Supplemental Material

DEAD box RNA helicases are pervasive protein kinase interactors and activators

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Supplementary Methods

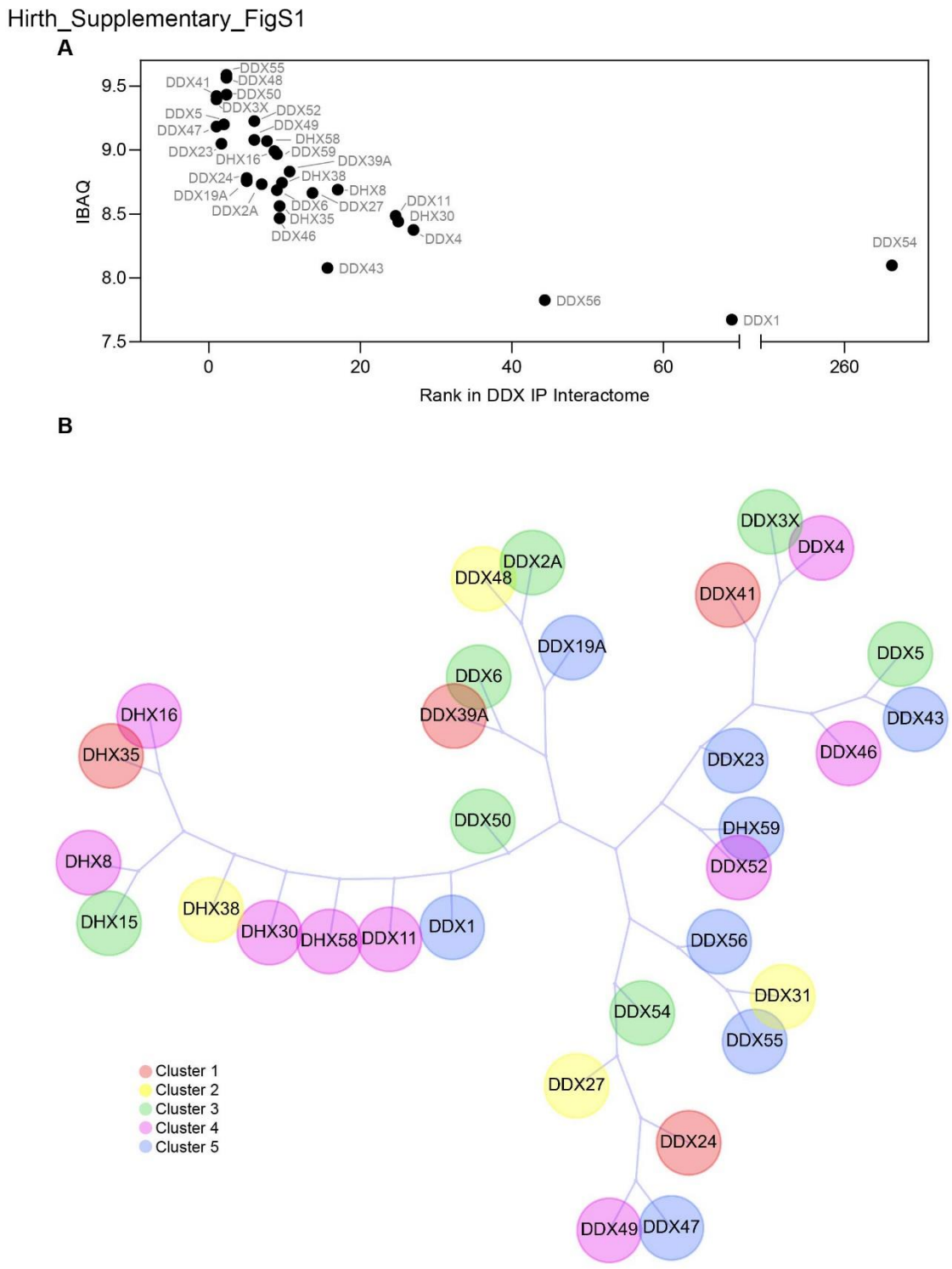

Fig. S1 | DDX/DHX interactome clusters do not correlate with phylogeny

(A) Intensity Based Absolute Quantification (IBAQ) values for all DDX proteins and their corresponding rank within the DDX specific interactome. The IBAQ values are a proxy for transfected DDX protein expression levels.

