Supplementary Figures

Supplementary Figure 1: A) Intensity correlations for all phosphopeptide quantifications from HeLa. The phosphopeptide log base 2 intensities are plotted for all HeLa enrichments (E1,E2,E3) and their mass spectrometry replicate run analysis (MS1,MS2,MS3). B) Intensity correlation comparisons between each enrichment (average of three MS replicates). The average Pearson correlation was 0.90 and 0.92 for the MS and enrichment replicates, respectively.

Supplementary Figure 2: Phosphosite ratio versus significance plots of all PGE_2 stimulations versus control comparisons (two sample t-test). P-values (-log base 10) are plotted as a function of the phosphosite ratio (log base 2) for all the PGE_2 stimulations (5, 10, 20, 30, 60 min) versus control (0 min) comparisons. Regulated sites are colored in red (permutation based FDR=0.005, s0 adjusted for 2-fold regulation).

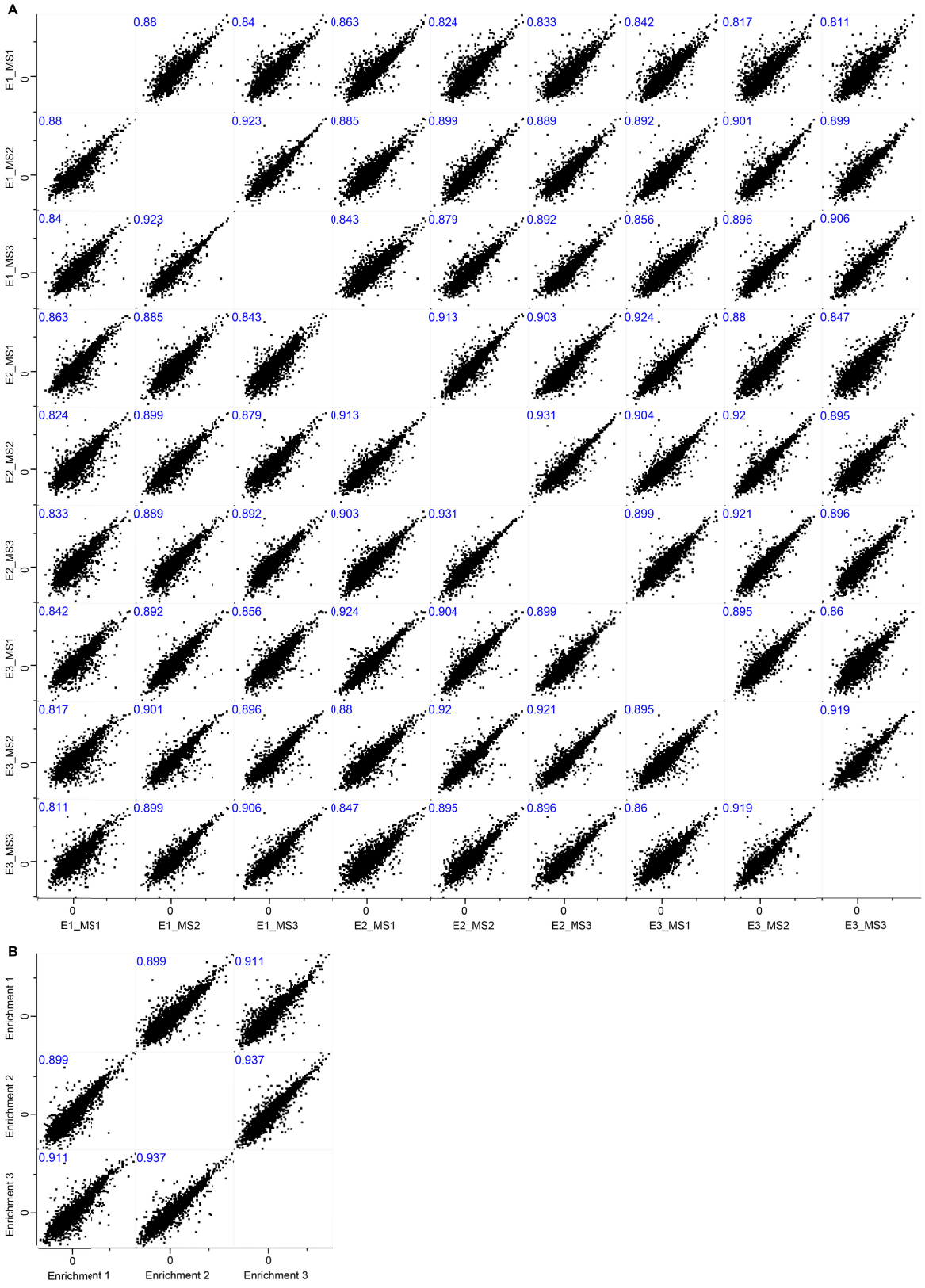
Supplementary Figure 3: Gene ontology enrichment analysis of all significantly regulated phosphosites. Panther ontologies were analyzed for enrichment using a fisher exact test (Benjamin-Hochberg FDR 0.02). Displayed are color coded Log2 enrichment factor to show high over and under representation of ontology terms.

