Supplemental Information for:

Multiplexed kinase interactome profiling quantifies cellular network activity and plasticity

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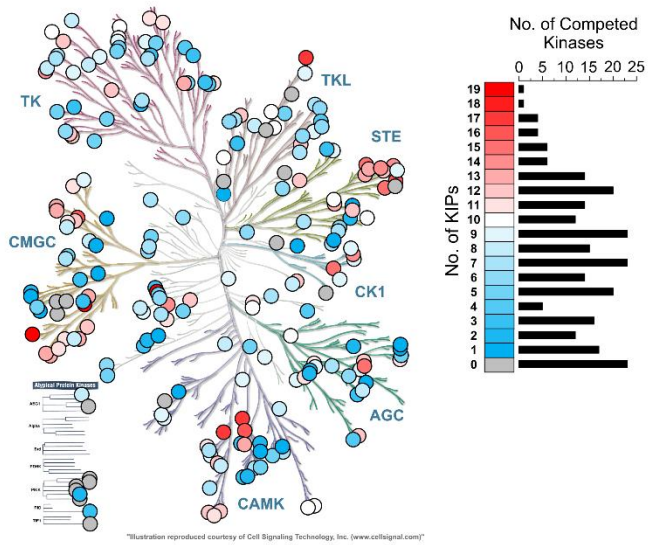
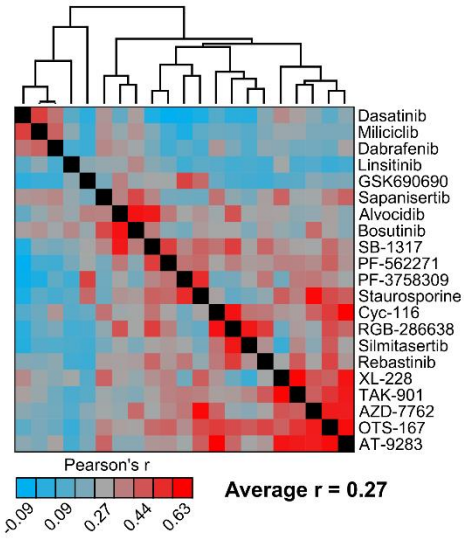
Golkowski et al., **Figure S1**

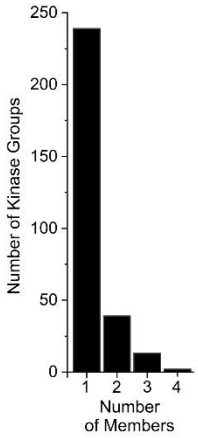
A Kinases Competed with our 21 Kinase Interactome Probes (KIPs)



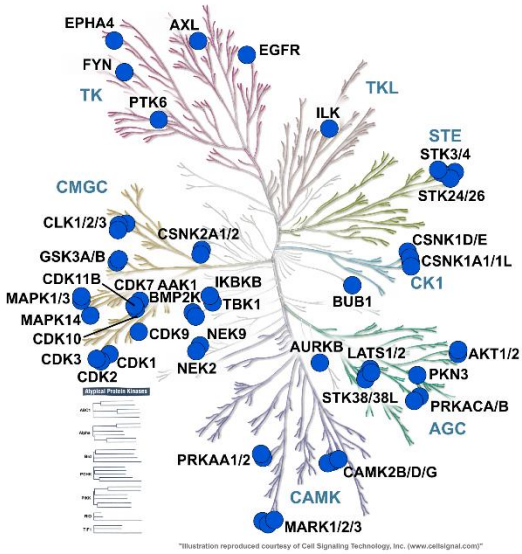
B KIPs Show Highly Dissimilar Kinome Profiles



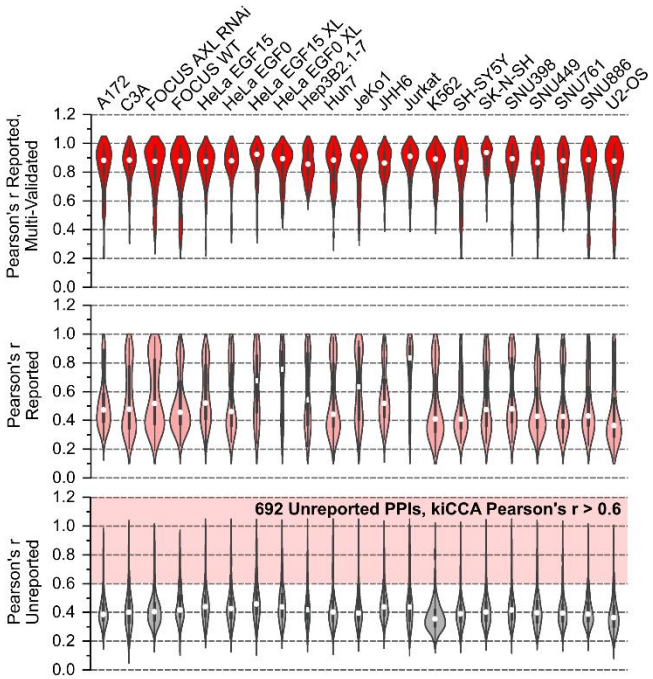
C kiCCA Kinase Groups by Number of Members



D Kinases with Previously Reported, Validated PPIs Identifed by kiCCA in HeLa Cells



E kiCCA Pearson's r-Value Distributions in the 18 Diverse Cancer Lines and HeLa Cells in Distinct Signaling States



Golkowski et al., **Figure S1**

Figure S1. Identifying a set of complementary kinome interactome probes (KIPs), pilot kiCCA experiment in HeLa cells, and kiCCA profiling of 18 diverse cancer lines. Related to Figure 2.

(A) Kinases significantly competed in our kinobead/LC-MS soluble competition assay using our 21 KIP panel (HeLa lysate, log2 MS intensity ratio >0.75 and t-test p-value < 0.1, n = 2).

(B) Pairwise Pearson correlation of KIP kinome profiles (kinase log2 MS intensity ratios), followed by unsupervised hierarchical clustering confirms high complementarity.

(C) Breakdown of kiCCA kinase groups by number of members.

(D) Overlaying kinases for which kiCCA identified previously reported PPIs in HeLa cell lysate with the human kinome dendrogram (n = 37 kinase groups).

(E) kiCCA Pearson's r-value distributions for reported and independently validated (BioGRID, top panel), reported but unvalidated (middle panel), and unreported interactions (bottom panel) across 18 diverse cell lines and HeLa cells in different signaling states.

