

Expanded View Figures

Figure EV1. Detailed workflows used in this study and overall data assessment.

- A Workflows used in this study for affinity purification coupled with mass spectrometry (AP-MS) and BioID approaches. AP-MS enables the capture of protein complexes and stoichiometries, whereas the complementary BioID method enables capture of direct, transient/proximal interactions.
 - B Network topologies of RTK subfamilies. Blue nodes indicate the bait protein used in the experiment and green nodes the detected HCl proteins. Interactions detected in AP-MS only are marked in blue, BioID only in green, and interactions detected with both approaches are shown in burgundy.
 - C Proportion of HCIs shared between RTK subfamilies. Values are calculated based on the number of shared HCIs and the number of HCIs in each subfamily.
 - D Anti-phosphotyrosine blot for 8 RTKs, showing bait RTK phosphorylation.
 - E Co-IP validation of RTK-RTK interactions detected via AP-MS method in the study. Spectral count values for the interactions are shown underneath.
- Source data are available online for this figure.

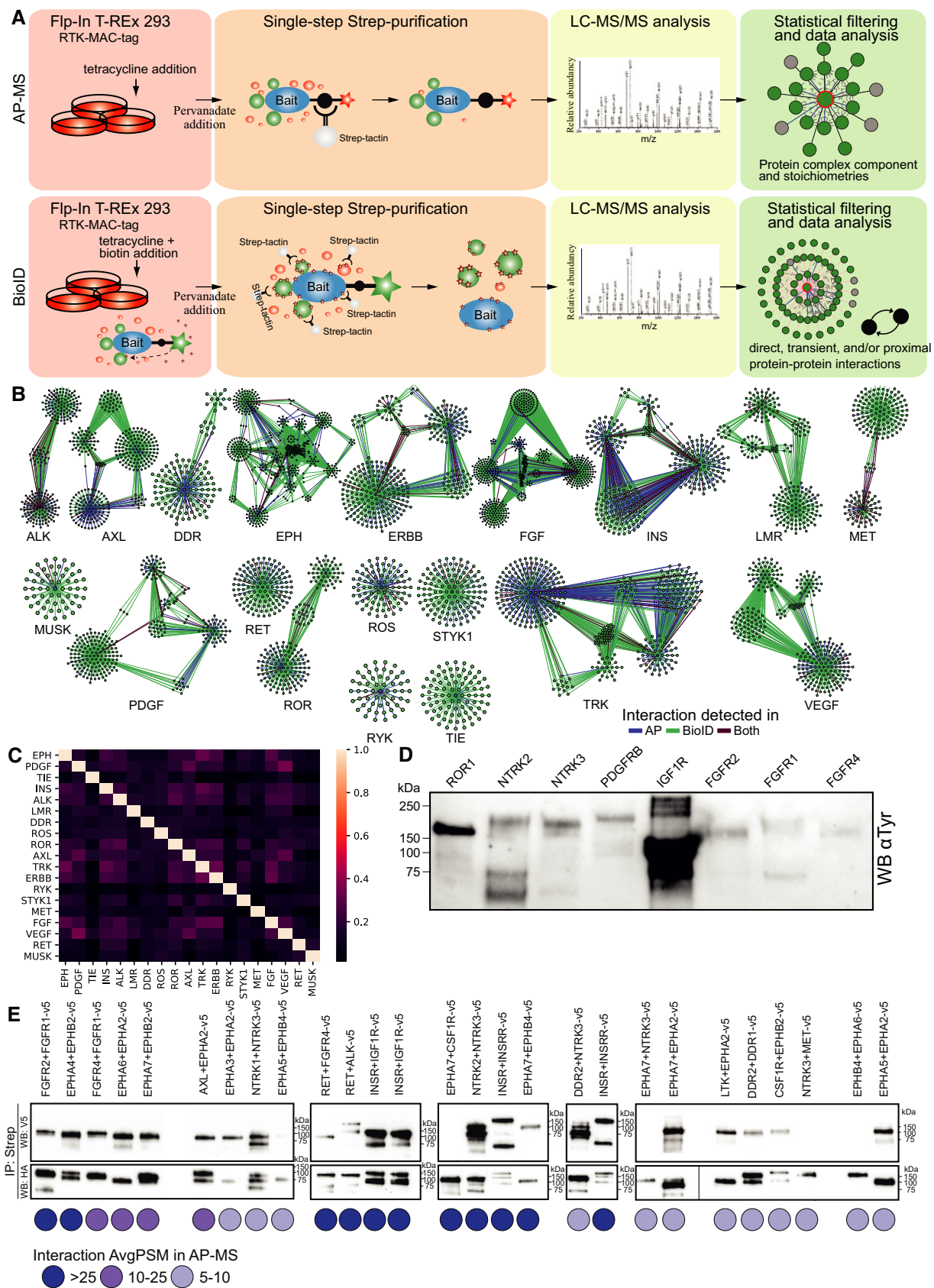


Figure EV1.

