The Cdk8 kinase module regulates Mediator interaction with RNA polymerase II

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Supplementary information

SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure S1, Overlayed gel filtration profiles and SDS-PAGE gels of chromatographic fractions of each of CKM(A) (top) and CKM (KD) (bottom), showing that the two complexes exhibit highly comparable biochemical behavior.

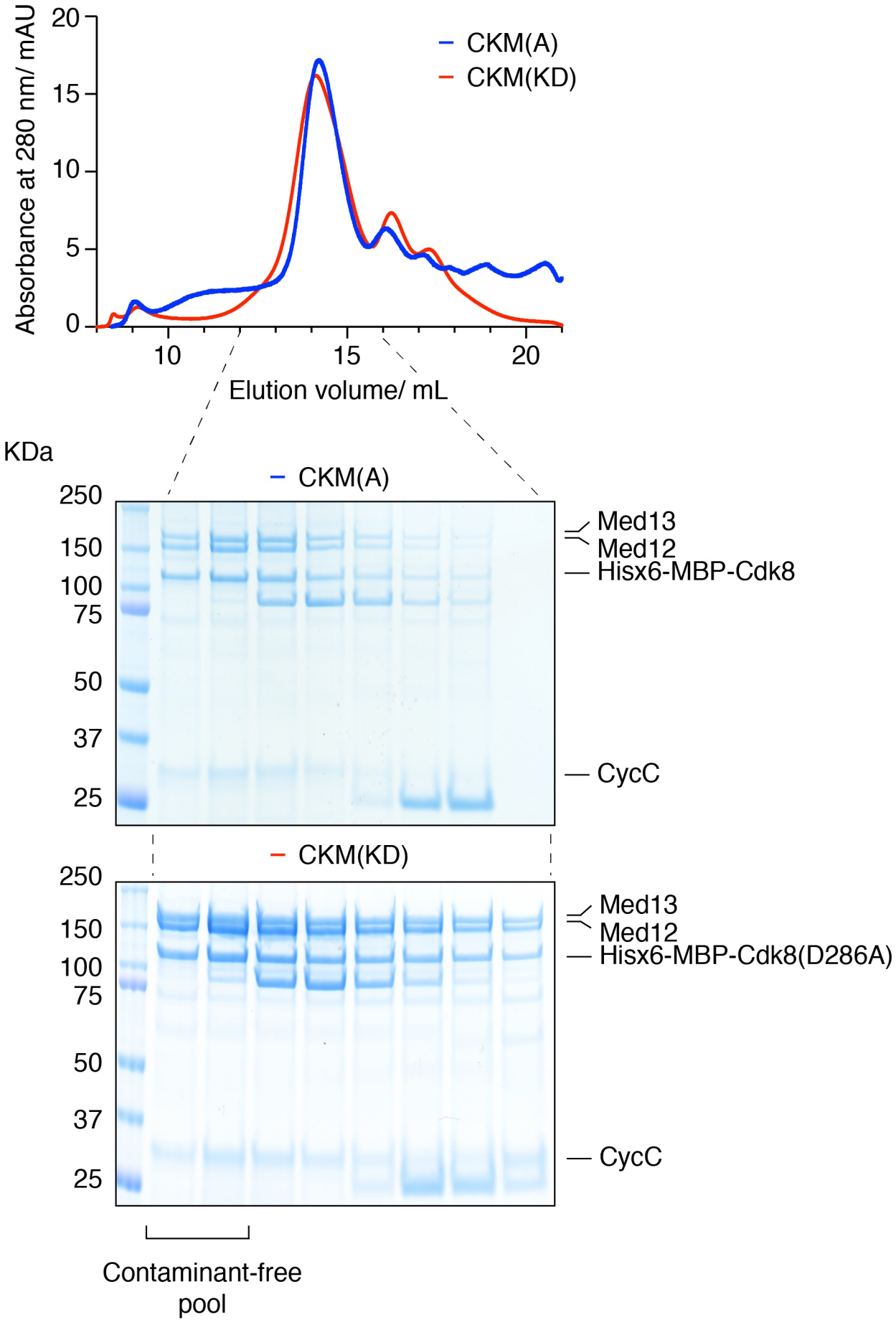
Supplemental Figure S2, a, b CKM phosphorylates the PIC components TFIIF and TBP. Phosphorylation sites are shown that have a localization probability greater than or equal to 0.75 in phosphopeptide enrichment mass spectrometry. Phosphorylation sites deposited by the CKM on the PIC components TFIIF and TBP plotted on the PIC structure (PDB ID 5OQM *S.cerevisiae* cMed-PIC showing TBP, TFIIA, TFIIB, TFIIF and Pol II with all other factors hidden) occur on unstructured regions that were not visible in the structure.