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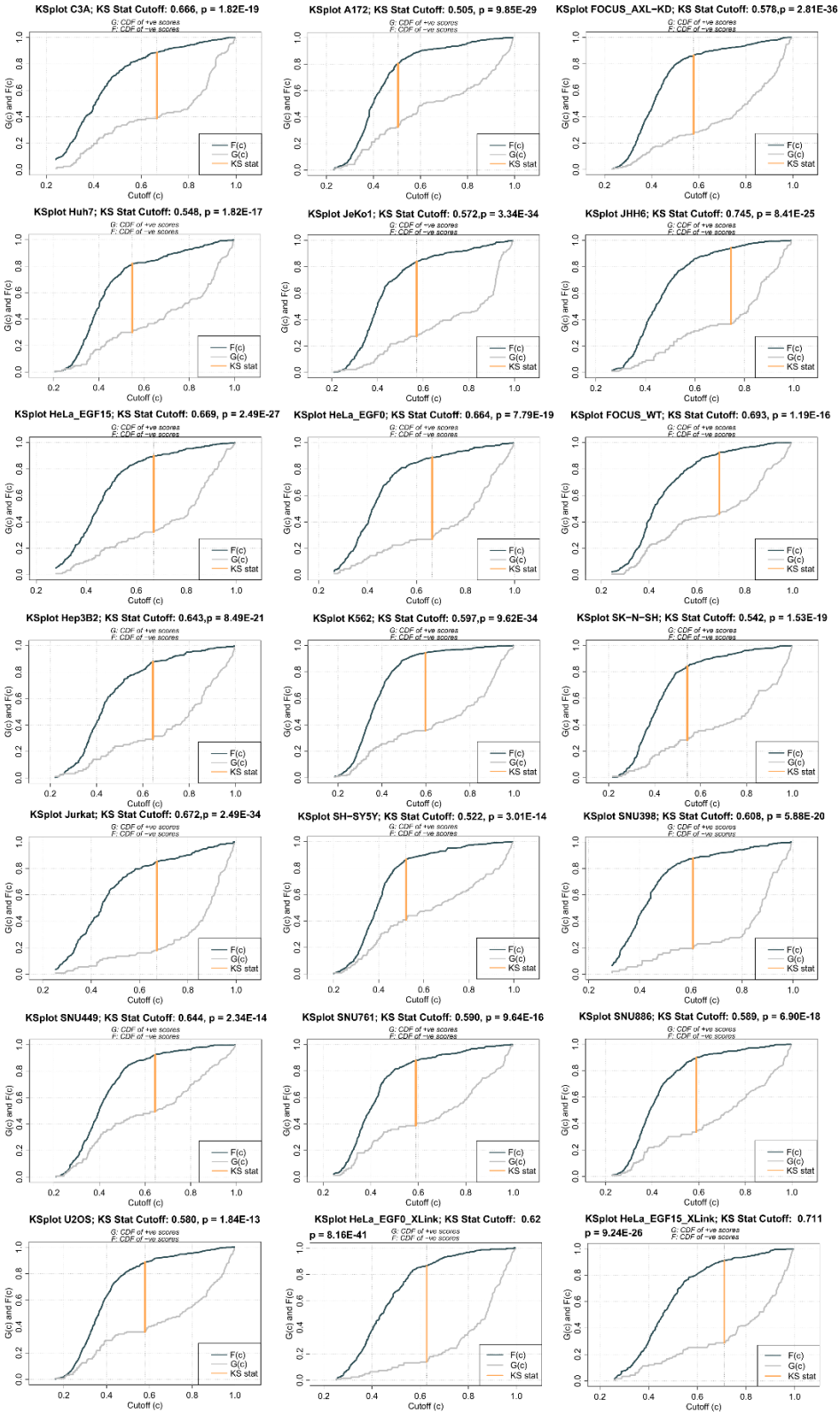

Figure S2. KS statistics for kiCCA profiling results of our 18-cancer cell line panel. Related to Figure 2.

