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Supplemental Information

Kinase Interaction Network Expands Functional and Disease Roles of Human Kinases

Marija Buljan, Rodolfo Ciuffa, Audrey van Drogen, Anton Vichalkovski, Martin Mehnert, George Rosenberger, Sohyon Lee, Markku Varjosalo, Lucia Espona Pernas, Vincent Spegg, Berend Snijder, Ruedi Aebersold, and Matthias Gstaiger

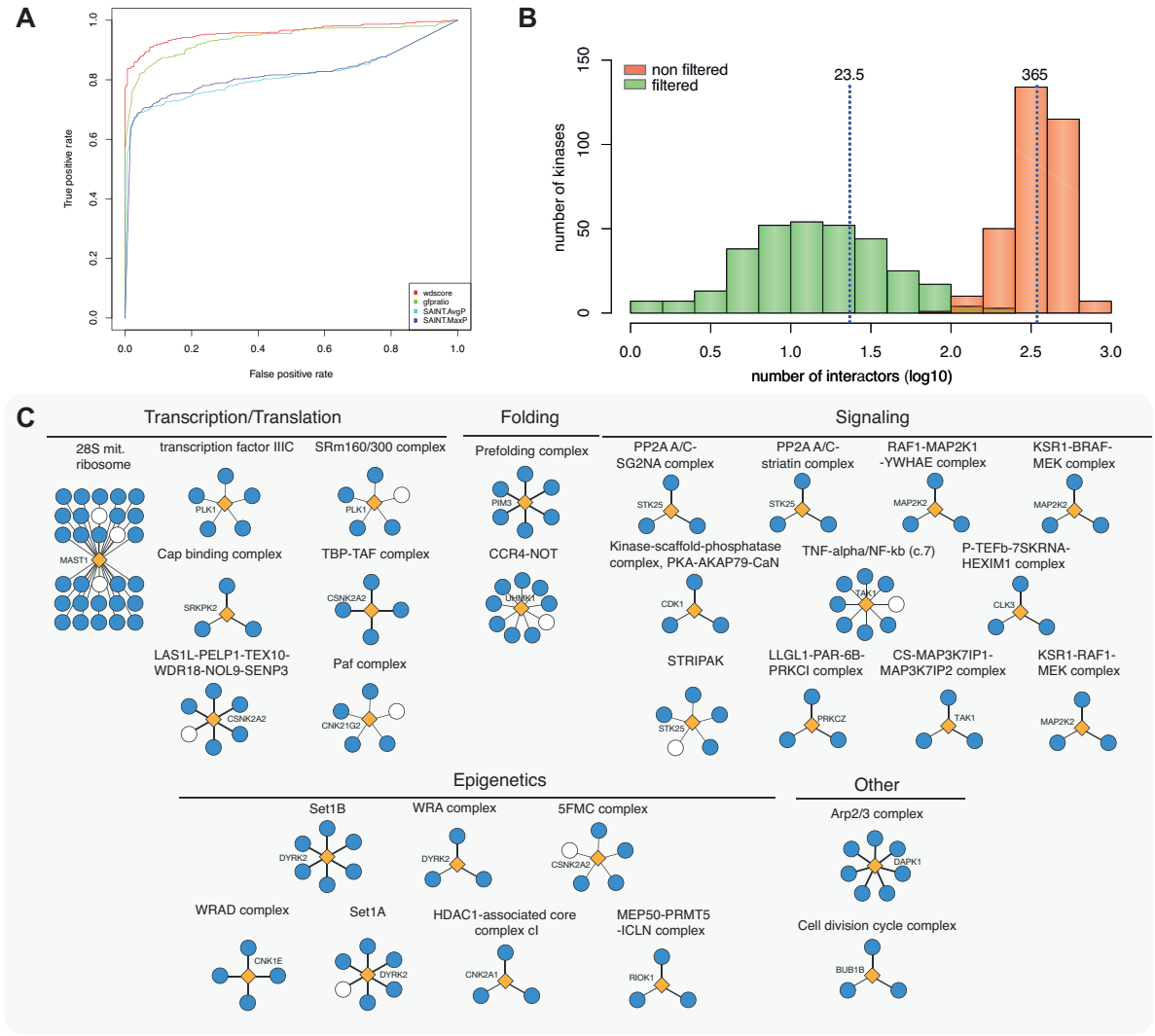

Figure S1. Data filtering and detection of known stable interactions, Related to Figure 1. **A**. Receiver operator characteristic curves for different filtering approaches that were applied on the initial interaction dataset. **B**. Histograms depicting the number of interactors before and after filtering show a reduction of nearly 95% of the interactors due to filtering. **C.** Proteins copurified with the same kinase bait can form stable complexes. CORUM protein complexes (>2 subunits) with at least 80% coverage that was achieved with a single pulldown. Some partially redundant complexes are not shown.

