Supplemental Information

Integrated Genomic and Proteomic Analyses Revealed Novel Mechanisms of SETD2 in Tumor Development

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Legends for Supplemental Figures

Supplemental Fig. S1. Comparison of H3K36me3 and H4K16ac occupancy on chromosomes.
A-B, The density of H3K36me3 and H4K16ac ChIP-seq reads across chromosomes 2 (A) and 3 (B) in 293T and SETD2 KO cells.

Supplemental Fig. S2. Measurement of creatine content in 293T and SETD2 KO cells.
A-D, Shown are the selected-ion chromatograms and corresponding MS/MS for the indicated transitions for creatine and its stable isotope-labeled standard (D_3-creatine).

Supplemental Fig. S3. Real-time quantitative PCR (RT-qPCR) for the confirmation of up-regulated genes in SETD2 KO cells.

Supplemental Fig. S4. CRISPR/Cas9-mediated knockout of SETD2 and gene expression levels in SETD2 KO-clone 20.
A, DNA sequencing confirms the deletion in *SETD2* genes generated by CRISPR/Cas9 genome editing method.
B, The levels of H3K36me3 and H4K16ac were decreased in SETD2 KO-clone 20, which is consistent with SETD2 KO-clone 5.
C, The expression levels of many genes were up-regulated in SETD2 KO-clone 20 cells.

Supplemental Fig. S5. Comparison of H3K36me3 and H4K16ac levels of differentially expressed genes in SETD2 KO cells. Up-regulated genes are correlated with higher levels of H3K36me3 and H4K16ac occupancy, and vice versa.
A, Average distribution of the H3K36me3 occupancy of up- or down-regulated genes around the TSS and TES.
B, Average distribution of the H4K16ac occupancy of up- or down-regulated genes around the TSS and TES.

Supplemental Fig. S6-S10. Representative mass spectra of peptides from several proteins.
Upper panels show the MS of the light and heavy arginine- or lysine-containing peptide.
Bottom panels show the MS/MS of the light or heavy arginine- or lysine-containing peptide. K or R in red indicates heavy labeled lysine or arginine.

Supplemental Fig. S11. The interaction network of up-regulated proteins in chromatin fraction of SETD2 KO cells and correlation analysis of proteomic data.
A, The interaction network of up-regulated proteins in the chromatin fraction of SETD2 KO cells. Lines indicate the interaction between proteins. The size and the color of each node are proportional to the interaction protein number of nodes.
B-D, Reproducibility of proteomic quantification were examined by correlation analysis between forward and reverse replicates.

E. Correlation between RNA-seq quantification data and chromatin proteomic data.

Supplemental Fig. S12. The mRNA expression levels and the SILAC ratios of several proteins in the chromatin fraction.
A-D, Expression levels of *ANXA2*, *CCNB1*, *CDK1* and *HMGB2* genes in 293T and SETD2 KO cells were confirmed by RT-qPCR.
E, The SILAC ratio of CDK1, CDK2, CCNB1, ANXA2 and HMGB2 in chromatin fraction of 293T and SETD2 KO cells.

Supplemental Fig. S13. Proliferation of 293T and SETD2 KO-20 cells after knockdown of *PLK1*, *CCNB1*, *CDK1* or treatment with a PLK1 inhibitor (GSK461364, GSK).

Supplemental Fig. S14. Knockdown efficiency of shRNA or siRNA used in this study.
A-C, Knockdown efficiency of shPLK1 (A), shCCNB1 (B) and siCDK1 (C) were confirmed by RT-qPCR.
D, Creatine levels after knockdown of CDK1 in SETD2 KO cells.
E, The relative mRNA expression levels of several oncogenes upon knockdown of *CDK1* gene in SETD2 KO cells.

Supplemental Fig. S15. The mRNA expression levels of *PLK1* gene cells derived from ccRCC patients with wild-type or mutated SETD2 gene.

Supplemental Table S1. Primers or sequences used in this study.