Golkowski et al., **Figure S3**

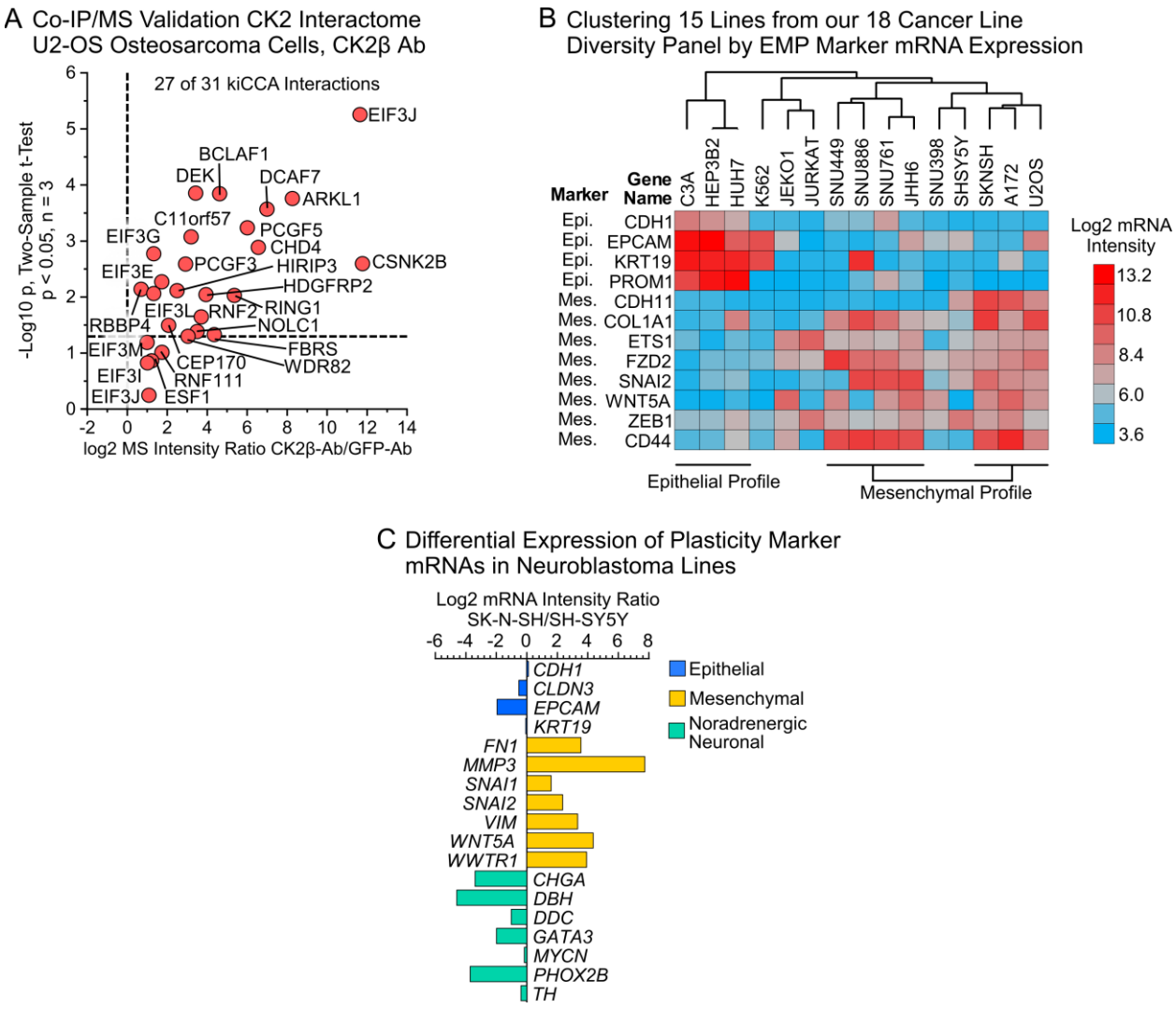

Figure S3. Validation of the CK2 interactome and marker mRNA expression in the 18-cancer line panel. Related to Figure 3 and 4.

(A) Co-IP/MS experiment in U2-OS osteosarcoma cells using an antibody specific to the casein kinase 2 regulatory subunit (CK2β) validates a CK2 interaction network identified by kiCCA (GFP antibody was control, two sample t-test, $p < 0.05$, $n = 3$)

(B) Clustering of 15 cancer cell lines included in our diversity panel by EMP marker mRNA expression ($n = 52$). Shown are the 12 most characteristic markers for the epithelial-like and mesenchymal-like phenotype.

(C) Comparing marker mRNA expression for EMO and noradrenergic neuronal differentiation between the SK-N-SH and SH-SY5Y neuroblastoma lines.

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Differential expression of kinases and their interaction partners between SK-N-SH and SH-SY5Y neuroblastoma cells

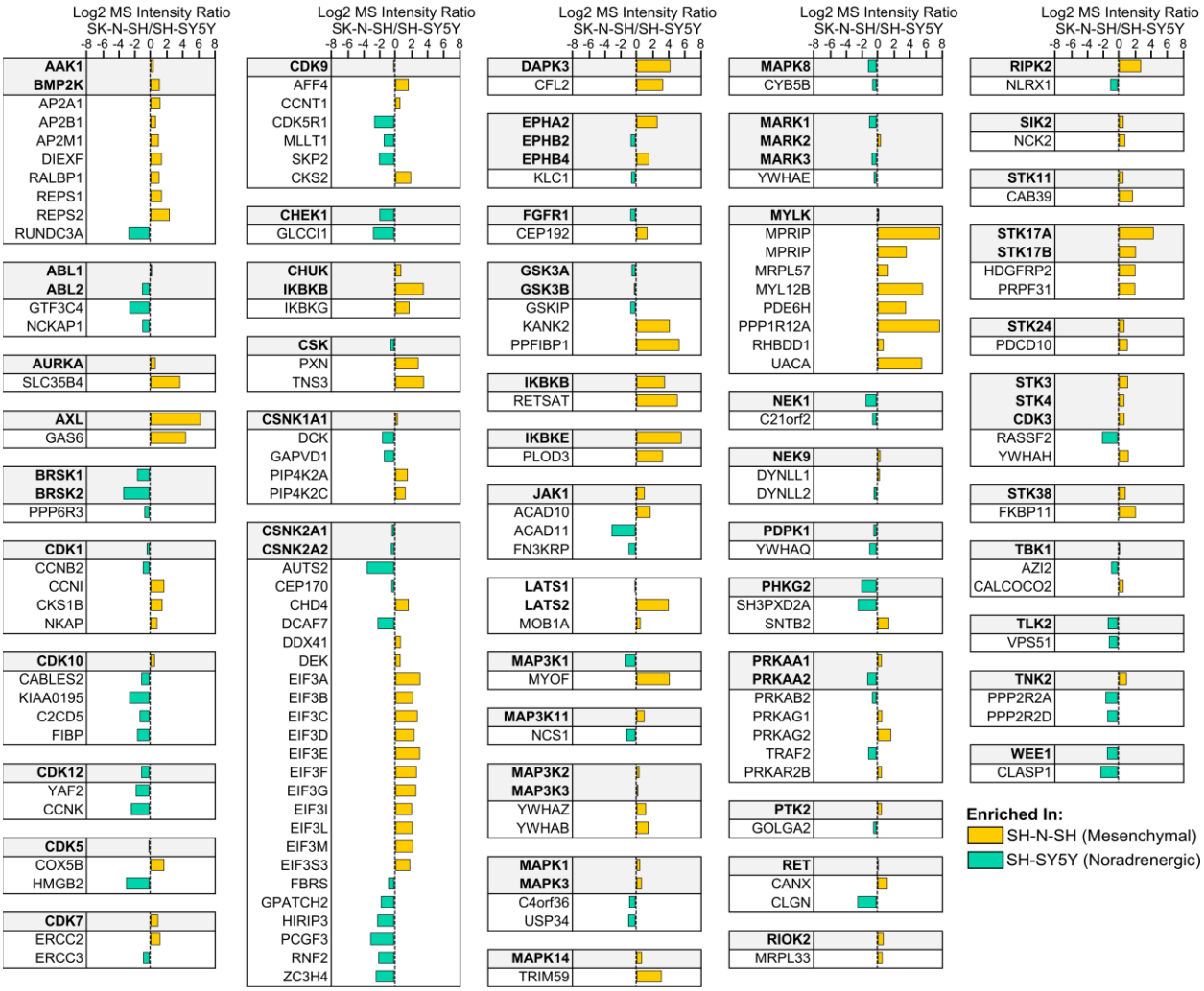
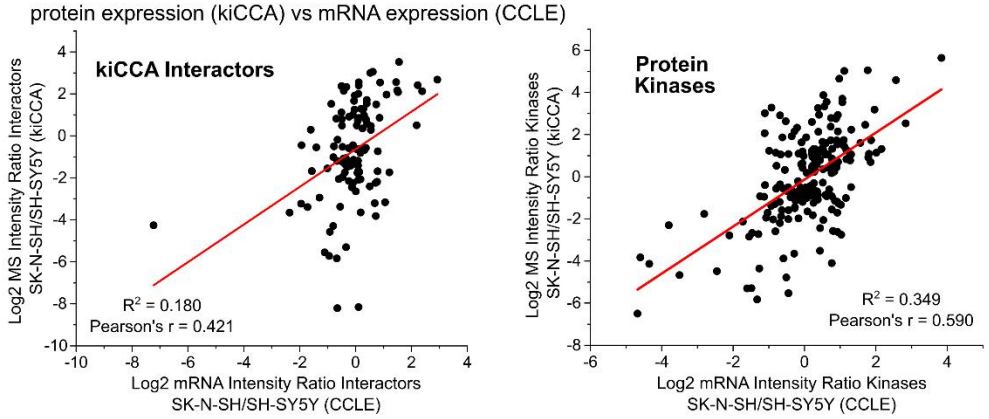