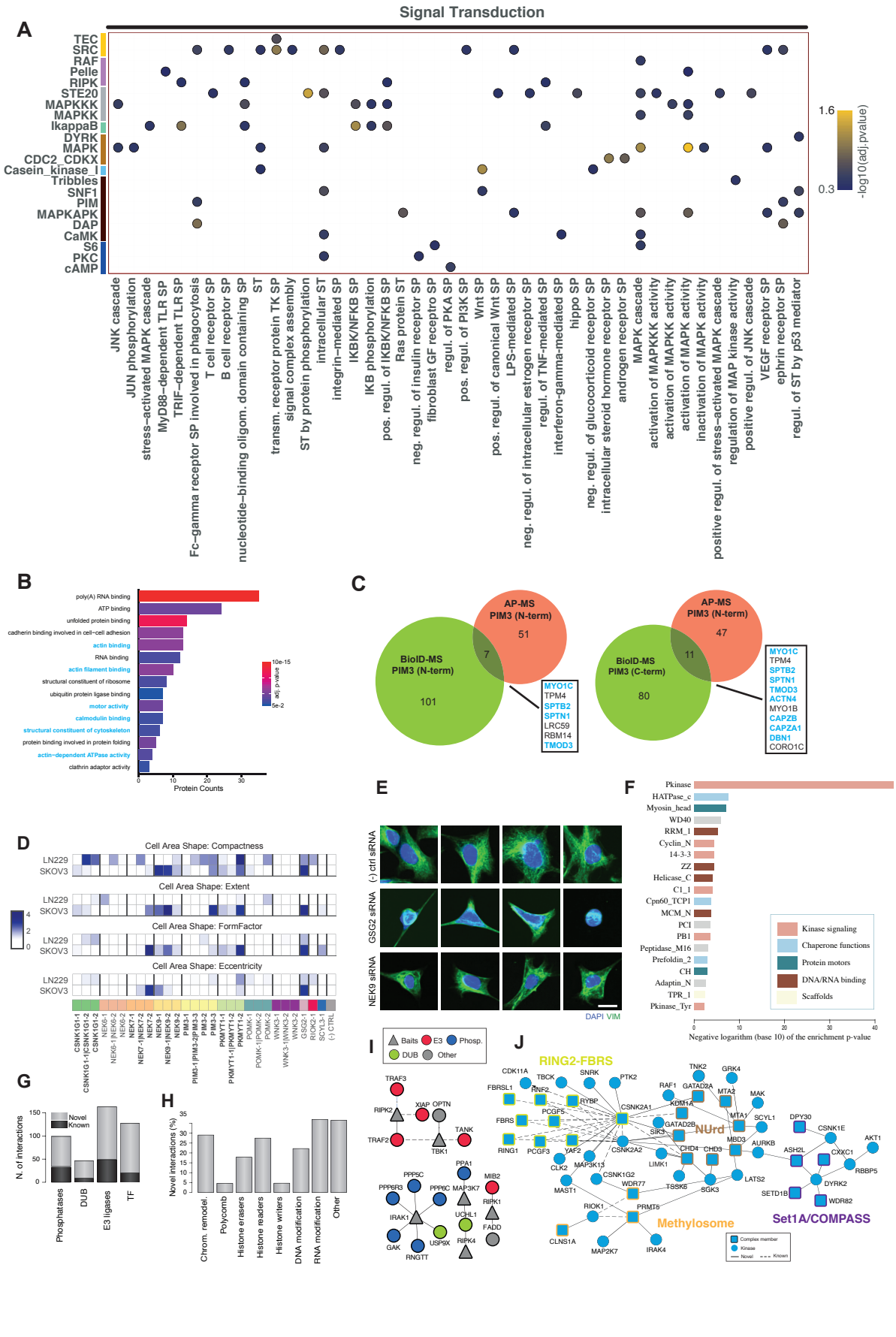
A
Signal Transduction
TEC
SRC
RAF
Pelle
RIPK
STE20
MAPKKK
MAPKK
IkappaB
DYRK
MAPK
CDC2_CDKX
Casein_kinase_I
Tribbles
SNF1
PIM
MAPKAPK
DAP
CaMK
S6
PKC
cAMP
JNK cascade
JUN phosphorylation
stress−activated MAPK cascade
MyD88−dependent TLR SP
TRIF−dependent TLR SP
Fc−gamma receptor SP involved in phagocytosis
T cell receptor SP
B cell receptor SP
nucleotide−binding oligom. domain containing SP
ST
transm. receptor protein TK SP
signal complex assembly
ST by protein phosphorylation
intracellular ST
integrin−mediated SP
IKBK/NFKB SP
IKB phosphorylation
pos. regul. of IKBK/NFKB SP
Ras protein ST
neg. regul. of insulin receptor SP
fibroblast GF receptro SP
regul. of PKA SP
pos. regul. of PI3K SP
Wnt SP
pos. regul. of canonical Wnt SP
LPS−mediated SP
neg. regul. of intracellular estrogen receptor SP
regul. of TNF−mediated SP
interferon−gamma−mediated SP
hippo SP
neg. regul. of glucocorticoid receptor SP
intracellular steroid hormone receptor SP
androgen receptor SP
MAPK cascade
activation of MAPKKK activity
activation of MAPKK activity
activation of MAPK activity
inactivation of MAPK activity
positive regul. of stress−activated MAPK cascade
regulation of MAP kinase activity
positive regul. of JNK cascade
VEGF receptor SP
ephrin receptor SP
regul. of ST by p53 mediator
1.6
0.3
-log10(adj.pvalue)
B
poly(A) RNA binding
ATP binding
unfolded protein binding
cadherin binding involved in cell−cell adhesion
actin binding
RNA binding
actin filament binding
structural constituent of ribosome
ubiquitin protein ligase binding
motor activity
calmodulin binding
structural constituent of cytoskeleton
protein binding involved in protein folding
actin−dependent ATPase activity
clathrin adaptor activity
10e-15
5e-2
adj. p-value
0
10
20
30
Protein Counts
C
AP-MS
PIM3 (N-term)
51
7
BioID-MS
PIM3 (N-term)
101
MYO1C
TPM4
SPTB2
SPTN1
LRC59
RBM14
TMOD3
AP-MS
PIM3 (N-term)