Figure EV2. RTK localization and AP-MS validation via CO-IP.

- A Immunofluorescence microscopy images of each bait RTK. Images are divided based on the RTK subfamily. Green: Anti-HA, Blue: DAPI. Scale bar 10 μ m.
- B Verification of several AP-MS high-confidence interactions with Co-IP and dot blot. 83 interactions were tested, of which 69 were detected via Co-IP. The prey proteins were tagged with Strep-HA, and bait proteins with V5 and coexpressed in HEK293 cells. For negative controls, Strep-HA-tagged GFP and V5-tagged RTKs were used. The Strep-HA tagged proteins were immunoprecipitated with Strep-Tactin sepharose. The immunoprecipitated protein complexes were then dot blotted with anti-V5 and anti-HA antibodies.

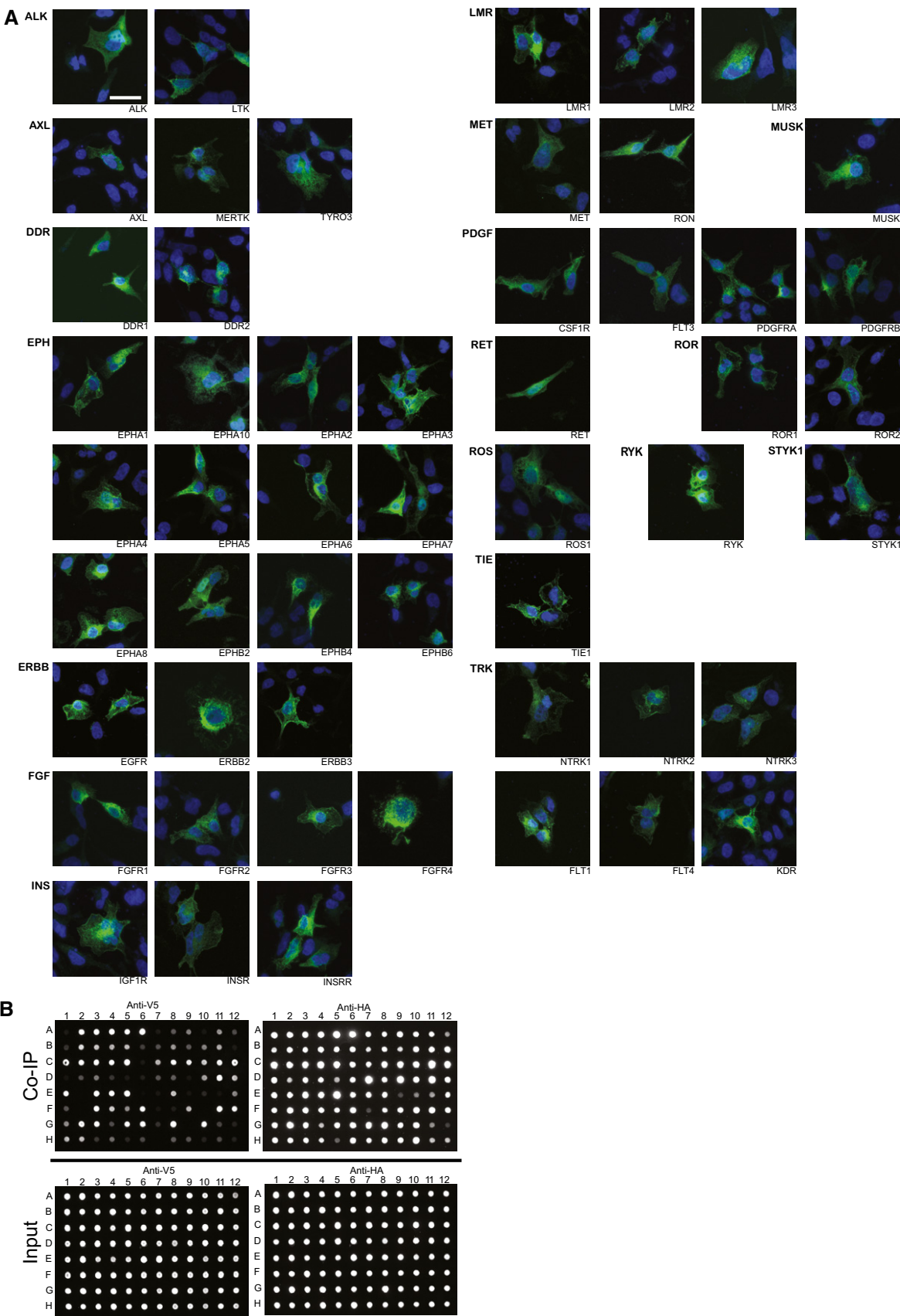


Figure EV2.