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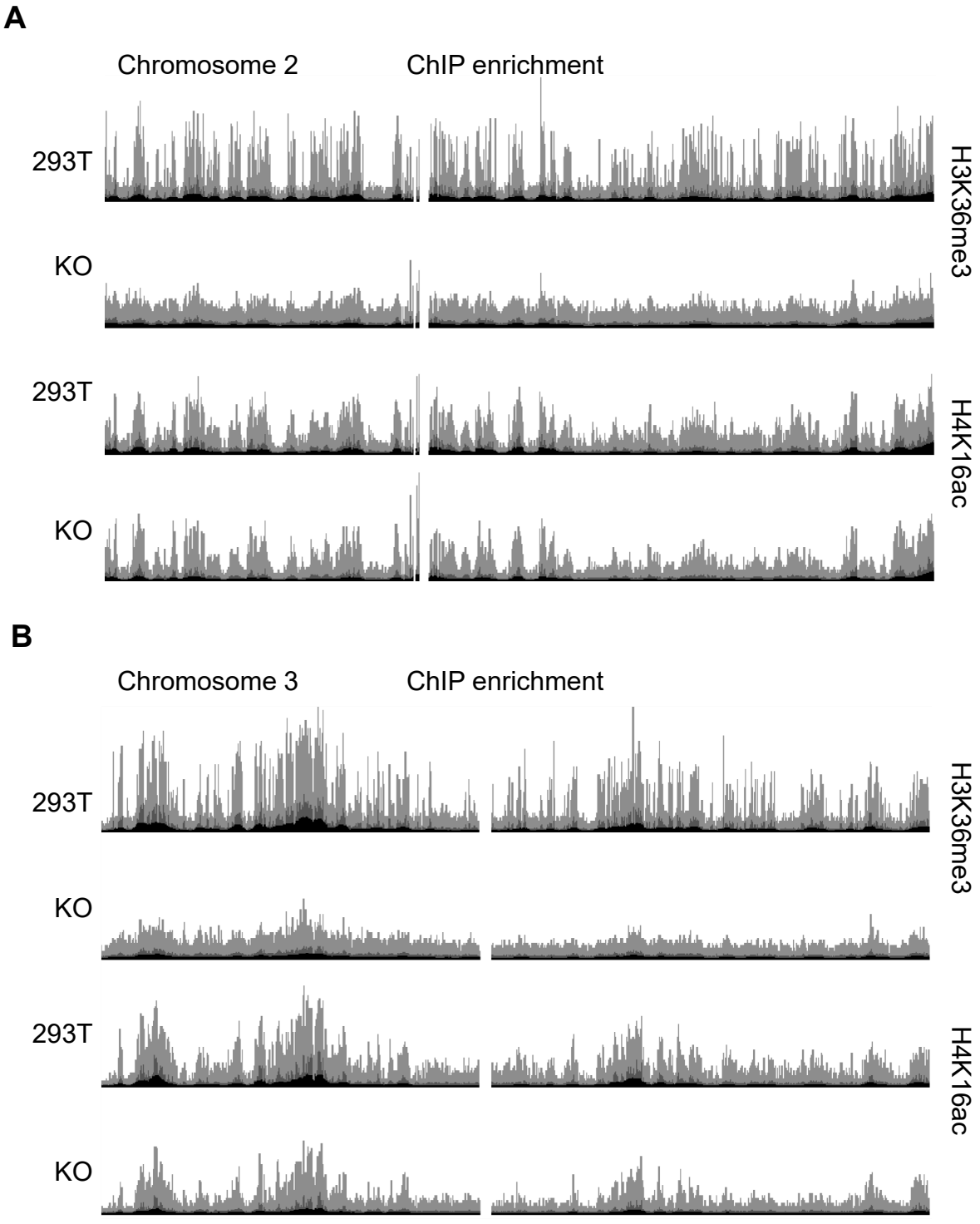
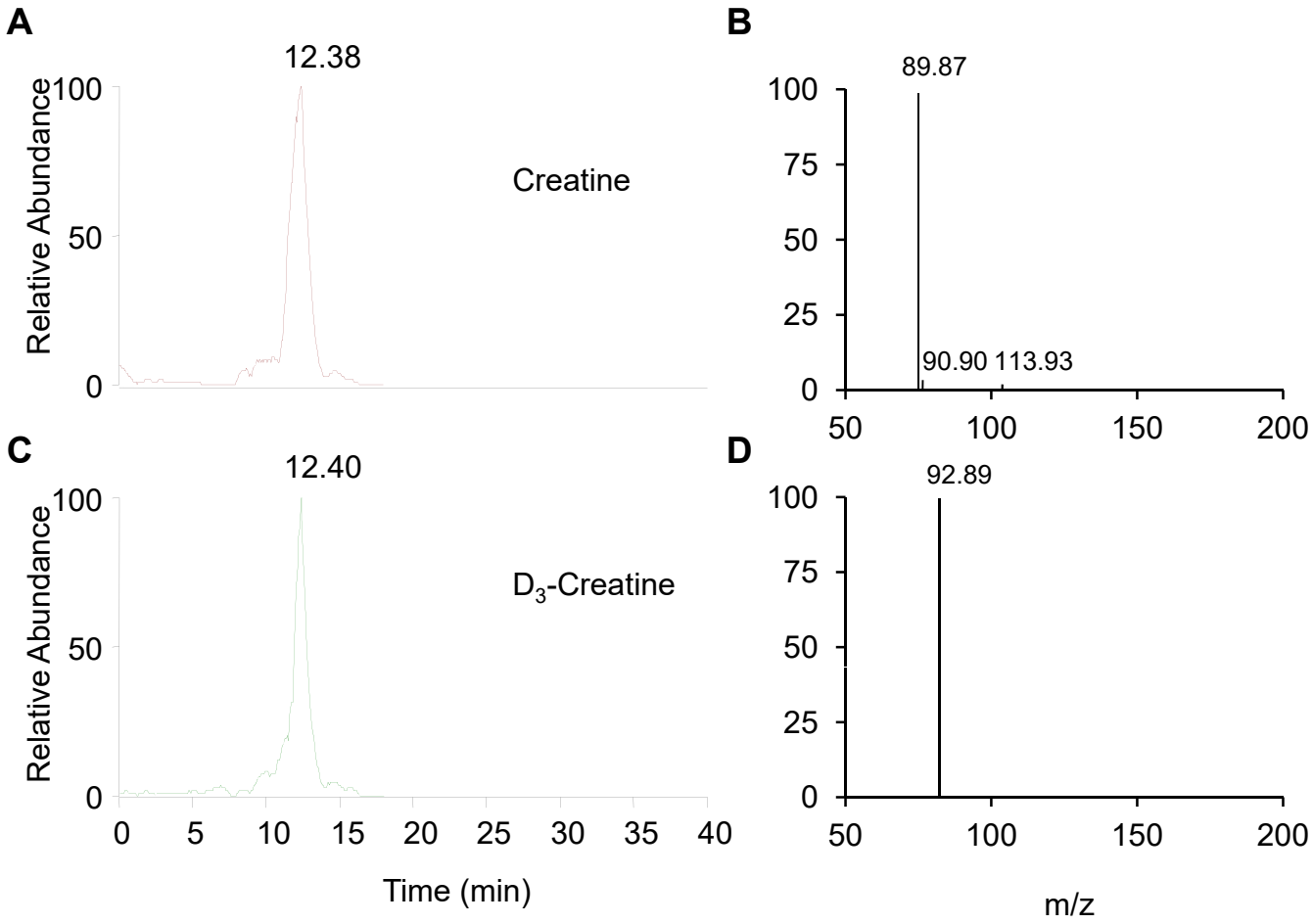
Sequences used for shRNA or siRNA	
Name	Sequence
shPLK1 targeted sequence	5'-CCCGAGGTGCTGAGCAAGAAA-3'
shCCNB1 targeted sequence	5'-CTTGAGTTGGAGTACTATATT-3'
siCDK1 targeted sequence	5'-ACTTCGTCATCCAAATATA-3'
Primers used for real-time quantitative PCR	
Gene	Sequence
GAPDH	5'- TTCGACAGTCAGCCGCATCTTCTT -3' 5'- CAGGCGCCCAATACGACCAAATC -3'
JUN	5'- CAGGTGGCACAGCTTAAACA -3' 5'- AACTGCTGCGTTAGCATGAG -3'
RCN3	5'- GGGACATCGTGATTGCTGAA -3' 5'- CAGATCCGCGATGTACTCCT -3'
CCNB1	5'- TGCCTATGAAGAAGGAAGCA -3' 5'- CTCAGGTTCTGGCTCAGGTT -3'
CDK1	5'- TGTGGCCAGAAGTGGAATCT -3' 5'- TGCCAGAAATTCGTTTGGCT -3'
GAS1	5'- CCCTCATTCAGCTCAACCAC -3' 5'- GTGGACTTGCAGTTCTCGTC -3'
GLI2	5'- TGGACGTGTCCCGTTTCTC -3' 5'- CTGACAGATGCCCGTAGGAA -3'
IRS2	5'- ACTGCTGCCTGAACATCAAC -3' 5'- AGGGCGATCAGGTACTTGTG -3'
PLK1	5'- GAGGTGCTGAGCAAGAAAGG -3' 5'- GGGTTGATGTGCTTGGGAAT -3'
ANXA2	5'- TGAGCGGGATGCTTTGAAC -3' 5'- ATCCTGTCTCTGTGCATTGCTG -3'
HMGB2	5'- ATGTCCTCGTACGCCTTCTT -3' 5'- CATGGTCTTCCATCTCTCCGA -3'