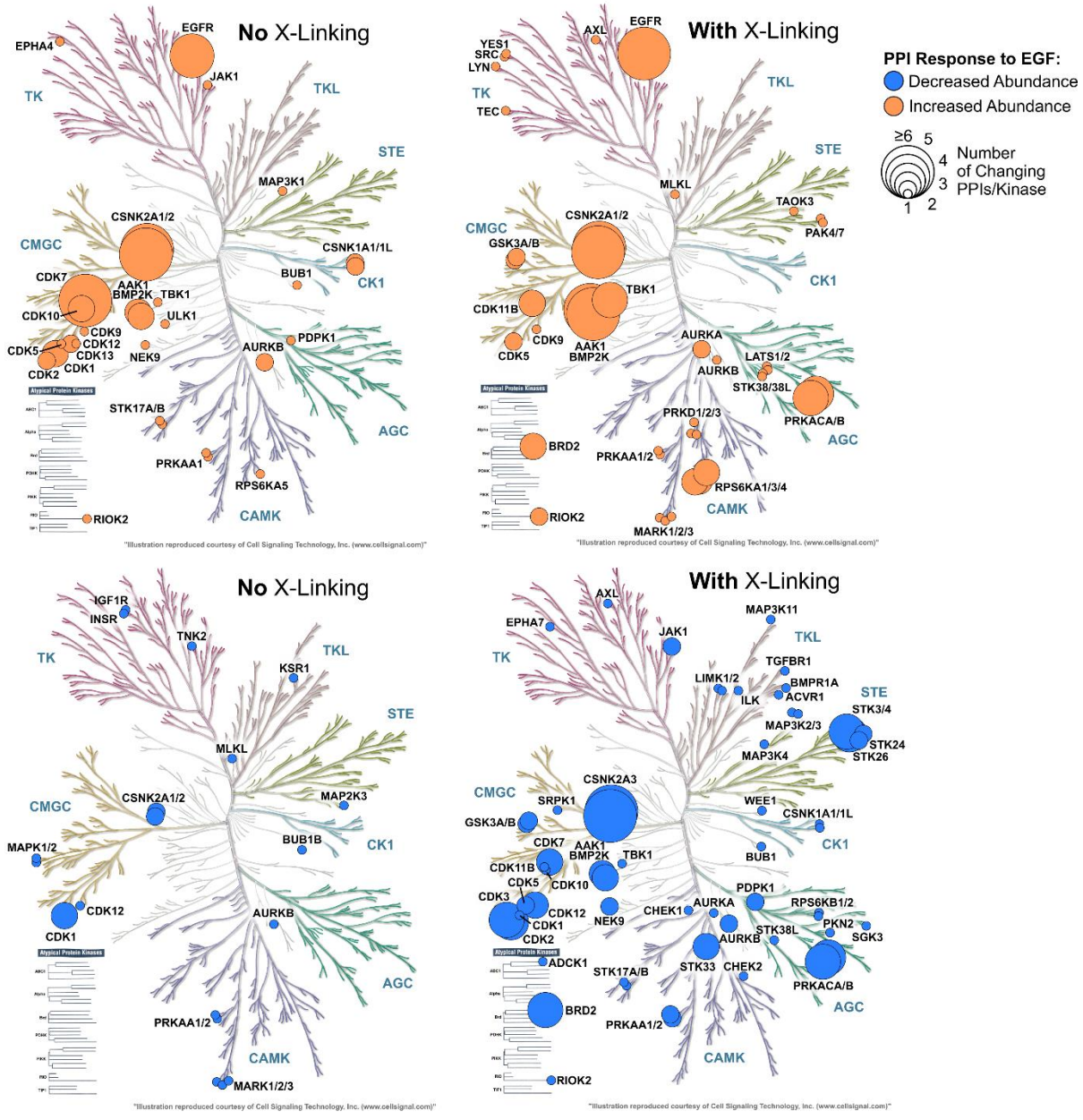
Figure S4. Kinases and their kiCCA interaction partners that were found to be differentially expressed between the mesenchymal-like SK-N-SH and the noradrenergic SH-SY5Y neuroblastoma lines (Student's t-test, BH-FDR = 0.05, n = 22). Related to Figure 3.

Golkowski et al., **Figure S5**

A Correlation, DEA ratios kinases and their interactors SK-N-SH/SH-SY5Y protein expression (kiCCA) vs mRNA expression (CCLE)



B Kinases whose interaction partners change in abundance in response to EGF treatment, kiCCA ± FA crosslinking



Golkowski et al., **Figure S5**

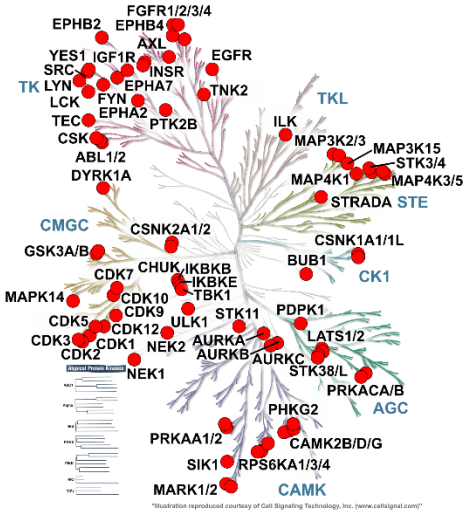
Figure S5. Correlation of differentially expressed kiCCA interactors and kinases at the protein and mRNA level, and results from kiCCA profiling of EGF-stimulated HeLa cells. Related to Figure 3.