47
11
BioID-MS
PIM3 (C-term)
80
MYO1C
TPM4
SPTB2
SPTN1
TMOD3
ACTN4
MYO1B
CAPZB
CAPZA1
DBN1
CORO1C
D
Cell Area Shape: Compactness
Cell Area Shape: Extent
Cell Area Shape: FormFactor
Cell Area Shape: Eccentricity
LN229
SKOV3
4
2
0
CSNK1G1-1
CSNK1G1-1|CSNK1G1-2
CSNK1G1-2
NEK6-1
NEK6-1|NEK6-2
NEK6-2
NEK7-1
NEK7-1|NEK7-2
NEK7-2
NEK9-1
NEK9-1|NEK9-2
NEK9-2
PIM3-1
PIM3-1|PIM3-2|PIM3-3
PIM3-2
PIM3-3
PKMYT1-1
PKMYT1-1|PKMYT1-2
PKMYT1-2
POMK-1
POMK-1|POMK-2
POMK-2
WNK3-1
WNK3-1|WNK3-2
WNK3-2
GSG2-1
RIOK2-1
SCYL3-1
(-) CTRL
E
(-) ctrl siRNA
GSG2 siRNA
NEK9 siRNA
DAPI VIM
F
Pkinase
HATPase_c
Myosin_head
WD40
RRM_1
Cyclin_N
14-3-3
ZZ
Helicase_C
C1_1
Cpn60_TCP1
MCM_N
PC1
PB1
Peptidase_M16
Prefoldin_2
CH
Adaptin_N
TPR_1
Pkinase_Tyr
Kinase signaling
Chaperone functions
Protein motors
DNA/RNA binding
Scaffolds
0
10
20
30
40
Negative logarithm (base 10) of the enrichment p-value
G
N. of interactions
150
100
50
0
Novel
Known
Phosphatases
DUB
E3 ligases
TF
H
Novel interactions (%)
35
30
25
20
15
10
5
0
Chrom. remodel.
Polycomb
Histone erasers
Histone readers
Histone writers
DNA modification
RNA modification
Other
I
Baits
E3
Phosp.
DUB
Other
TRAF3
RIPK2
XIAP
OPTN
TRAF2
TANK
TBK1
PPA1
PPP5C
MIB2
PPP6R3
PPP6C
MAP3K7
RIPK1
IRAK1
UCHL1
FADD
GAK
USP9X
RIPK4
RNGTT
J
RING2-FBRS
CDK11A
TBCK
SNRK
PTK2
FBRSL1
RNF2
RYBP
PCGF5
FBRS
RING1
PCGF3
YAF2
CSNK2A1
CSNK2A2
CLK2
MAP3K13
CSNK1G2
MAST1
WDR77
PRMT5
RIOK1
CLNS1A
MAP2K7
IRAK4
TNK2
GRK4
RAF1
GATAD2A
MTA2
MAK
KDM1A
MTA1
SCYL1
GATAD2B
NURd
SIN3
MBD3
AURKB
CHD4
CHD3
LIMK1
LATS2
TSSK6
SGK3
DPY30
CSNK1E
AKT1
ASH2L
CXXC1
RBBP5
DYRK2
SETD1B
WDR82
Methylosome
Set1A/COMPASS
Complex member
Kinase
Novel
Known