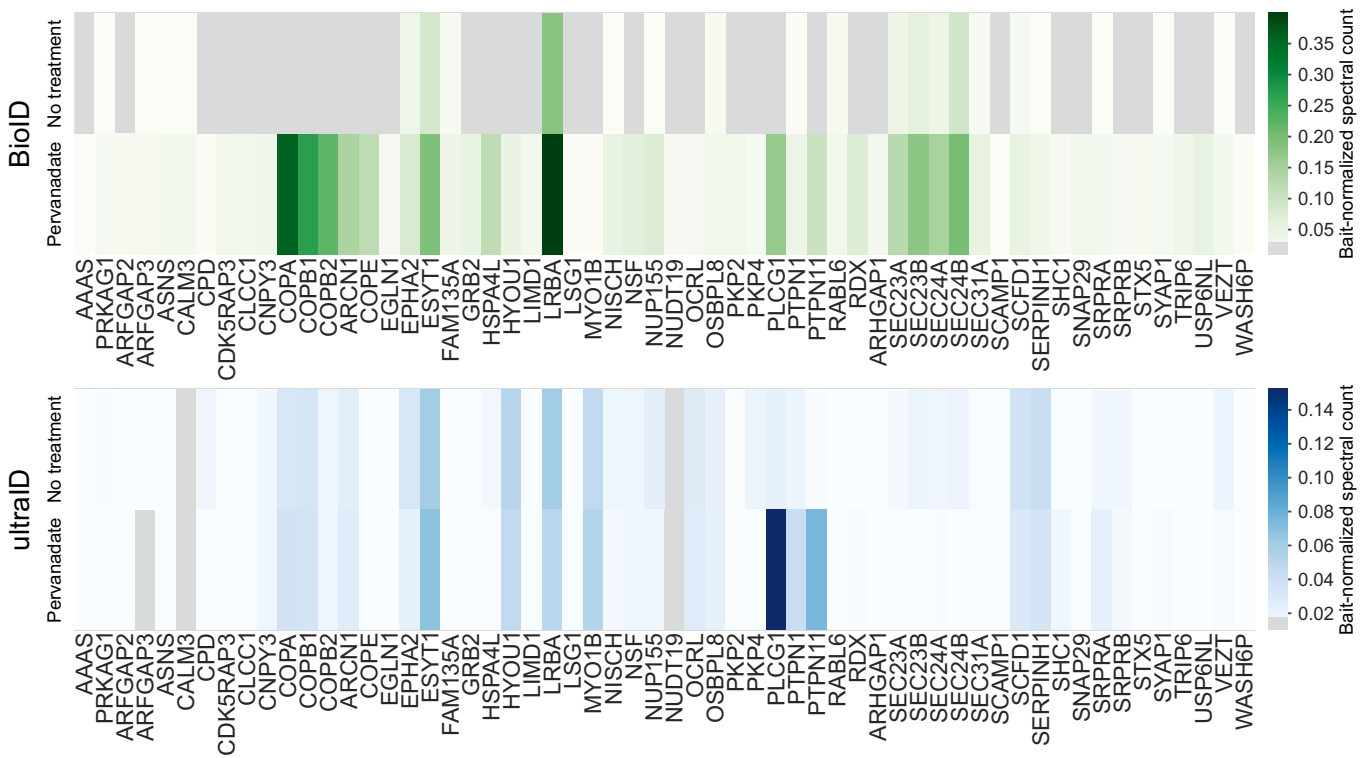


Figure EV3. 15-min pervanadate treatment compared to no treatment with NTRK3 BioID and ultraID experiments.

HCl proteins of pervanadate-treated NTRK3 detected by proximity labeling approaches (BioID or UltraID) are shown to highlight what interactions are treatment-dependent. With ultraID (preprint: Zhao *et al*, 2021), 10-min biotinylation time was used for identifying interactions at the time of activation. For visualization purposes, only proteins with significant changes (bait-normalized average spectral count difference > 2 fold, or exclusively identified in pervanadate treatment) were selected to be presented here.