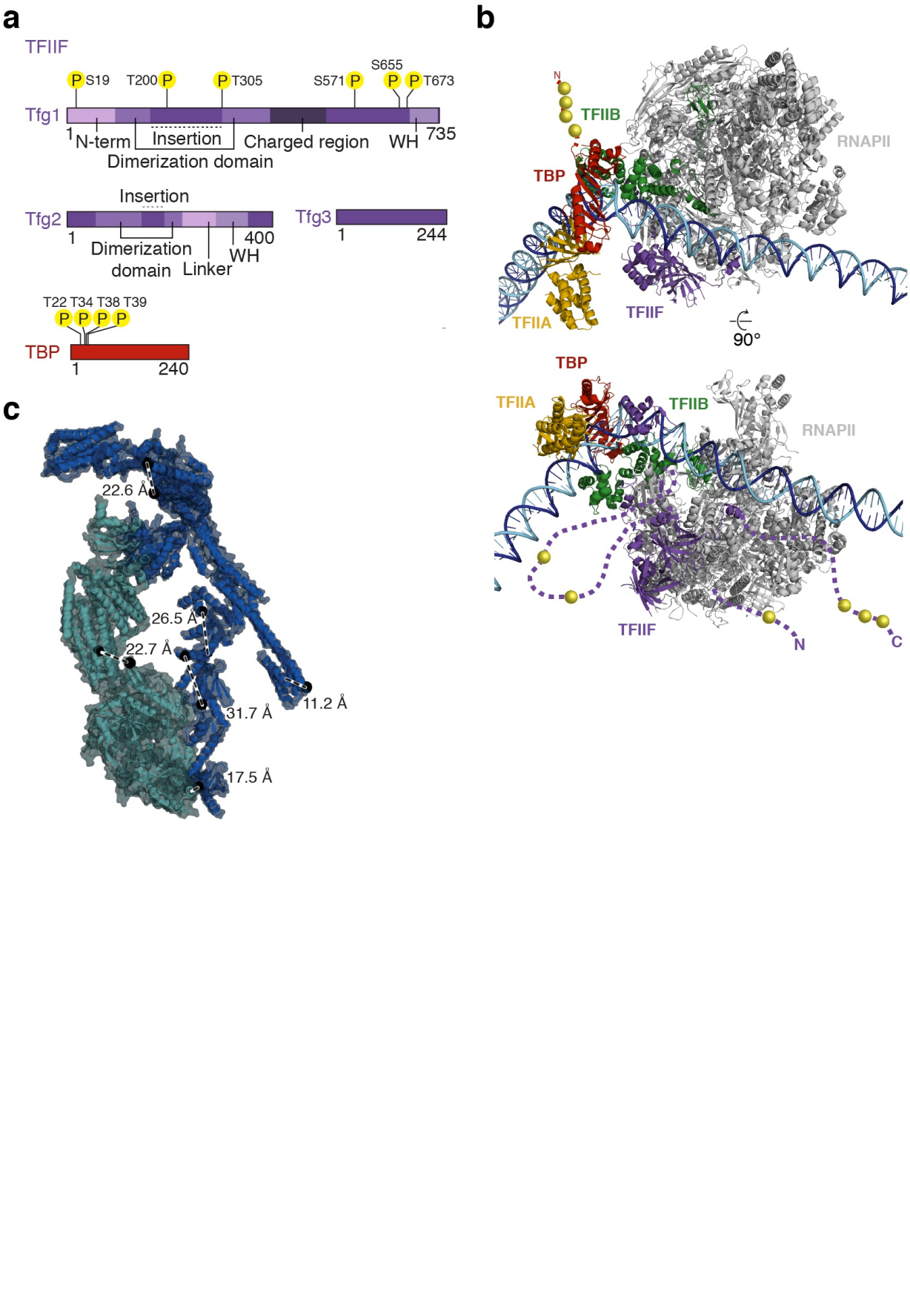
Supplemental Figure S2, c Sample crosslinks from the CKM-cMed XL-MS network that lie within cMed were plotted on the cMed structure (PDB ID 5OQM *S.cerevisiae* cMed-PIC with other factors hidden) as black spheres connected with dashed lines, with atomic distances indicated.