Figure S2. Functional landscape of proteins included in the Kinase interaction network, Related to Figure 3. A. Dotplot diagram of all terms which were collapsed to the GO term "signal transduction" in Figure 3A. SP=Signaling/Signaling Pathway; ST=Signal Transduction. **B**. Functional GO-term analysis ("Molecular Function_direct") of the combined (N- and C-terminal) BioID-MS interaction network of PIM3. GO-terms that involve actin related molecular functions are shown in blue. **C**. Venn diagram is illustrating the overlap of the filtered AP-MS interaction network of PIM3 and the identified BioID-MS network for N-terminal (left panel) or C-terminal (right panel) FLAG-BirA*-tagged PIM3. Proteins highlighted in blue are assigned with the GO-term "actin binding" (GO: 0003779). **D**. Heat maps depict significant changes in cell phenotypes after gene silencing of target genes relative to the negative siRNA control. Example data for four different area and shape features, which were defined and measured by CellProfiler are shown. Rows represent measurements on two independent cell lines LN299 and SKOV3 and columns correspond to individual siRNA gene targets where different siRNAs for the same gene are indicated with -1 or -2 and combinations of siRNAs are denoted by the symbol '|'. Color-coded bars represent –log10 of the calculated p-values. Data is derived from n=3 replicate wells. siRNA targets that elicit significant phenotypes following siRNA depletion are indicated in bold at the bottom. **E.** Representative images of cells treated with negative control siRNAs and with siRNA constructs for knockdown of the GSG2 and NEK9 genes. **F**. Barplot of most significantly enriched domains among kinase prey proteins **G.** Stacked barchart with the fraction of novel vs already characterized interactions for the indicated protein classes. **H.** Fraction of novel interactions in the defined subclasses of epigenetic factors (see (Medvedeva et al., 2015) for classification) **I.** NfKB activation-related modules with previously reported (dashed line) and novel (solid line) interactions. **J.** Novel vs known interactions involving epigenetic complexes.