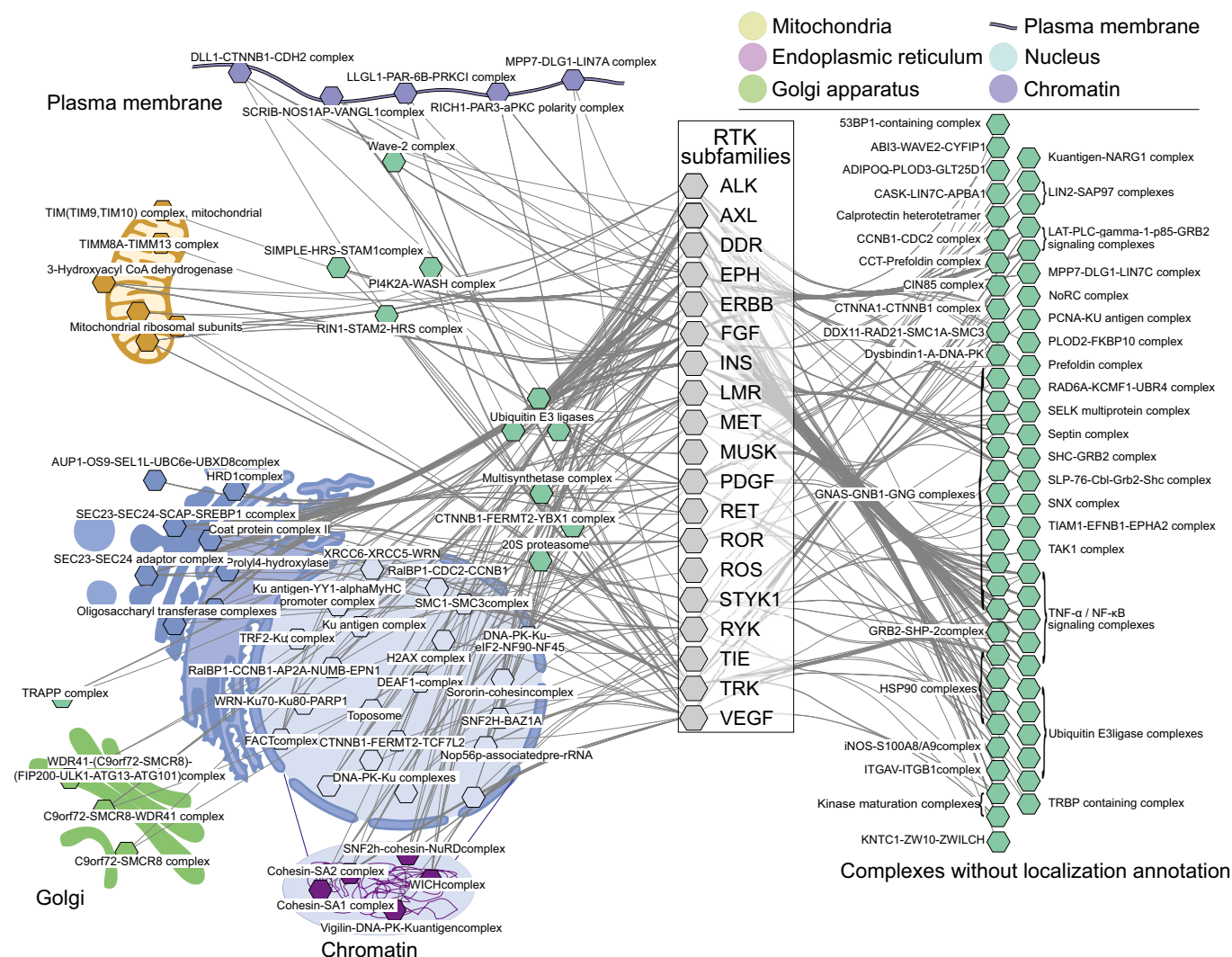


Figure EV4. Enriched complexes in the RTK interactome data.

Significantly ($q < 0.05$, calculated with Fisher exact test followed by Benjamini–Hochberg multiple-testing correction) enriched CORUM complexes in the interactomes of the RTK subfamilies. The cellular localization was assigned to each complex with available GO cellular component in CORUM. Connections from subfamilies to complexes denote significant enrichment of the complex with one or more members of the subfamily. On the right side, complexes without localization information are grouped based on their protein composition.

Figure EV5. Analysis of EGFR interactome generated in this study.