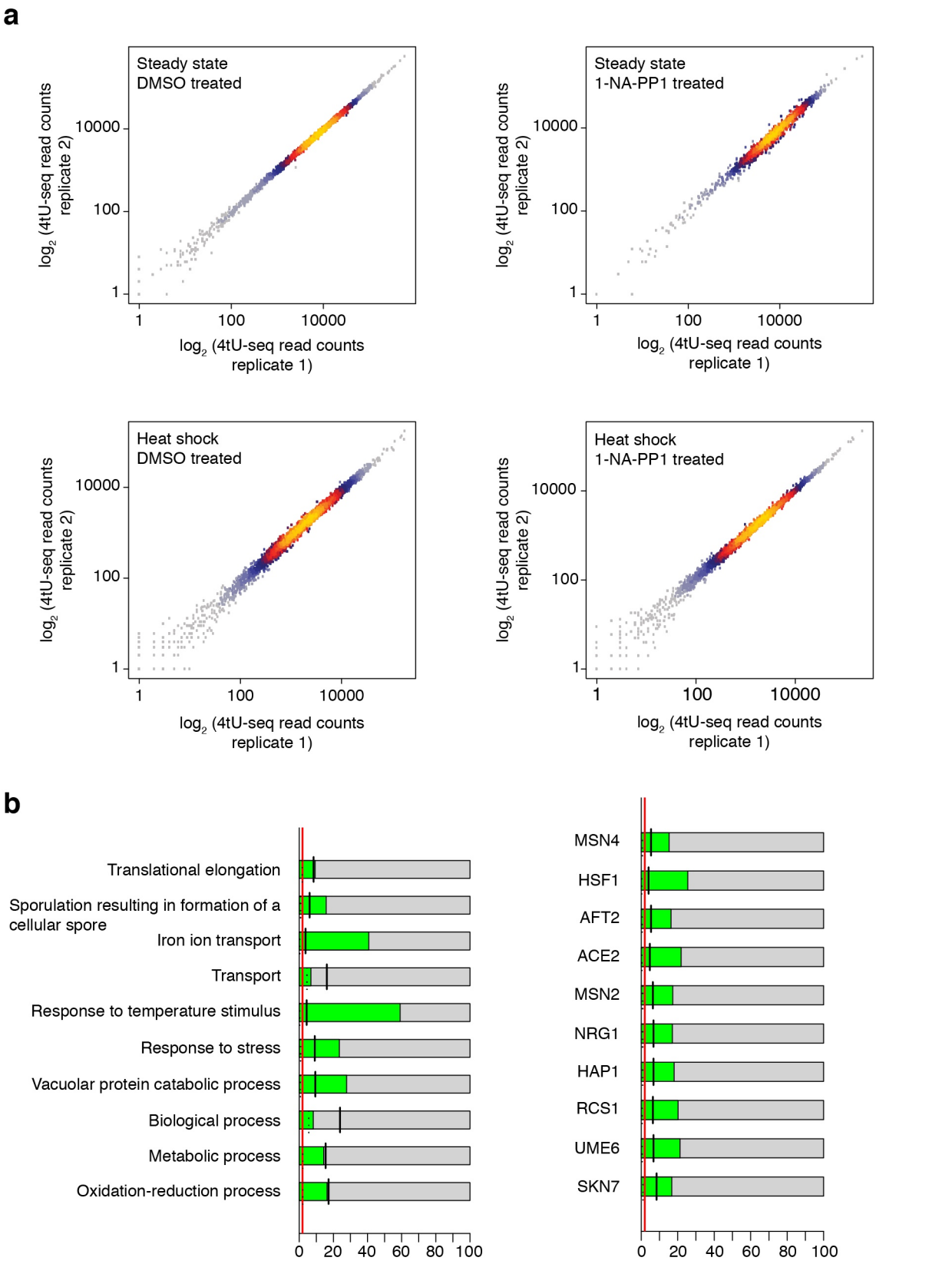
Supplemental Figure S3, a Assessment of Reproducibility of 4tU-Seq Data. (upper left panel) Comparison of replicate measurements for 4tU-seq of the DMSO sample under steady state conditions. The scatterplot compares read counts of ORF-Ts. Spearman correlation is 1. (upper right panel) Comparison of replicate measurements for 4tU-seq of the 1-NA-PP1 sample under steady state conditions. The scatterplot compares read counts of ORF-Ts. Spearman correlation is 0.99. (lower left panel) Comparison of replicate measurements for 4tU-seq of the DMSO sample under heat shock conditions. The scatterplot compares read counts of ORF-Ts. Spearman correlation is 1. (lower right panel) Comparison of replicate measurements for 4tU-seq of the 1-NA-PP1 sample under heat shock conditions. The scatterplot compares read counts of ORF-Ts. Spearman correlation is 1.