(A) Correlation of log2 ratio of mRNA intensity (CCLE data) and log2 ratio of MS intensity differences of high confidence kiCCA interactors and protein kinases between the mesenchymal-like SK-N-SH and the noradrenergic SH-SY5Y neuroblastoma lines.

(B) Overlay of the human kinome dendrogram with kinases whose interaction partners show altered abundance in response to EGF treatment (Student's t-test, BH-FDR < 0.05, n = 22). Kinase interactions that were identified by kiCCA without protein crosslinking are shown on the left, interactions identified only when using formaldehyde-mediated protein crosslinking are shown on the right.

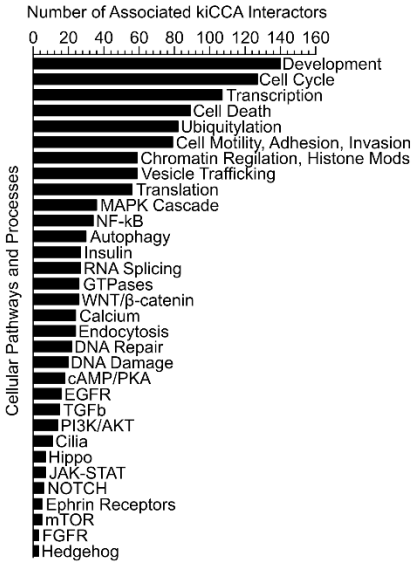
Golkowski et al., **Figure S6**

A Kinases for which kiCCA identified PPIs that inform about their functional state

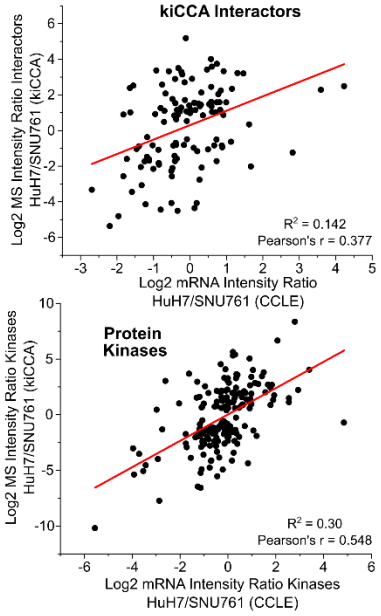


n = 85 Kinases from 56 Kinase Groups

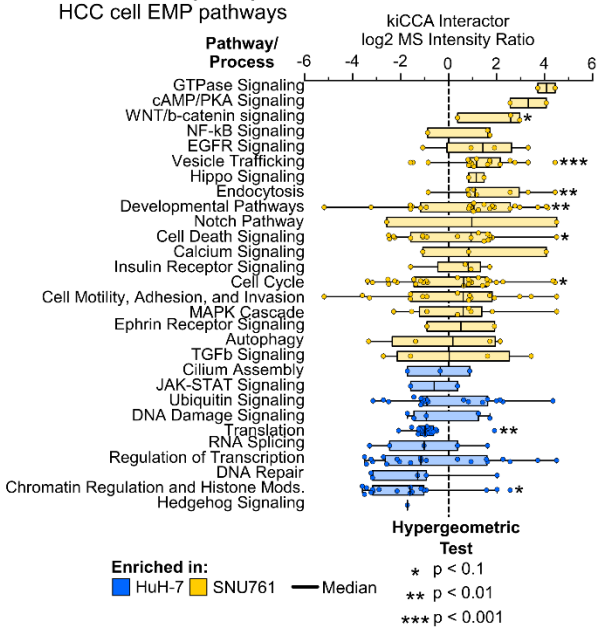
B Mapping kiCCA interactors to 32 disease-relevant signaling pathways and processes



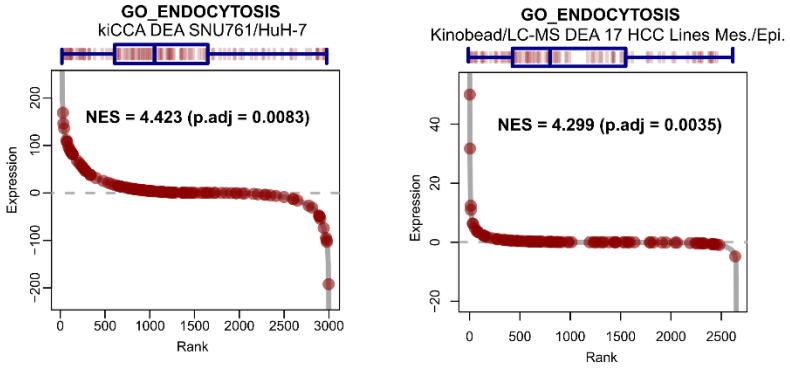
C Correlation, differential expression kinases and their interactors HuH7 and SNU761protein expression (kiCCA) vs mRNA expression (CCLE)



D kiCCA Pathway Analysis of HuH-7 and SNU761Cells Identifies HCC cell EMP pathways



E GSEA analysis validates enrichment of endocytosis pathways in mesenchymal HCC cells



Golkowski et al., **Figure S6**

Figure S6. Defining functional kiCCA PPIs and pathway associations, correlation of differentially expressed kiCCA interactors and kinases at the protein and mRNA level, and kiCCA and GSEA signaling pathway analysis of the HuH-7 and SNU761 HCC lines. Related to Figure 4.

(A) Kinases with identified PPIs indicating kinase activation states and/or localization, i.e., functional marker PPIs (fmPPIs), in the human kinome dendrogram (n = 82 kinase groups).

(B) The 32 pathway and process terms that we associated with high confidence kiCCA interactors, including the number of members.

(C) Correlation of log2 mRNA intensity (CCLE data) and log2 MS intensity differences of high confidence kiCCA interactors and protein kinases between the mesenchymal-like SNU761 and the epithelial-like HuH-7 hepatocellular carcinoma (HCC) lines.

(D) Box plots of MS intensity ratios of high confidence kiCCA interactors with significant abundance differences between SNU761 and HuH-7 cells (Student's t-test BH-FDR < 0.05, n = 22). Every dot represents a kiCCA interactor, and interactors were grouped by their association with 32 representative gene ontology-biological process (GOBP) terms (see Table S3, Tabs 'Pathway Associations' and 'GOBP Search Strings'). Significant enrichment of interactor associated GOBP terms in either cell line was determined using a hypergeometric test and significant terms marked with an asterisk (* $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$). The 29 of 32 plotted GOBP terms had interactors which changed in abundance.

(E) Gene set enrichment analysis (GSEA) of proteins expressed in SNU761 compared to HuH-7 cells using our kiCCA Student's t-test data (see (D) and STAR Methods).