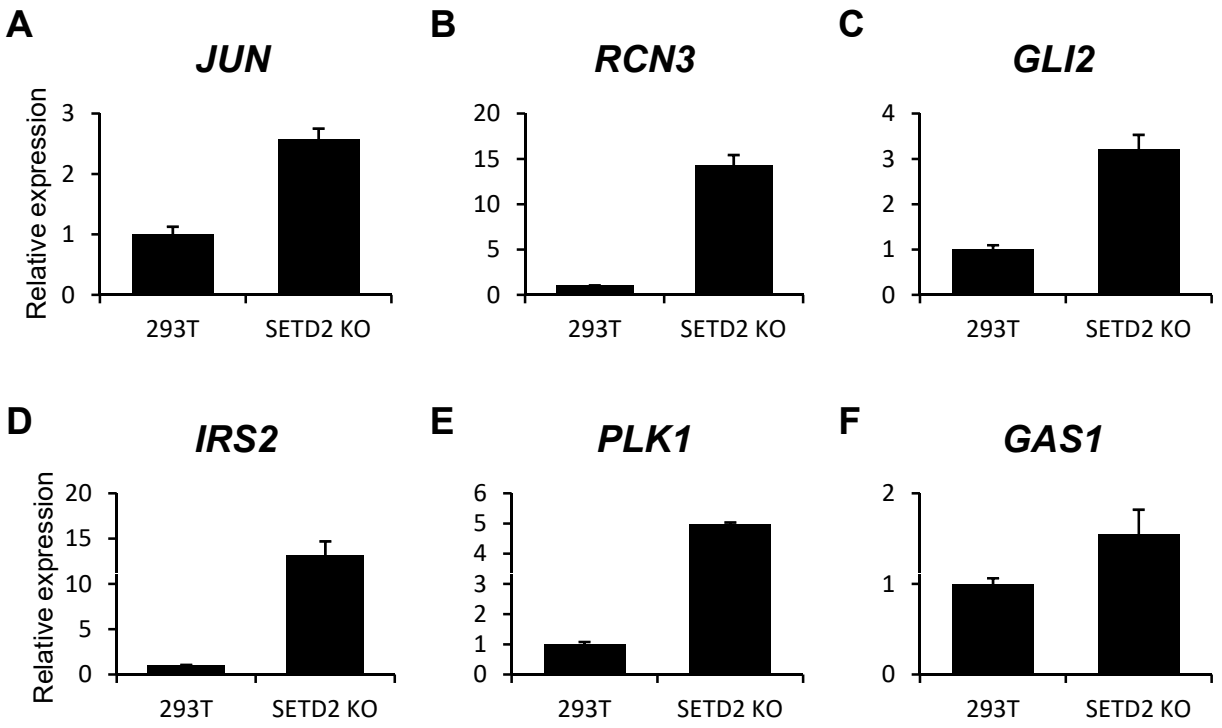
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