Supplementary Figure 4: A) Western blot validation of the direct PKA substrate FLNA (Ser2152), indirect PKA substrate CFL (Ser3) and the MAPK substrate ATF2 (Thr71). α-Tubulin was used as a loading control. B) Following 3 independent PGE_2 induced activations, western blot quantitative analysis nicely reveal agreement with the MS-based label-free phosphoproteomics screening, with the latter possessing overall higher accuracy.

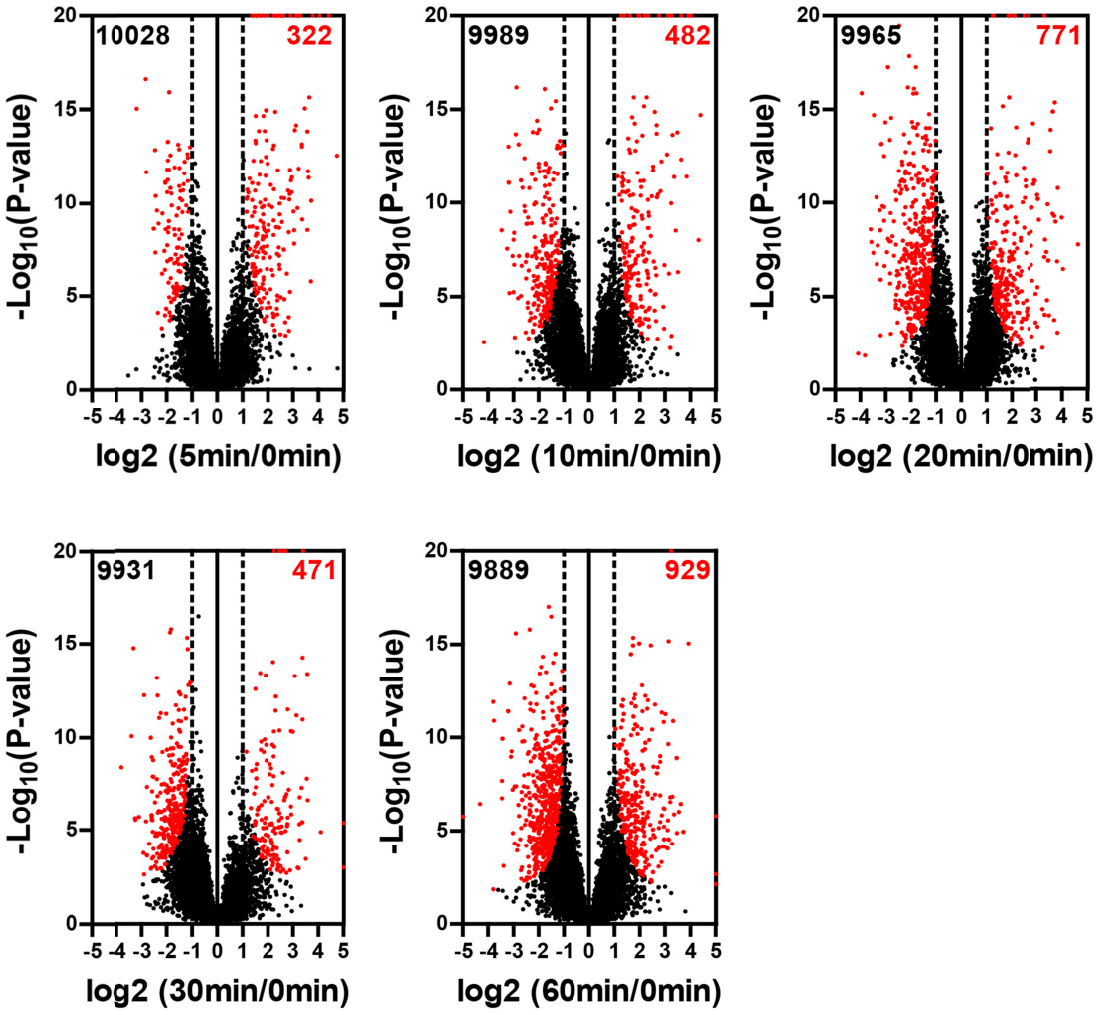
Supplementary Figure 5: Protein complexes and/or networks of predicted PKA substrates. Significantly regulated substrates for each cluster and additional proteins belonging to the same protein complex are shown in red and green, respectively. Interaction data and common protein features were retrieved from the STRING and Uniprot database, respectively.