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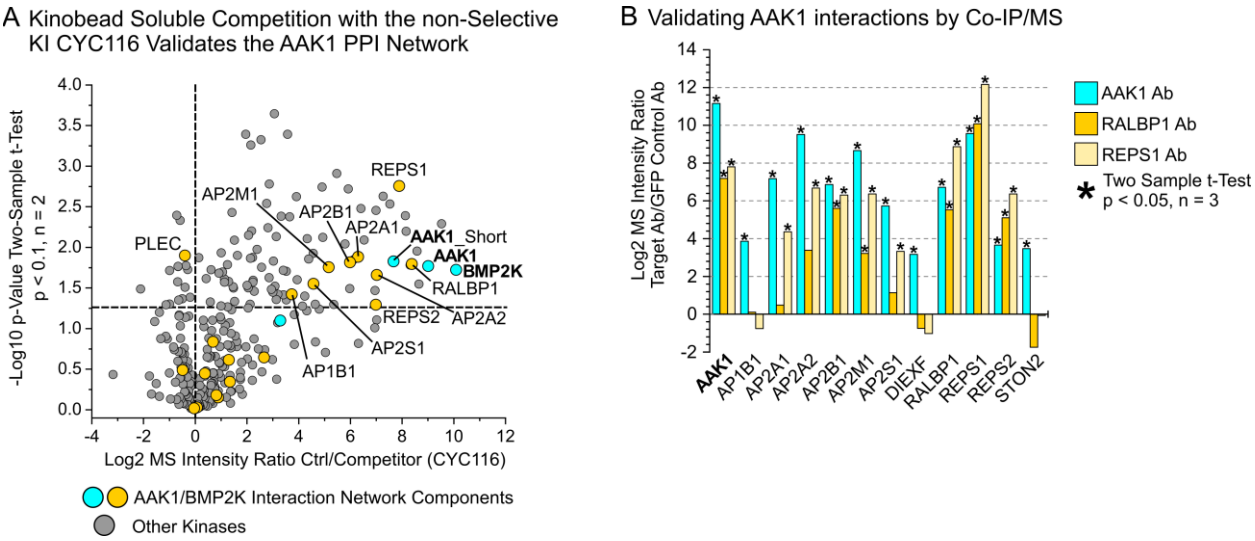

Figure S7. Validating the composition of the AAK1 interactome. Related to Figure 5.

(A) Kinobead competition data using the KIP CYC116 in FOCUS cell lysate, showing competition of AAK1 and BMP2K and their putative interaction partners, as well as dozens of unrelated kinases (two sample t-test, $p < 2$, $n = 2$)

(B) Co-IP/MS experiments in FOCUS cell lysate using specific antibodies targeting AAK1, RALBP1, and REPS1, validating the composition of an AAK1 interaction network identified by kiCCA (two sample t-test, $p < 0.05$, $n = 3$).

Golkowski et al., **Figure S8**

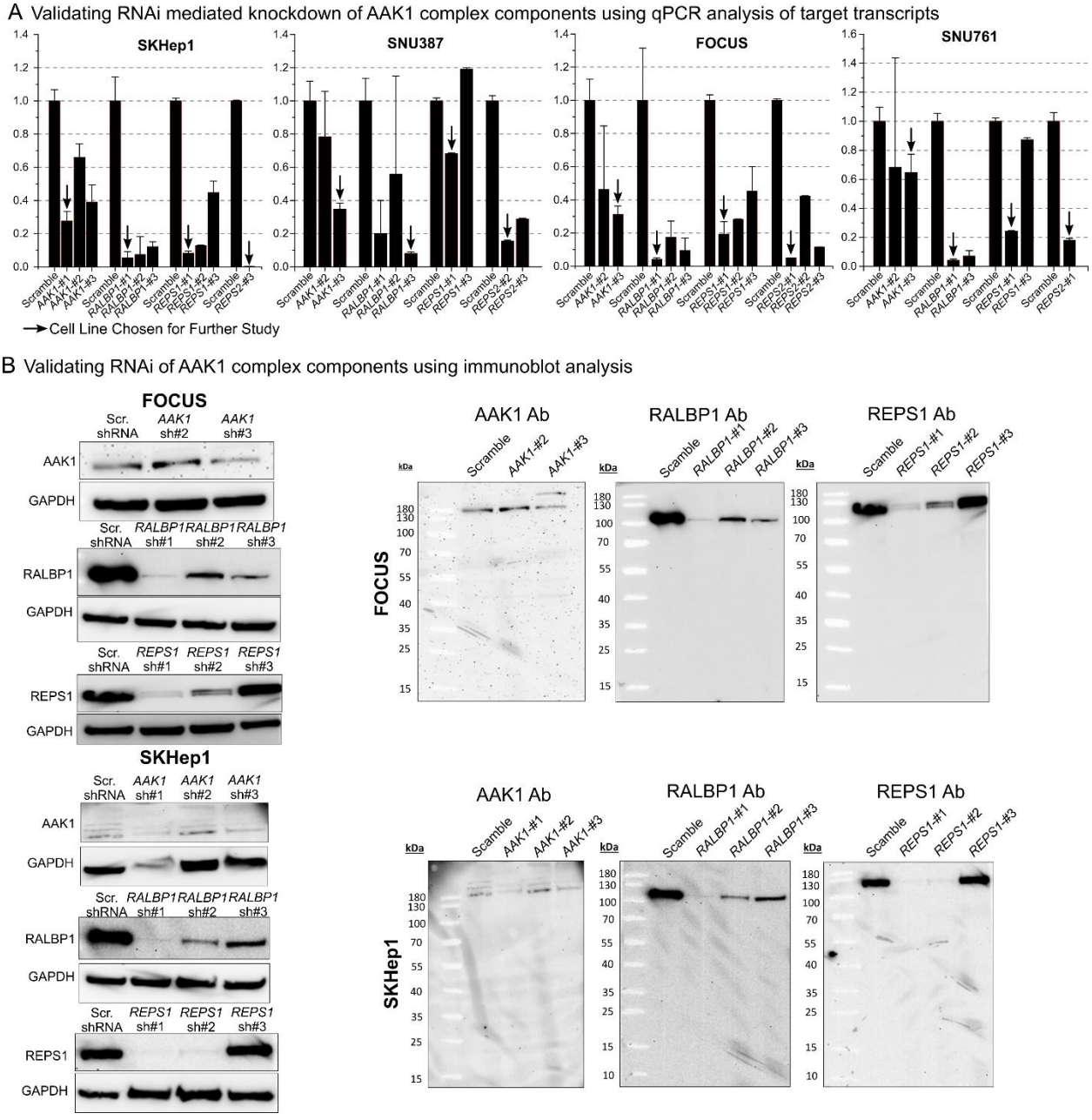

Figure S8. Validating AAK1 complex RNAi knockdown in FOCUS, SKHep1, SNU761, and SNU387. Related to Figure 5.