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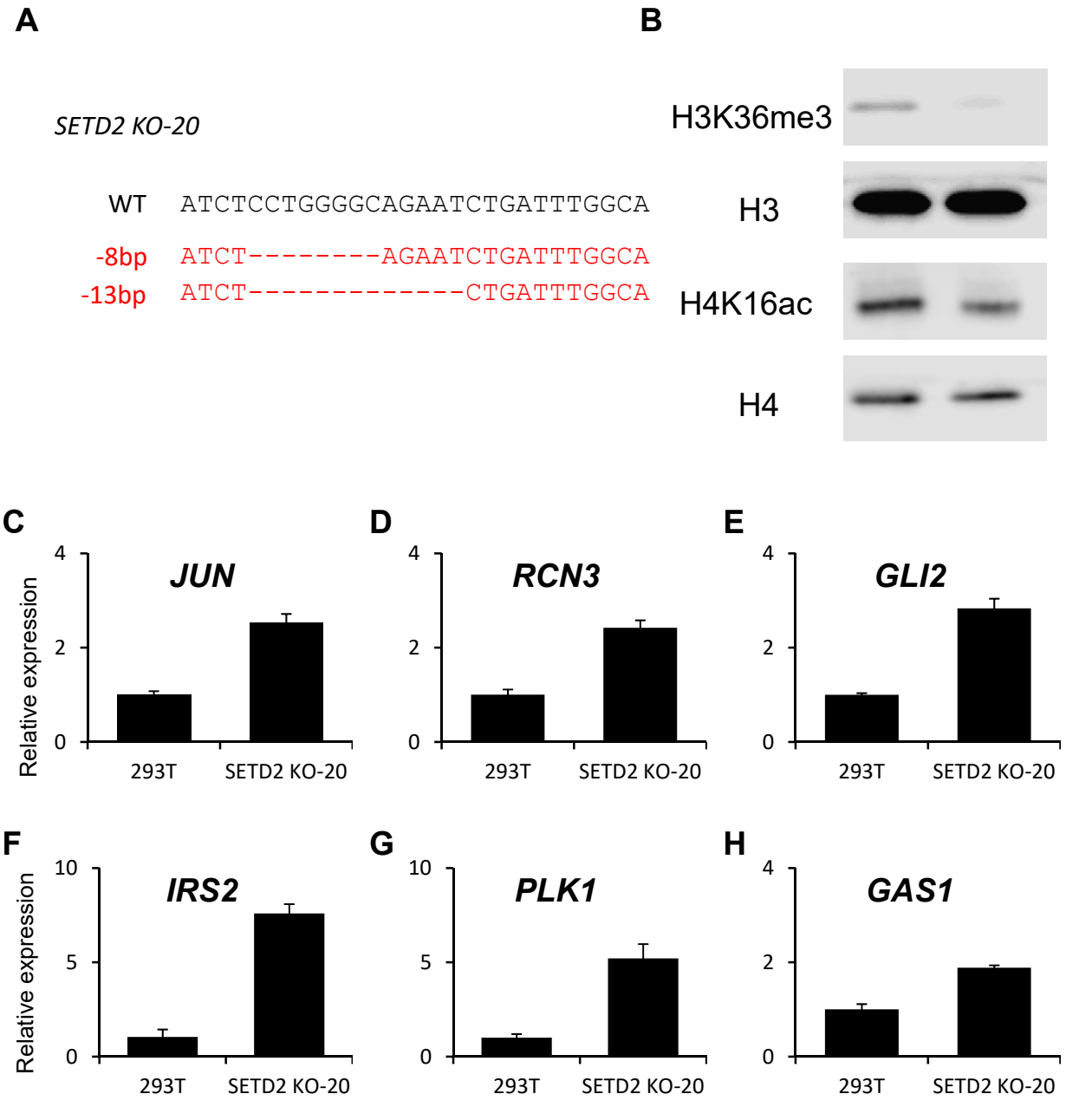
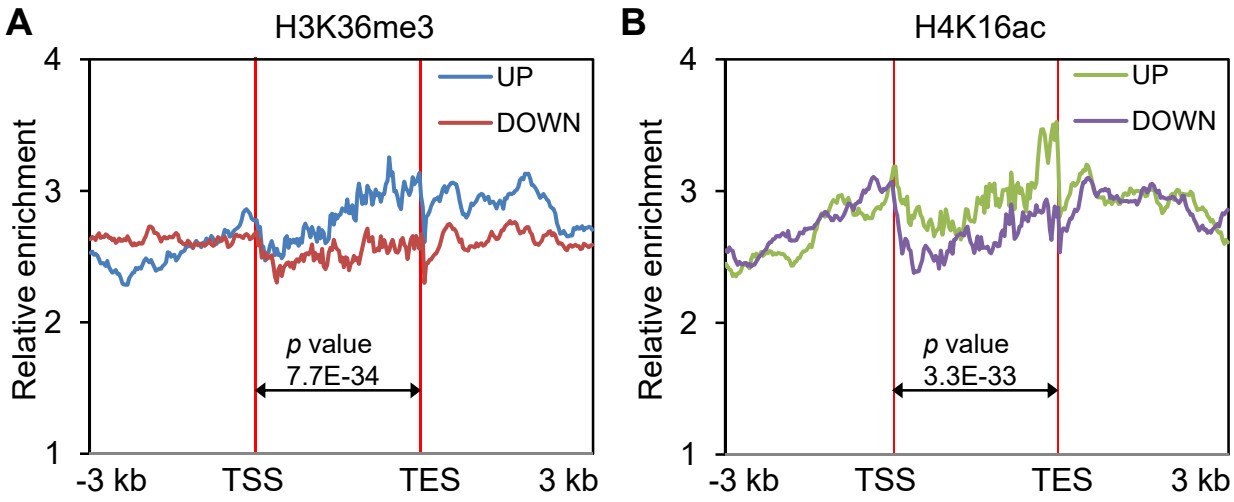