(B) A phylogenetic tree of the DDX/DHX baits used in the proteomics screen. The colors of the nodes represent the 7 clusters from spectral biclustering of the interactome against the DDX/DHX baits.

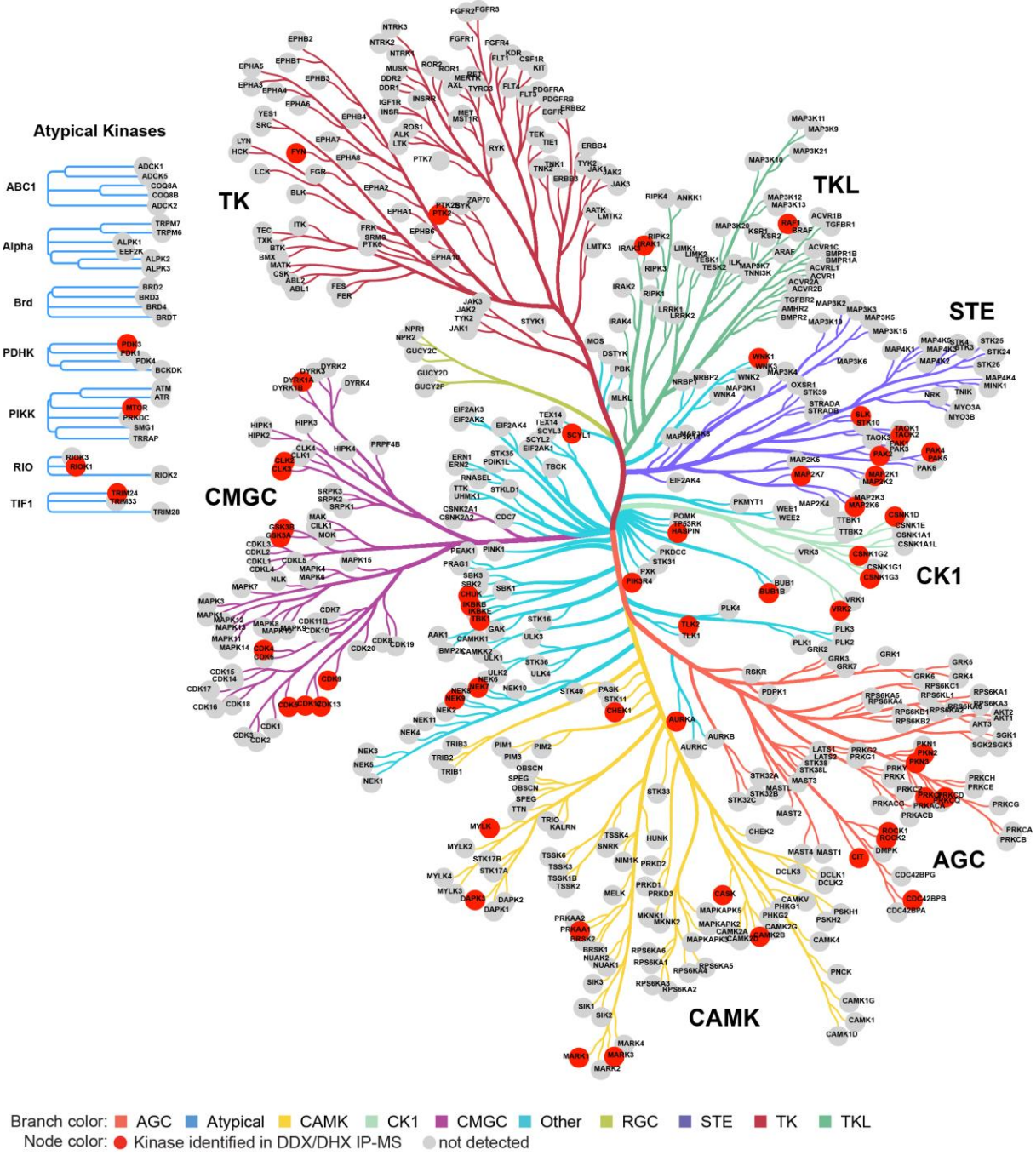

Fig. S2 | DDX/DHX proteins interact with kinases of all kinase groups in HEK293T cells

Phylogenetic kinome tree highlighting kinases (red) identified in the DDX/DHX interactome screen.