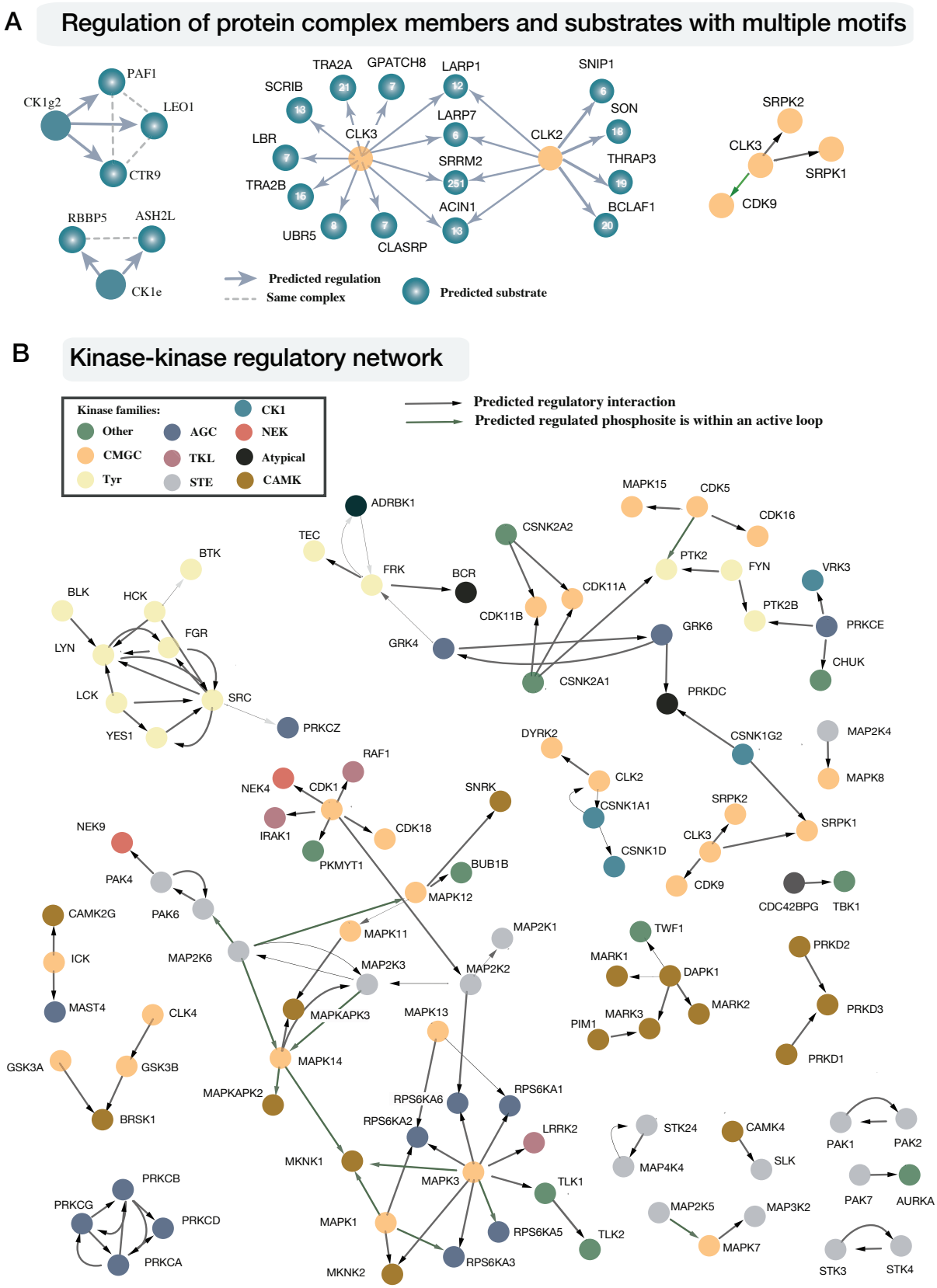
A
Regulation of protein complex members and substrates with multiple motifs
PAF1
CK1g2
LEO1
CTR9
RBBP5
ASH2L
CK1e
TRA2A
GPATCH8
LARP1
SCRIB
CLK3
LARP7
LBR
SRRM2
TRA2B
ACIN1
UBR5
CLASRP
SNIP1
SON
CLK2
THRAP3
BCLAF1
SRPK2
CLK3
SRPK1
CDK9
Predicted regulation
Same complex
Predicted substrate
B
Kinase-kinase regulatory network
Kinase families:
CK1
Other
AGC
NEK
CMGC
TKL
Atypical
Tyr
STE
CAMK
Predicted regulatory interaction
Predicted regulated phosphosite is within an active loop
ADRBK1
TEC
FRK
BCR
BTK
BLK
HCK
FGR
LYN
LCK
SRC
YES1
PRKCZ
CSNK2A2
CDK11B
CDK11A
CSNK2A1
GRK4
GRK6
MAPK15
CDK5
CDK16
PTK2
FYN
VRK3
PTK2B
PRKCE
CHUK
PRKDC
DYRK2
CLK2
CSNK1A1
CSNK1D
CSNK1G2
MAP2K4
MAPK8
SRPK2
CLK3
SRPK1
CDK9
CDC42BPG
TBK1
RAF1
NEK4
CDK1
IRAK1
CDK18
PKMYT1
SNRK
BUB1B
MAPK12
NEK9
PAK4
PAK6
CAMK2G
ICK
MAST4
CLK4
GSK3A
GSK3B
BRSK1
MAP2K6
MAPK11
MAP2K3
MAPKAPK3
MAP2K1
MAP2K2
MAPK13
MAPK14
MAPKAPK2
TWF1
MARK1
DAPK1
MARK3
MARK2
PIM1
PRKD2
PRKD3
PRKD1
RPS6KA6
RPS6KA1
RPS6KA2
LRRK2
MKNK1
MAPK3
TLK1
MAPK1
RPS6KA5
TLK2
MKNK2
RPS6KA3
STK24
MAP4K4
CAMK4
SLK
MAP2K5
MAP3K2
MAPK7
PAK1
PAK2
PAK7
AURKA
STK3
STK4
PRKCB
PRKCG
PRKCD
PRKCA

Figure S3. **Regulatory interactions in the kinase network, Related to Figure 4**. **A.** Highlighted regulatory examples: Similar to CK2 substrates, several predicted substrates of casein kinases CK1e and CK1g2 also had a role in transcription regulation. Three proteins from the Paf complex, which is involved in transcription elongation, were predicted to be regulated by the CK1g2 kinase. Furthermore, another example for kinases that had substrates with multiple corresponding phosphomotifs were CDC-like kinases CLK2 and CLK3. There, 12 out of 15 predicted substrates with multiple CLK2/3 phosphomotifs were associated with the GO term "poly(A) RNA binding". **B.** Predicted kinase-kinase regulatory network. Dark arrows represent directed phosphorylation events, green arrows instances where a phosphosite maps to an activation loop. Kinase families are colored according to the scheme in **Figure 1**. Only regulatory modules with at least three kinases are included.