Supplementary Figure S5

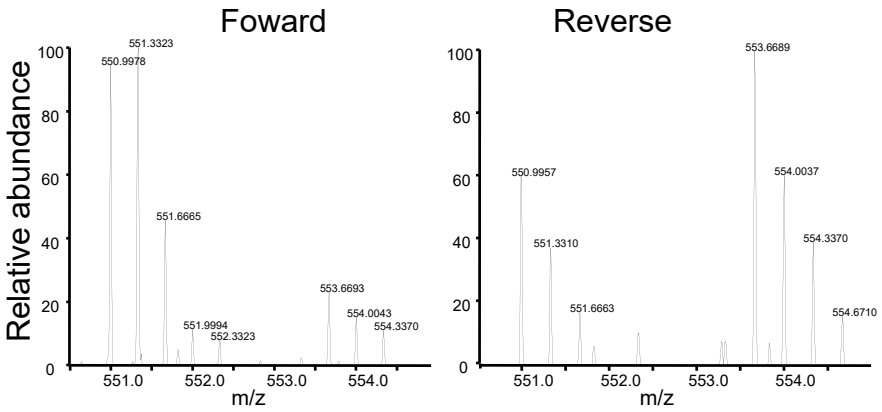


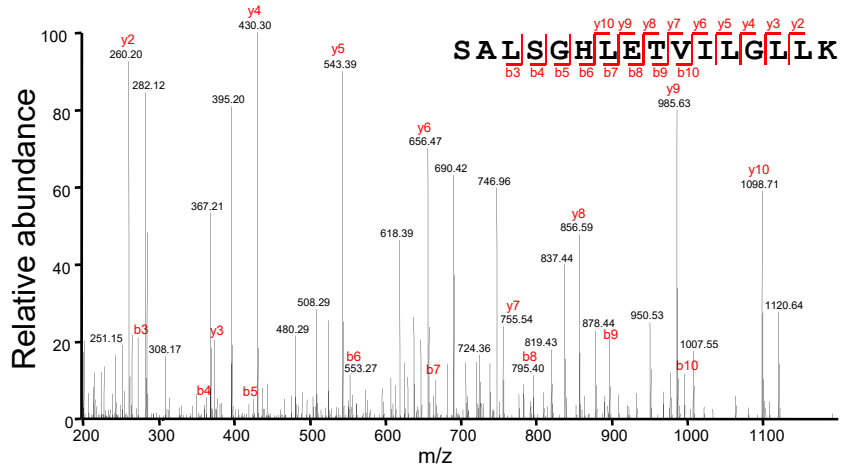
Supplementary Figure S6

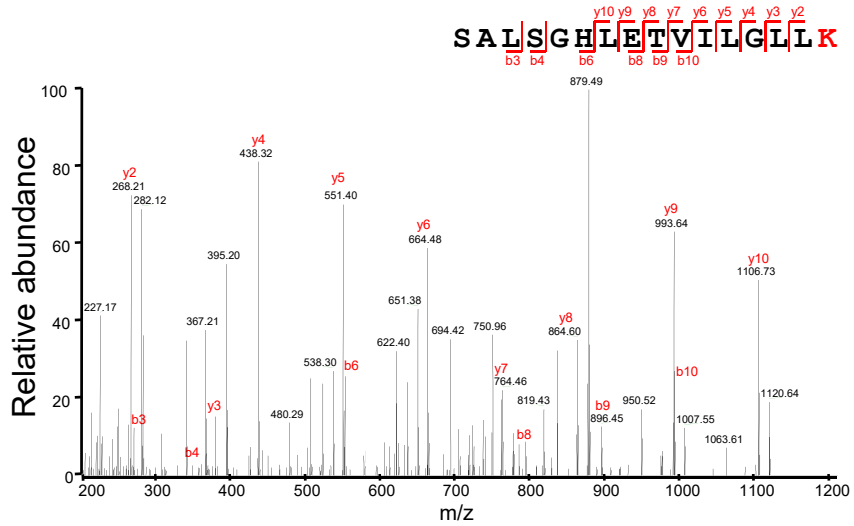
A

ANXA2 SALSGHLETVILGLLK

Light 550.9979
Heavy 553.6645