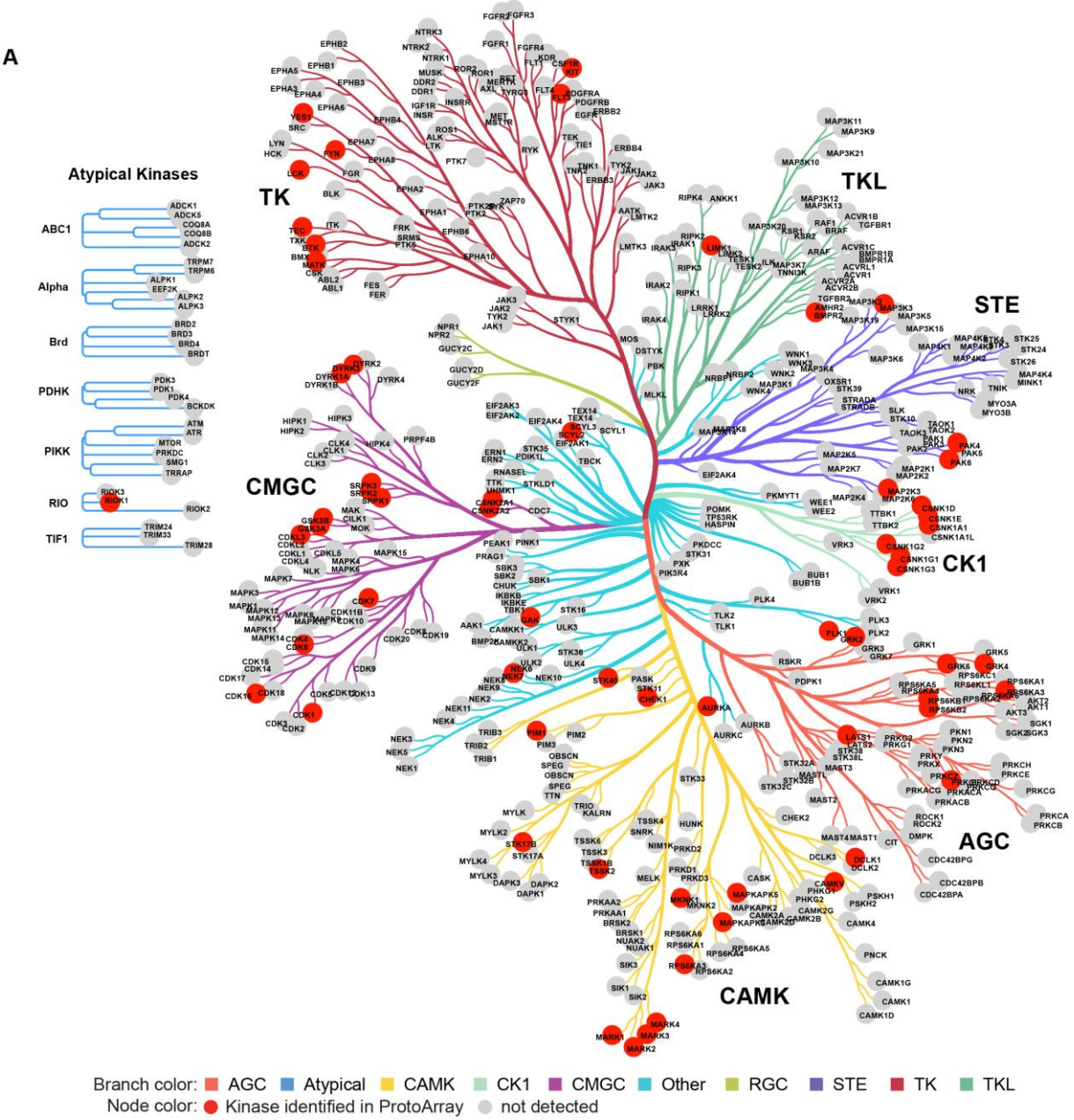

Fig. S3 | DDX proteins interact on ProtoArray with kinases of all kinase groups

(A) Phylogenetic kinome tree highlighting kinases (red) identified in the protein array screen.