Supplementary Figure 6: Protein complexes and/or networks of predicted CK2 substrates. Significantly regulated substrates for each cluster and additional proteins belonging to the same protein complex are shown in red and green, respectively. Interaction data and common protein features were retrieved from the STRING and Uniprot database, respectively.

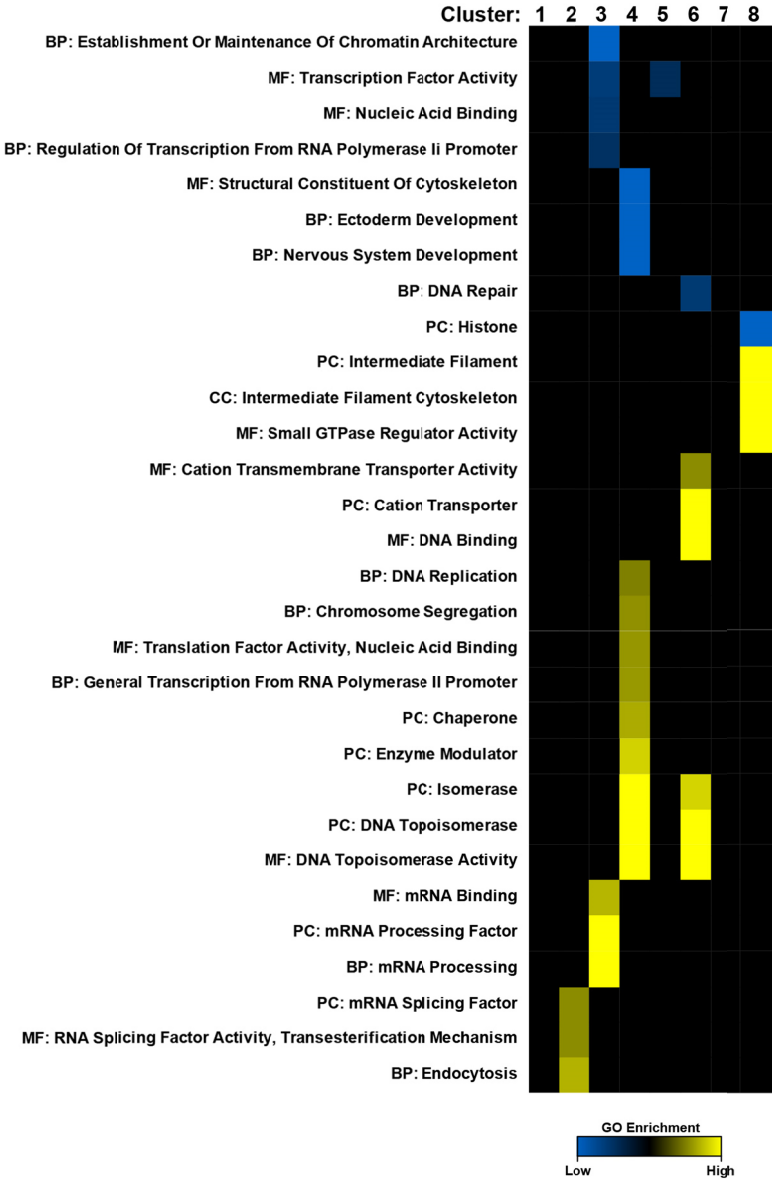
