

Upon 2 unique
peptide matches to
prevent false



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Upon EGF stimulation, EGFR in Her14 cells will be activated by the formation of dimers, tetramers, or higher-order oligomers. This could either induce several downstream signal transduction pathways or it may induce endocytosis. According to Seedham et al, higher-order oligomerization starts by the formation of dimers by a back-to-back dimerization interface located on domain II, followed by repeated incorporation of additional dimers using the face-to-face interface at EGFR ectodomain I and III. The clustering of EGFR oligomers leads to a negative membrane curvature organized by the dimer transmembrane helices, which will function as hydrophobic wedges in the plasma membrane. This EGFR clustering-induced membrane invagination will recruit curvature sensitive proteins, such as F-BAR domain-containing FCHO proteins. By isolating these activated EGFR oligomer clusters using SMA copolymer, we are able to determine the relative abundance of proteins recruited in the first steps of endocytosis.

As previously mentioned, the EGF stimulated EGFR-enriched nanodiscs showed enhanced Tyr-1068 phosphorylation indicating trans-phosphorylation of kinase domains (figure 3A). Because EGFR was used as bait during the immunoprecipitation, the NSAF of each protein was normalized by the NSAF of EGFR (see methods for further details). The relative protein abundance in the stimulated EGFR interactome was based on the ratio between the EGF-induced NSAF of proteins against the control NSAF of proteins (supplementary table 2 and 3). Table 1 provides an overview for a total of 72 proteins with an enrichment factor of ≥ 1.5 containing at least 2 unique peptide matches to prevent false positives in the EGF stimulated

sample. Here, MYB-binding protein P160 1a (Mybbp1a) shows the highest enrichment value of 4.375.

The enrichment value could not be calculated for twenty proteins due to the absence of unique peptide matches in the control sample. Nonetheless, they are taken into account based on the presence of ³² unique peptide matches in the EGF-stimulated sample.