(A) qPCR analysis of AAK1 complex RNAi lines, validating successful knockdown.

(B) Immunoblot analysis of AAK1 complex RNAi lines, validating successful knockdown. REPS2 blots are not shown because the antibody used is likely not specific.

Golkowski et al., **Figure S9**

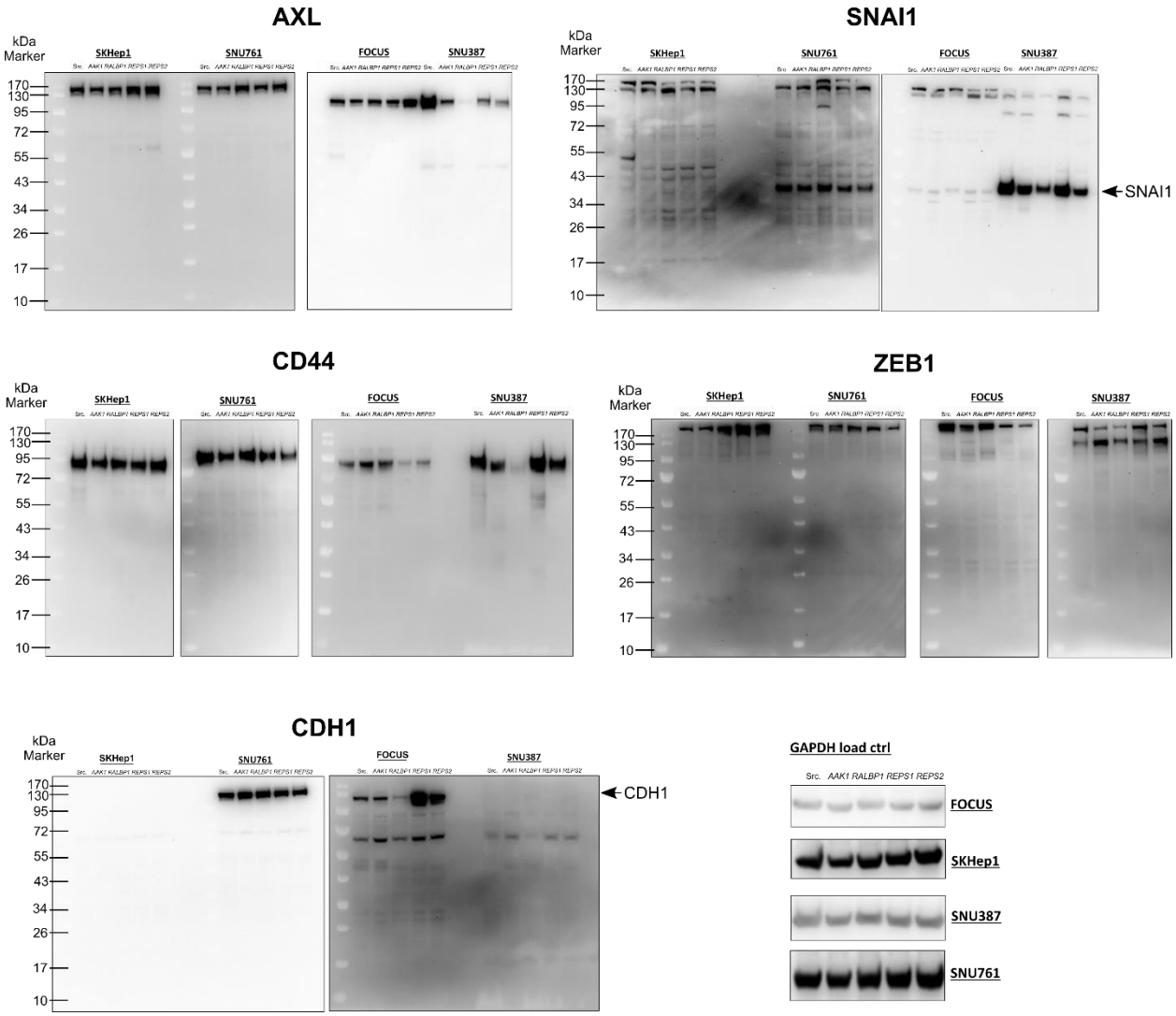

Figure S9. Immunoblot analysis of EMP marker expression in AAK1 complex RNAi cell lines. Related to Figure 5.