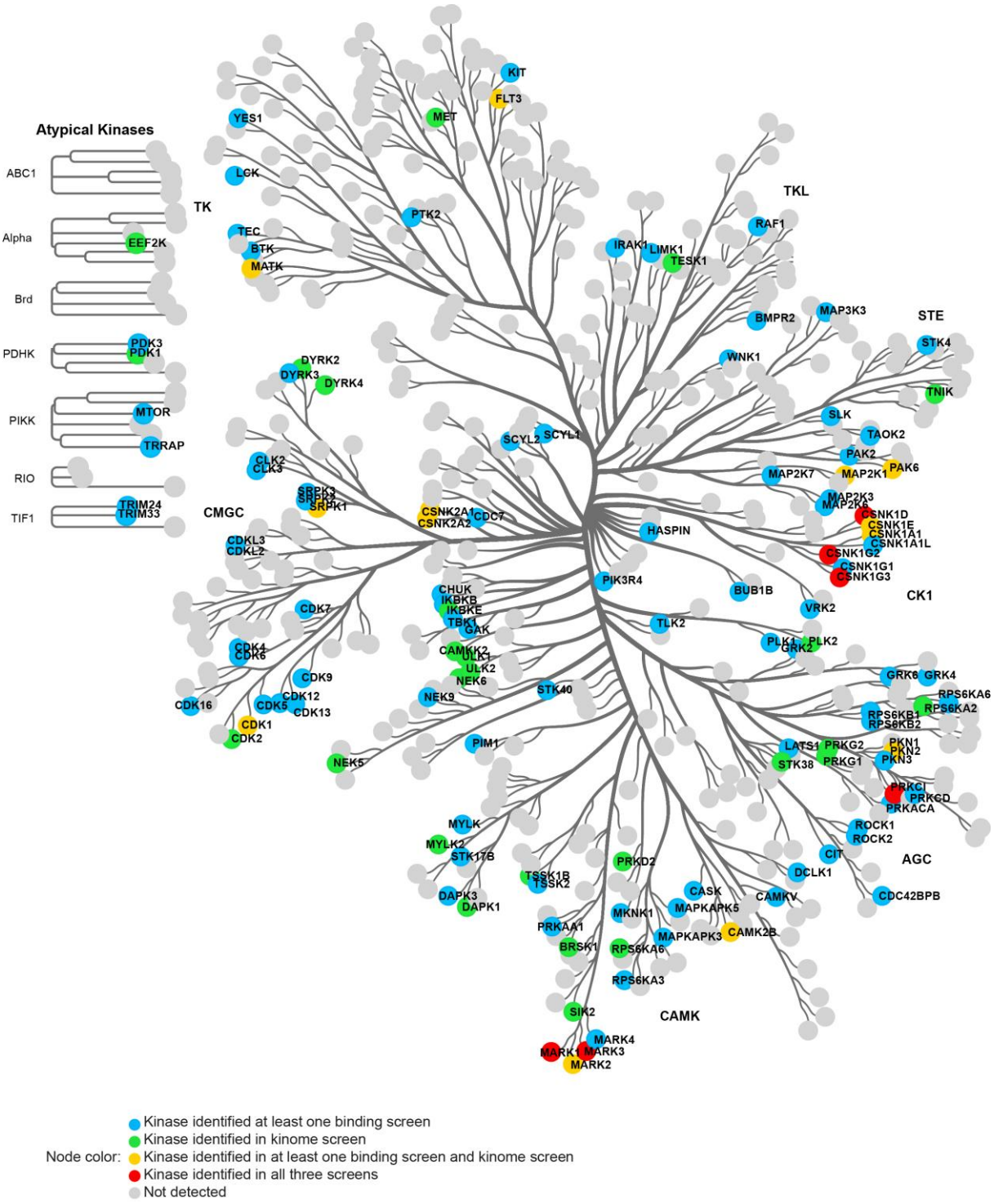

Fig. S4 | Summary of all screens: DDX proteins bind to- and stimulate kinases throughout all kinase groups

Blue: Kinases identified in the interactome and/or the ProtoArray screen. Green: Kinases with up-regulated activity upon DDX3X, -39A, -50 or -56 addition. Yellow: Kinases identified in at least one binding screen and with up-regulated kinase activity. Red: Kinases detected independently in all three screens.