Supplemental Figure S3, b Gene ontology analysis of genes induced upon application of heat shock shows that temperature stimulus response genes (GO:0009266) are enriched in this group (left). Specific transcription factor target gene groups that were found to be enriched are shown on the right.

Supplemental Figure S3, c Technical replicate of sucrose density ultracentrifugation experiment shown in figure 2c to exclude errors of loading confirms the observed pattern, which shows that presence of CKM (represented by anti-MBP antibody signal) is more prominent in lower density fractions when CKM(A) is used under phosphorylation reaction conditions (top), than when CKM(KD) is used under the same conditions (bottom).


Absorbance at 280 nm/ mAU
20
15
10
5
0
CKM(A)
CKM(KD)
10
15
20
Elution volume/ mL
KDa
CKM(A)
250
150
100
75
50
37
25
Med13
Med12
Hisx6-MBP-Cdk8
CycC
CKM(KD)
250
150
100
75
50
37
25
Med13
Med12
Hisx6-MBP-Cdk8(D286A)
CycC
Contaminant-free
pool


a
TFIIF
P S19
T200 P
P T305
S571 P
S655
P P T673
Tfg1
1
N-term
Insertion
Dimerization domain
Charged region
WH
735
Insertion
Tfg2
1
Dimerization domain
Linker
WH
400
Tfg3
1
244
T22 T34 T38 T39
P P P P
TBP
1
240
b
N
TFIIB
RNAPII
TBP
TFIIA
TFIIF
90°
TBP
TFIIA
TFIIB
RNAPII
TFIIF
N
C
c
22.6 Å
26.5 Å
22.7 Å
31.7 Å
11.2 Å
17.5 Å


a
Steady state
DMSO treated
Steady state
1-NA-PP1 treated
Heat shock
DMSO treated
Heat shock
1-NA-PP1 treated
log2 (4tU-seq read counts replicate 2)
log2 (4tU-seq read counts replicate 1)
1
100
10000
b
Translational elongation
Sporulation resulting in formation of a cellular spore
Iron ion transport
Transport
Response to temperature stimulus
Response to stress
Vacuolar protein catabolic process
Biological process
Metabolic process
Oxidation-reduction process
0 20 40 60 80 100
MSN4
HSF1
AFT2
ACE2
MSN2
NRG1
HAP1
RCS1
UME6
SKN7
c
Fraction from top
6 7 8 9 10 11 12
150
75
CKM(A) + cMed
CKM(KD) + cMed
Replicate 1
Replicate 2

Supplementary table guide

Supplementary table 1 Crosslinks within the CKM-cMed complex crosslinked with BS3 (shown in main figure 3) showing a list of all obtained crosslinks using an FDR cutoff of 0.01. All raw mass spectrometry data used in this manuscript have been deposited in the PRIDE repository as detailed under “Data Availability”.

Supplementary table 2 Phosphorylation sites used for PIC phosphoproteomic analysis in making main Figure 4 and supplementary Figure S2, showing all identified phosphosites after filtering for localization probability (p(STY)) above 0.75, showing the protein, phosphorylated residue, localization probability in the indicated fractions (Methods) and surrounding sequence. The complete raw unfiltered data from this experiment with all additional mass spectrometry parameters have been deposited in the PRIDE repository as detailed under “Data Availability”.

Supplementary table 3 Phosphorylation sites on CKM(A) and CKM(KD) used for making main Figure 4 showing the protein, phosphorylated residue, localization probability and surrounding sequence of all identified sites. The complete raw unfiltered data from this experiment with all additional mass spectrometry parameters have been deposited in the PRIDE repository as detailed under “Data Availability”.