Lys-6, (*B*) Lys-11, (*C*) Lys-48 and (*D*) Lys-63 specific polyubiquitin linkages. K_{GG} indicates the characteristic diglycine signature at the corresponding lysine residue.

Supplemental Figure 6

A



B

Protein	Argenzio, E., et al. Protein ratio 10 min EGF	Akimov, V., et al. Protein ratio 6 min EGF	Akimov, V., et al. Protein ratio 9 min EGF	Akimov, V., et al. Protein ratio 15 min EGF
EGFR	29.37	37.9 ± 3.22	15.79 ± 1.65	12.76 ± 1.54
CBL	9.82	4.38 ± 0.82	3.14 ± 0.51	2.67 ± 0.34
STS1	6.13	3.03 ± 0.13	1.69 ± 0.03	2.27 ± 0.26
AP2B1	1.85	1.12 ± 0.11	1.55 ± 0.14	1.55 ± 0.05
AP2A1	1.49	1.71 ± 0.2	1.78 ± 0.1	1.74 ± 0.23
NEDD4L	2.51	1.3 ± 0.26	1.36 ± 0.13	1.7 ± 0.11
PCM1	0.68	0.96 ± 0.08	2.12 ± 0.22	2.51 ± 0.29
AZI1	0.20	0.98 ± 0.1	2.19 ± 0.24	2.3 ± 0.11
RPA1	1.11	1.72 ± 0.27	1.58 ± 0.06	1.5 ± 0.27
MIB1	-	0.9 ± 0.17	1.24 ± 0.09	1.52 ± 0.2
WWP2	-	0.99 ± 0.2	1.03 ± 0.15	1.53 ± 0.07
TPX2	-	1.46 ± 0.14	1.25 ± 0.34	1.37 ± 0.22

Comparison of proteins identified in the current proteomic study using GST-UBDs with the proteins identified by Argenzio et al.* using anti-ubiquitin antibodies. (A) Overlap of the identified proteins between the two studies. (B) Comparison of the SILAC ratios for the EGF regulated proteins identified in the current study and the study by Argenzio et al.

* Argenzio, E., et al., Mol Syst Biol, 2011. 7: p. 462.