Golkowski et al., **Figure S10**

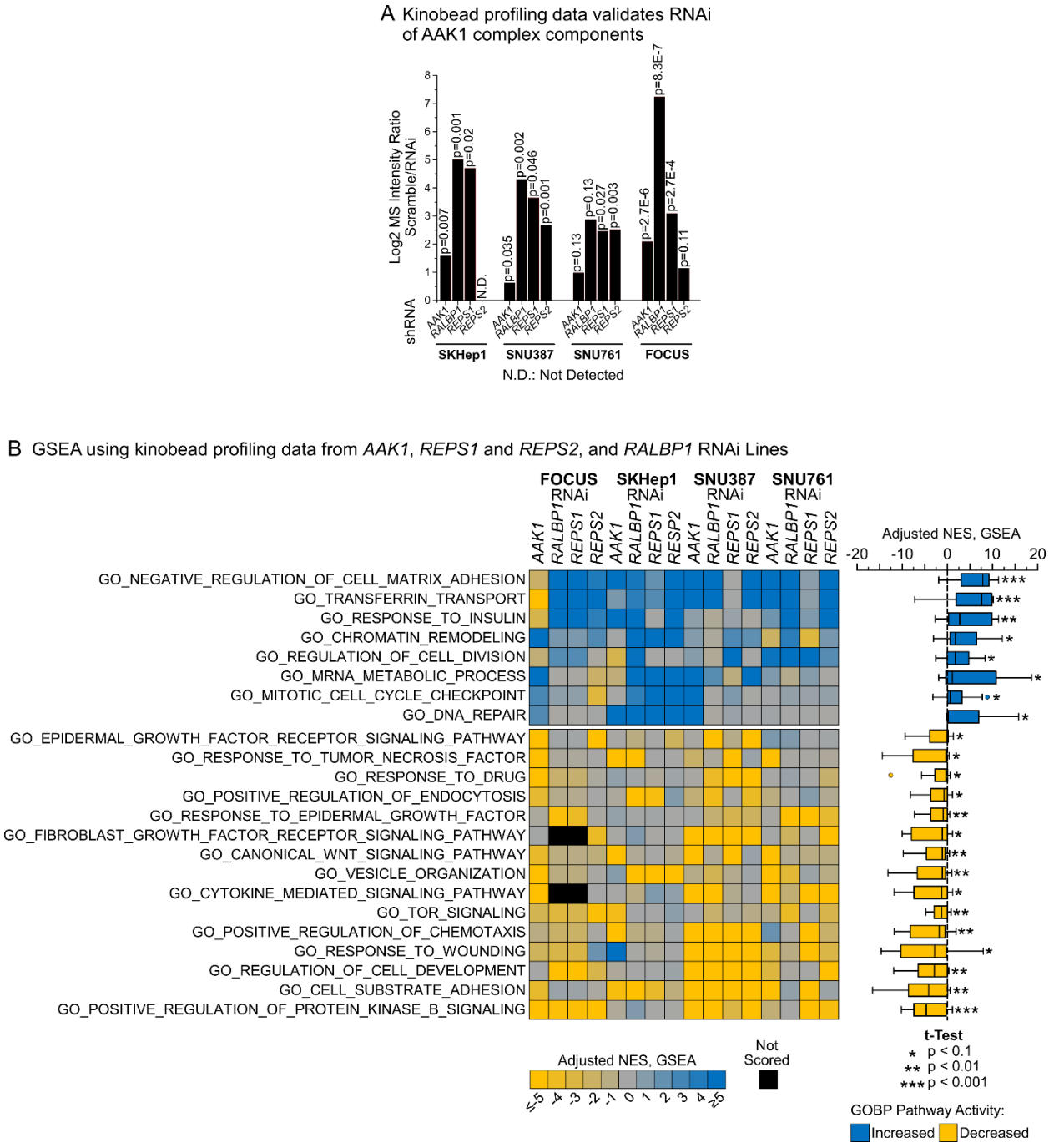

Figure S10. Kinobead/LC-MS analysis of AAK1 complex RNAi lines validates target knockdown, following GSEA identifies signaling pathways associated with each RNAi target. Related to Figure 5.

(A) Kinobead/LC-MS analysis of AAK1 complex RNAi lines, validating successful knockdown.

(B) GSEA pathway analysis of AAK1 complex RNAi lines using GOBP terms (STAR Methods).

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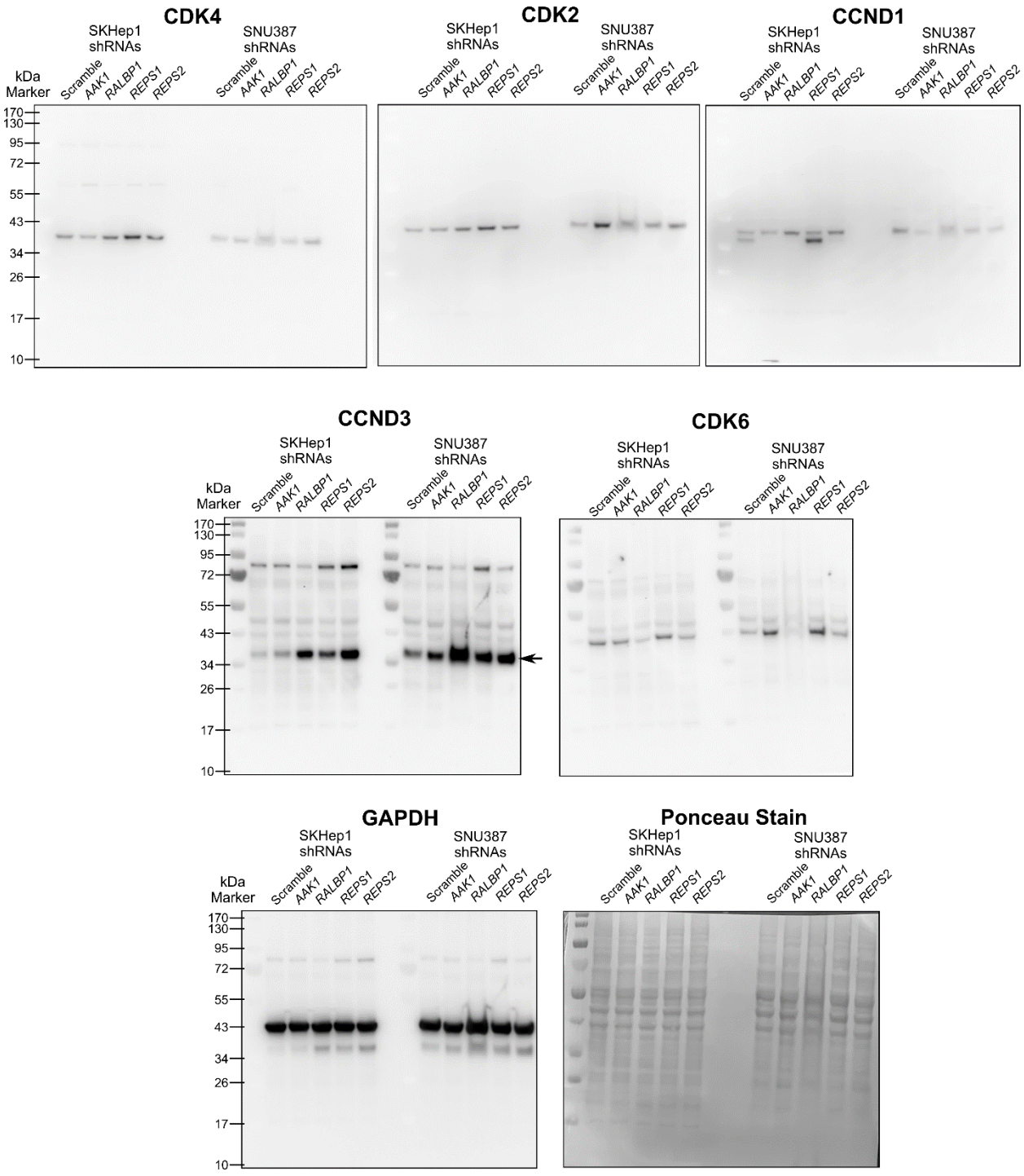

Figure S11. Immunoblot analysis of cell cycle marker expression in AAK1 complex RNAi cell lines. Related to Figure 5.