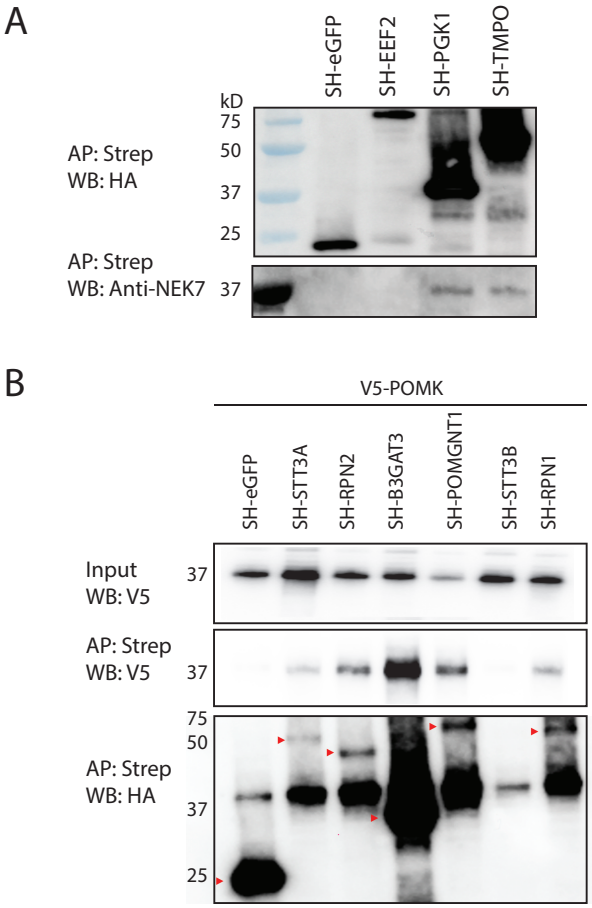

Figure S4. Validation of novel interactions by copurification and Western blotting, Related to Figure 6. A. Reciprocal validation of newly identified interactions with endogenous NEK7 in A549 cells. Proteins indicated at the top were transiently expressed as SH-tagged proteins for subsequent Streptactin affinity purification. Purified proteins were detected with anti HA antibodies (upper panel, bait expression control) or with anti NEK7 antibodies (lower panel). Lower panel validates the novel interaction of PGK1 and TMPO **B.** Validation of POMK interactors by reciprocal copurification and Western blotting in A549 cells. V5-tagged POMK kinase were transiently expressed with indicated SH-tagged proteins for subsequent copurification and Western blotting. Expression of V5-POMK5 was confirmed by anti V5 Western blotting of the transfected lysates (upper panel). Proteins purified by Streptactin affinity purification were detected by Western blotting with anti V5 antibodies for the detection of copurified V5-POMK (middle panel) and anti HA for the detection of purified SH tagged bait proteins (lower panel). Red arrow indicates expected size of SH tagged proteins. The detected V5-POMK signal confirms the known interaction with the POMGNT1 protein from the O-glycosylation pathway, as well as the novel interactions with STT3A, RPN2, RPN1 from the N-glycosylation pathway and with the B3GAT3 protein that is linked to multiple diseases. Of note, these are all membrane proteins (residing either in the ER or Golgi membranes). POMK (protein O-mannose kinase) itself is localized in the ER membrane, where it is involved in building protein-linked glycosyl chains.