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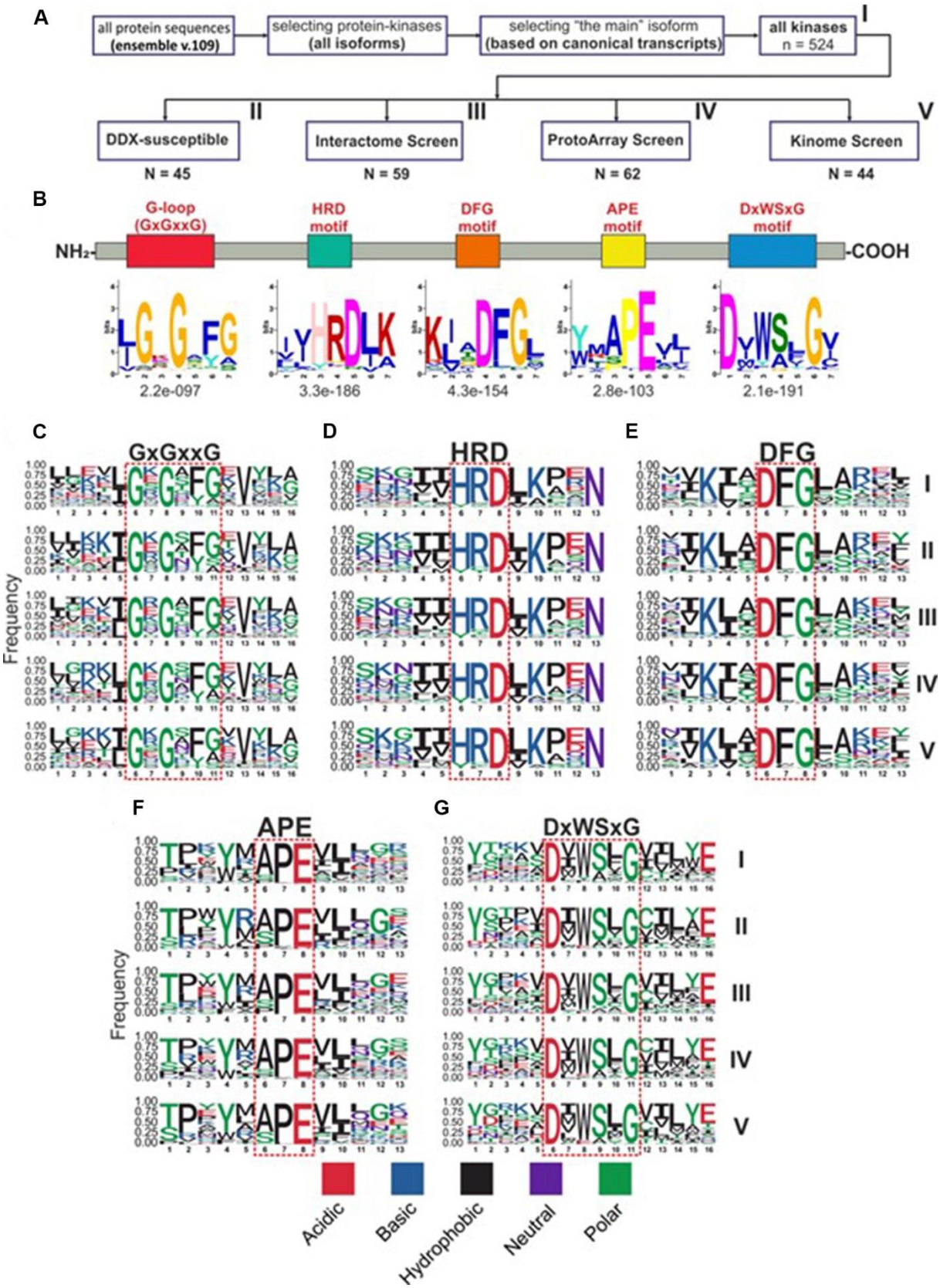
A
all protein sequences (ensemble v.109)
selecting protein-kinases (all isoforms)
selecting "the main" isoform (based on canonical transcripts)
all kinases n = 524
I
II
III
IV
V
DDX-susceptible
N = 45
Interactome Screen
N = 59
ProtoArray Screen
N = 62
Kinome Screen
N = 44
B
G-loop (GxGxxG)
HRD motif
DFG motif
APE motif
DxWSxG motif
NH2
-COOH
2.2e-097
3.3e-186
4.3e-154
2.8e-103
2.1e-191
C
GxGxxG
D
HRD
E
DFG
Frequency
F
APE
G
DxWSxG
Acidic
Basic
Hydrophobic
Neutral
Polar

Fig S5. | Motif analysis in DDX-regulated protein kinase candidates.