Supplementary Figure 1



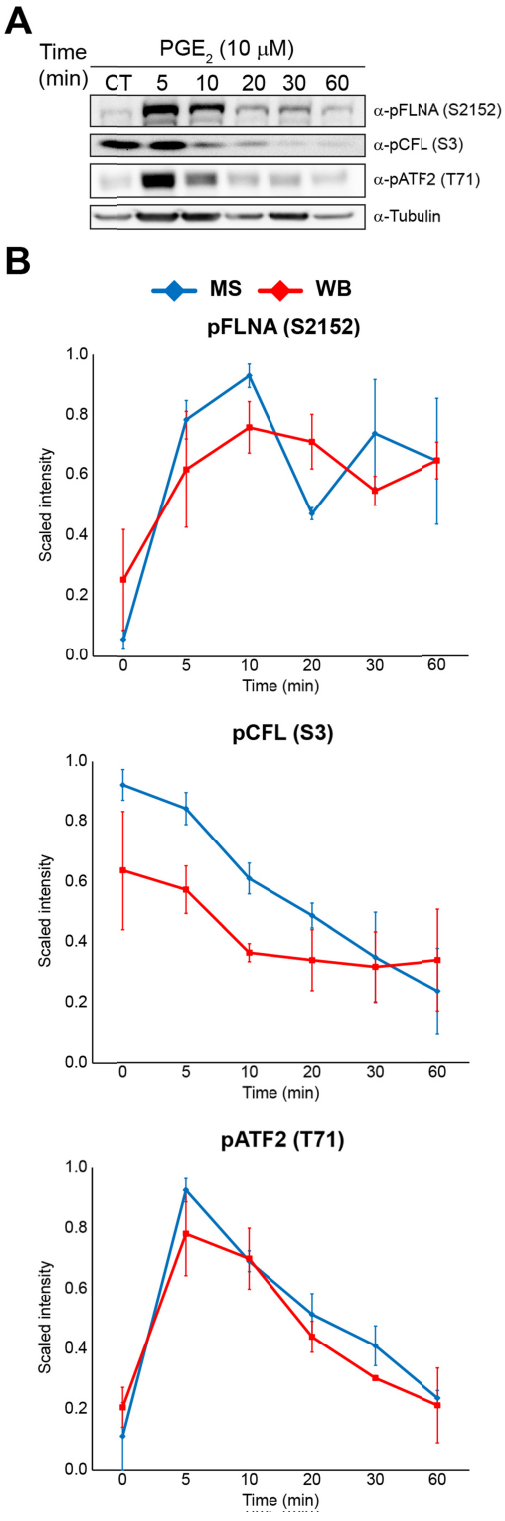
Supplementary Figure 2



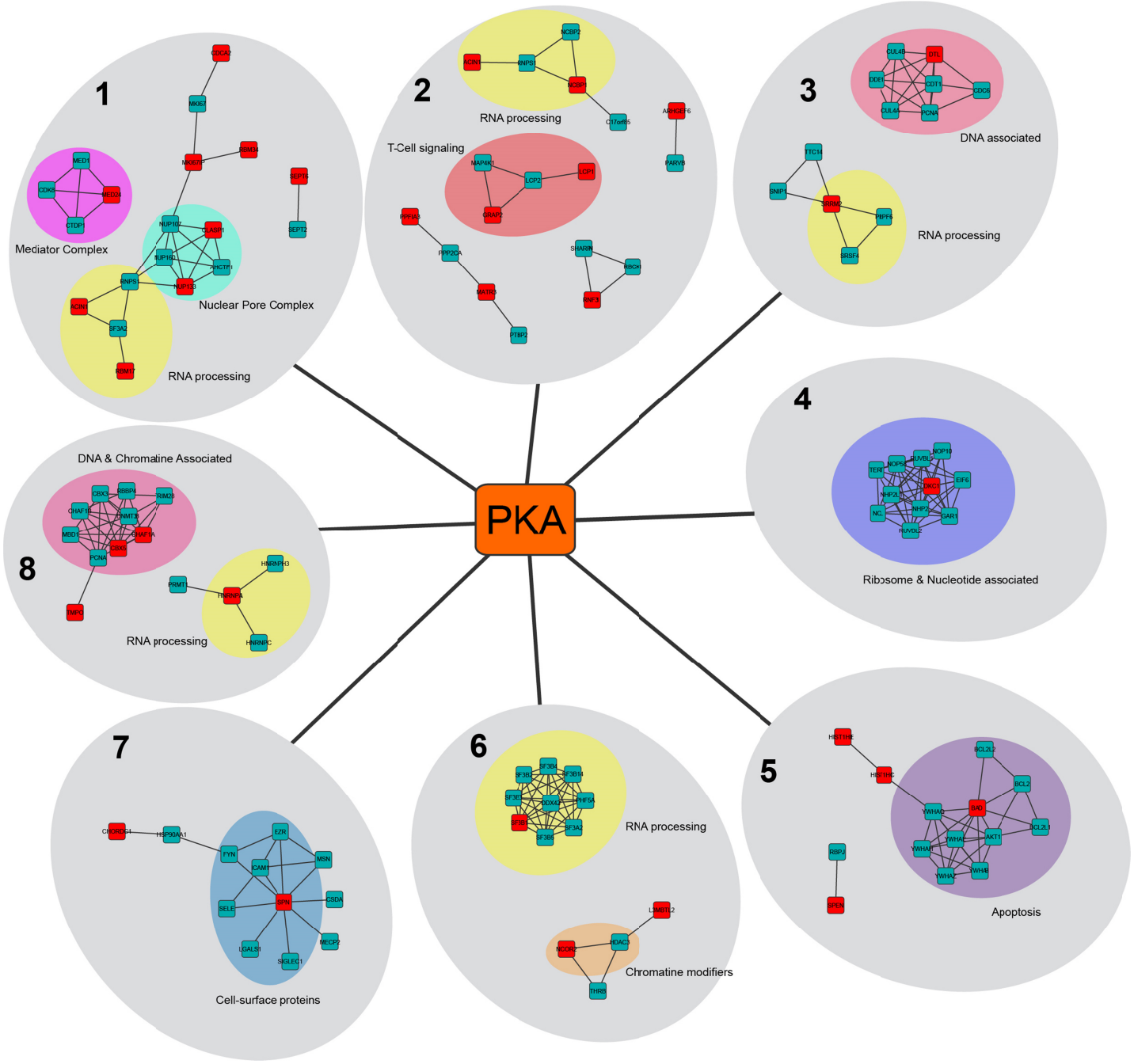
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6