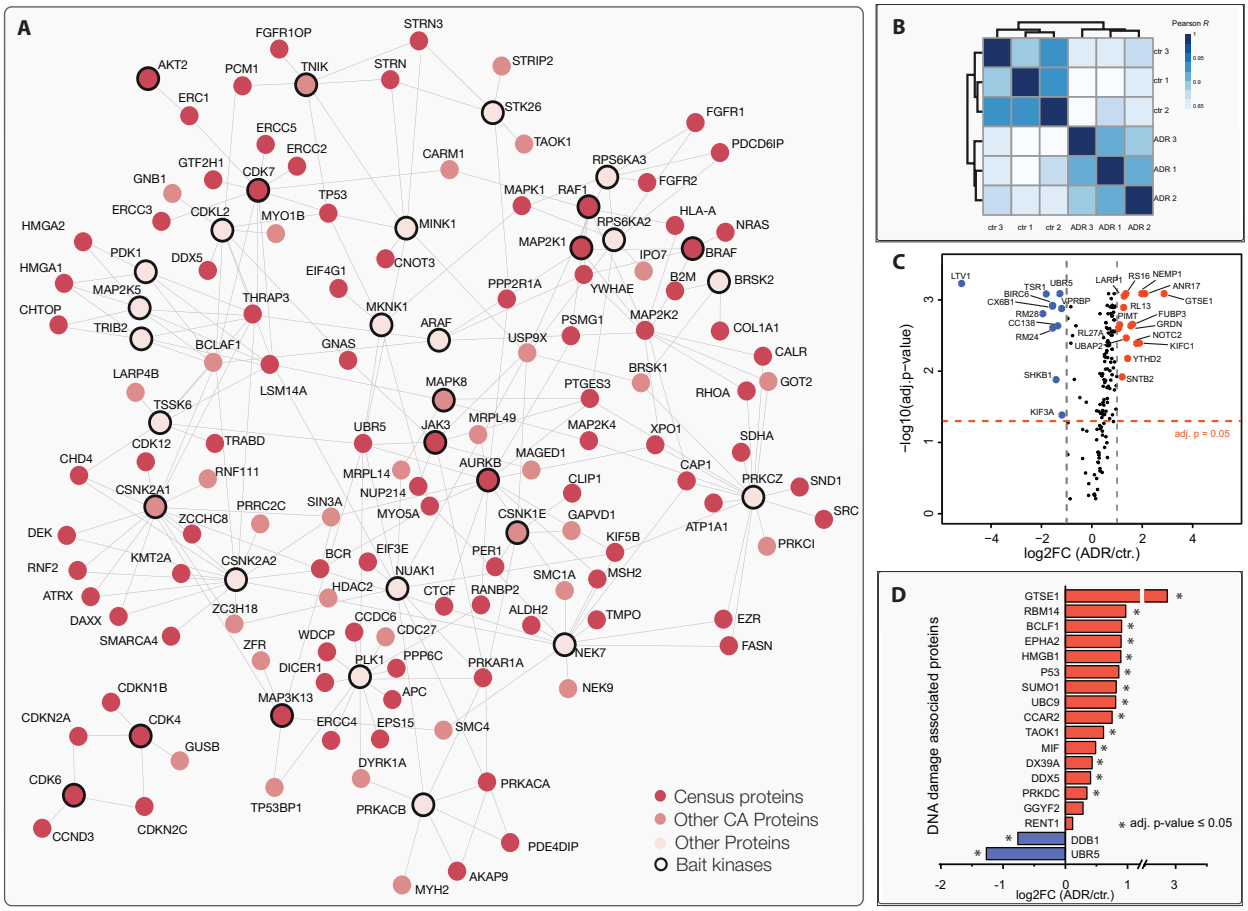

Figure S5. Cancer-associated kinase network, Related to Figure 7. A. All significant modules detected at a p-value < 0.05 are shown. This includes the modules depicted in **Figure 7** as well as additional examples. Bold circles indicate kinases with a significant enrichment of CA proteins. Coloring corresponds to the scheme introduced in **Figure 7**. This network highlights several kinases that are not in the Cancer Gene Census, but whose interaction neighborhoods are enriched in CA proteins. **B.** Correlation of the bait-normalized precursor peptide MS1 intensity of the different conditions and three replicates of Dyrk2 BioID-MS experiments shown in Figure 7 indicated a high reproducibility of the measurements. **C.** Volcano plot is illustrating interaction changes in the Dyrk2 network upon induction of genotoxic stress with Adriamycin (ADR). Upregulated interactions are presented as red dots and downregulated ones as blue dots (log2FC (ADR/ctr) ≥ 1 and adj. p-value ≤ 0.05). **D.** Barplot is showing all significant interaction changes to proteins associated with DNA damage related cellular processes (GO: 0006974; "cellular response to DNA damage stimulus") observed with BioID upon ADR treatment.