(A) Analysis workflow.

(B) Top-5 motifs obtained by MEME analysis of human protein kinase sequences (n = 524) with relative localization of corresponding conserved motifs across kinase sequence.

(C – G) The sequence LOGOs with 'frequency' Y-axis obtained with ggseqlogo package for sequences contexts (±5 aminoacids up- and downstream) around conservative GxGxxG, DxWSxG, HRD, DFG, and APE motifs (highlighted by dashed red rectangles), correspondingly. Different groups of kinases are denoted according to: I – all, II – DDX-susceptible, III – identified by Interactome screen, IV – identified by Protoarray screen, and V – identified by Kinome screen. Amino acids are color-encoded: red – acidic, blue – basic, black – hydrophobic, purple – neutral, and green – polar. Note that DDX-regulated kinase candidates fail to show distinct motifs.

Methods

Immunoprecipitation of FLAG-DDX proteins

HEK293T cells were seeded at a density of 1×10^6 cells per 15 cm dish. Cell transfection was immediately followed using polyethylenimine (PEI) and 10 ng of plasmid DNA per plate. 72 hours post transfection, the cells were harvested and three to five plates were pooled per sample. Cell pellets were stored at -80 °C until being processed for immunoprecipitation. Cells were lysed in 10 ml RIPA lysis buffer (30 mM Tris-HCl, 150 mM NaCl, 5 mM $MgCl_2$, 0.01 % SDS, 0.1 % Na-deoxycholate, 0.1 % Triton X-100, 1 mM DTT) supplied with 1:1,000 salt active nuclease (Sigma-Aldrich) and 1:1,000 Benzonase (Merck). Meanwhile, FLAG magnetic beads (Hölzel Diagnostika, HY-K0207) were blocked in 5 % BSA and 100 µg/ml salmon sperm (LIFE Technologies, 15632011) in PBS. After all the DNA has been sufficiently degraded (approx. 4 hours) the lysate was spun down and the supernatant was incubated with the FLAG beads at 4°C overnight. The beads were washed five times in lysis buffer, and twice in PBS. Subsequent sample processing was done directly on the beads.

Protein Digestion

Beads were resuspended in elution buffer (0.1% w/v SDS in 100 mM ammonium bicarbonate). DTT was added to a final concentration of 10mM and the samples were incubated at 56 ^{0}C for 30 min. Samples were brought to room temperature and 50 mM of chloroacetamide was added to a final concentration of 50 mM with a 30 min incubation at RT. The reaction was quenched by adding DTT again to 10 mM final concentration. Finally, proteins were digested overnight at 37°C by using 1 µg of Trypsin – LysC mix. The following day the peptide supernatant was transferred to new tubes and 10 µl of SP3 beads that were prepared as described (Muller et al. 2020) were added to each sample. Neat acetonitrile was added to every tube to reach a final concentration of acetonitrile higher than 95% (v/v). The tubes were placed on a rotating platform for 20 min at room temperature. Subsequently the supernatant was discarded, and the

beads were washed twice with neat acetonitrile. Finally, the tubes were airdried to remove the remaining acetonitrile completely. The beads were resuspended in 0.1% formic acid and stored in -20 ^{0}C until the time of the analysis.

Analysis of MS data

Raw files were processed using MaxQuant software (Tyanova et al. 2016) (version 2.0.3.0). The search was performed against the human UniProt canonical database (downloaded 04/2019) containing only reviewed entries. Enzyme digestion in MaxQuant settings was set to trypsin allowing for a maximum of up to three missed-cleavages. Protein N-term acetylation, methionine oxidation, as well as deamidation (NQ) were set as variable modifications and carbamidomethylation of cysteine as a fixed modification. Minimum unique peptides option was set to 1. Match between runs was enabled by setting the match time window at 0.4 min. Both intensity-based absolute quantification and label-free quantification values were calculated. Peptide and protein hits were filtered at a false discovery rate (FDR) of 1% with a minimal peptide length of seven amino acids. The reversed sequences of the target database were used as a decoy database. Second peptide search for the identification of the chimeric MS^2 spectra was enabled. All other MaxQuant options were left to their default settings. MaxQuant output tables were analyzed with Microsoft Excel and R (version 4.0.3) (R Core Team, 2020). To improve the specificity of the protein identification, proteins that were identified with only one unique peptide were filtered out of the dataset.