- EGFR HCIs identified in the study. Previously known interactors are denoted in brown, and novel in green.
- Most common significantly ($q < 0.05$, calculated with Fisher exact test and Benjamini–Hochberg multiple-testing correction) enriched GO Biological process annotation terms in the EGFR HCIs. The top chart shows unique protein counts, while the lower chart depicts log2 fold change over expected value calculated from the background set.
- Known interactions between previously known HCIs and novel HCIs.
- Co-IP validation of a subgroup of EGFR (left) and NTRK3 (right) interactors.

Source data are available online for this figure.

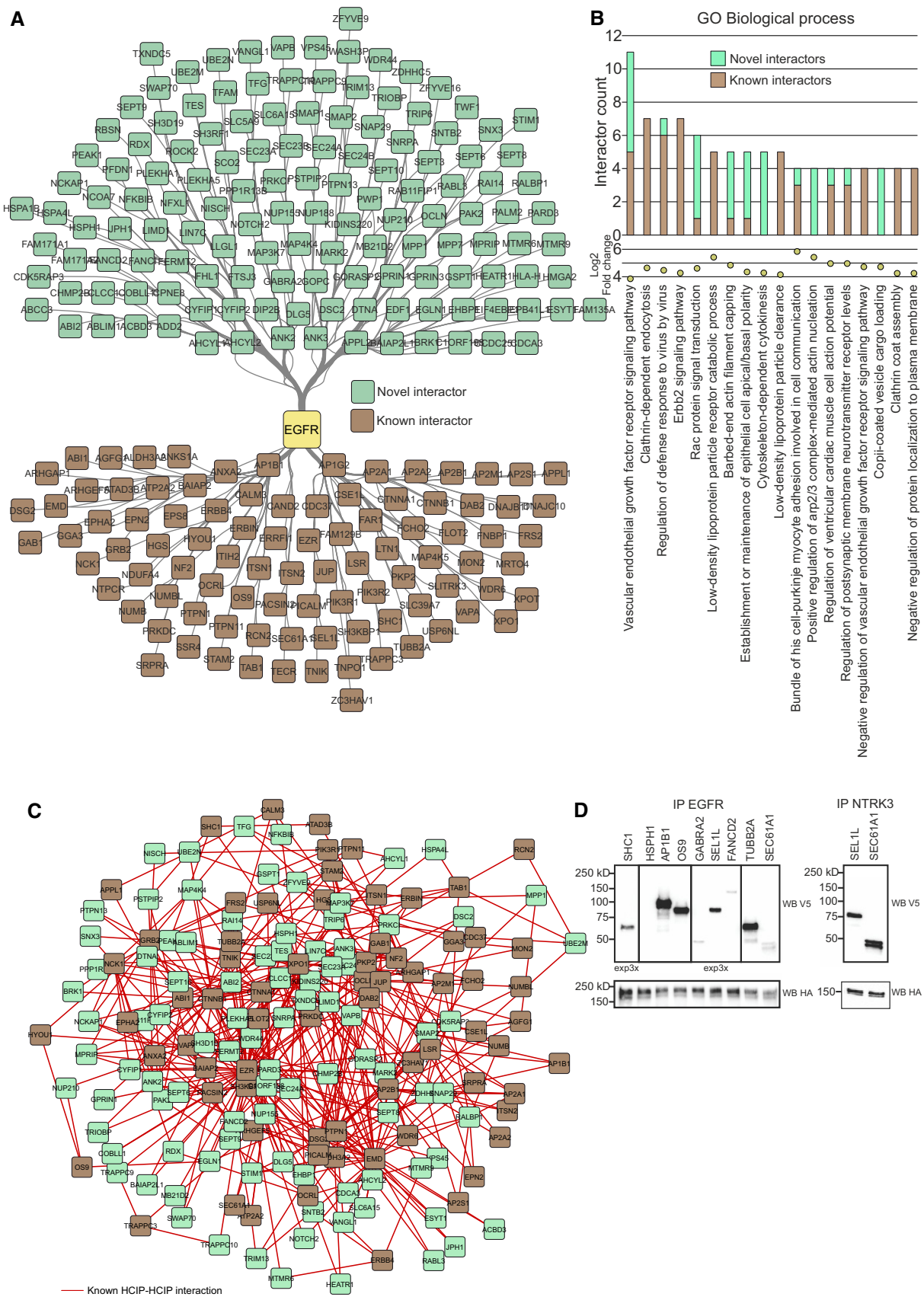


Figure EV5.