SUPPLEMENTARY INFORMATION

Systematic Identification of Protein Phosphorylation-Mediated Interactions

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- S-1: Supplemental Figure 1 Elution traces for additional protein complexes.
- S-2: Supplemental Figure 2 Comparison of functional protein phosphorylation studies.
- S-3: Supplemental Figure 3 Additional analysis of scoring methods used for determining the phospho-DIFFRAC hits.
- S-4: Supplemental Table 1 Elution profiles for all identified proteins.
- S-4: Supplemental Table 2 DA z-score and DIFFRAC scores for hits and all identified proteins.



Supplemental Figure 1. A) Elution traces for Hepatoma derived growth factor related protein 2 (HDGFL2), top, and histone 3 (H3C1), bottom. B and C) Elution traces for subunits of the ASAP complex from different biological replicates than that in Fig 4E. From top to bottom: Apoptotic chromatin condensation inducer in the nucleus (ACIN1), RNA-binding protein with serine-rich domain 1 (RNPS1), Histone deacetylase complex subunit SAP18 (SAP18).



Supplemental Figure 2. A) Protein functional score distribution for all proteins with functional scores (grey), hits from the study by Potel *et al.* (purple), hits from the study by Huang *et al.* (orange) and hits from this study (blue). B) Venn diagram of functional phosphoproteins identified in 3 different experimental studies.



Supplemental Figure 3. A) Abundance distribution for proteins identified in the 3 biological replicates in this study. B) Precision-recall curve of the two scoring methods used in this study and with points highlighted indicating the 90th (orange), 95th (magenta), and 99th (grey) percentile of each score.

Supplemental Table 1. Protein elution profiles for phosphatase-treated and control samples across all three replicates and fractions, reporting peptide spectral matches (PSMs) per protein per biochemical fraction.

Supplemental Table 2. DA z-score and DIFFRAC scores for phospho-DIFFRAC hits and all other proteins identified in the study, as well as per-fraction DA z-scores.