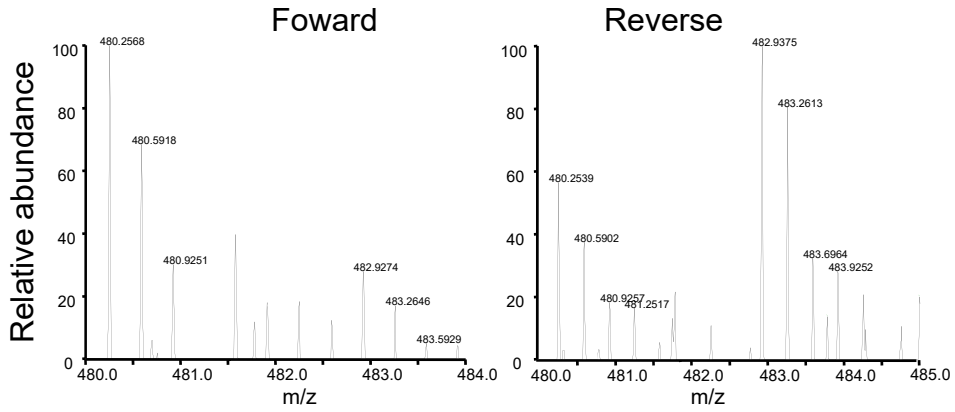


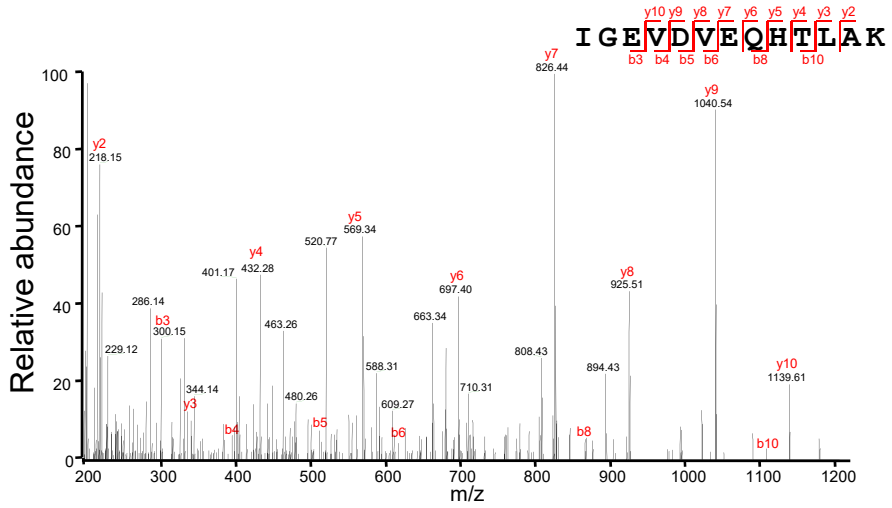


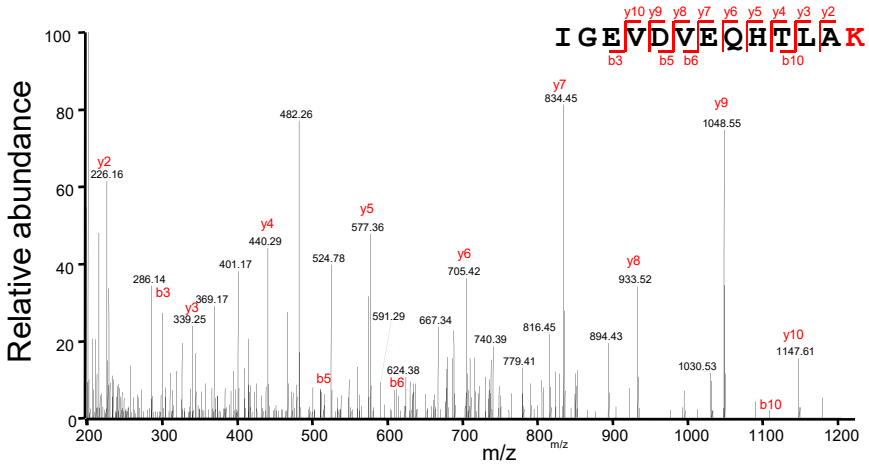
Supplementary Figure S7

A

CCNB1 IGEVDVEQHTLAK Light 480.2561 Heavy 482.9228



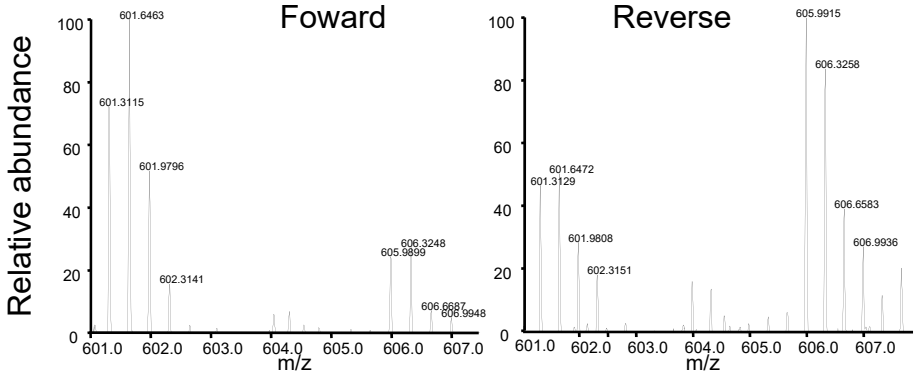


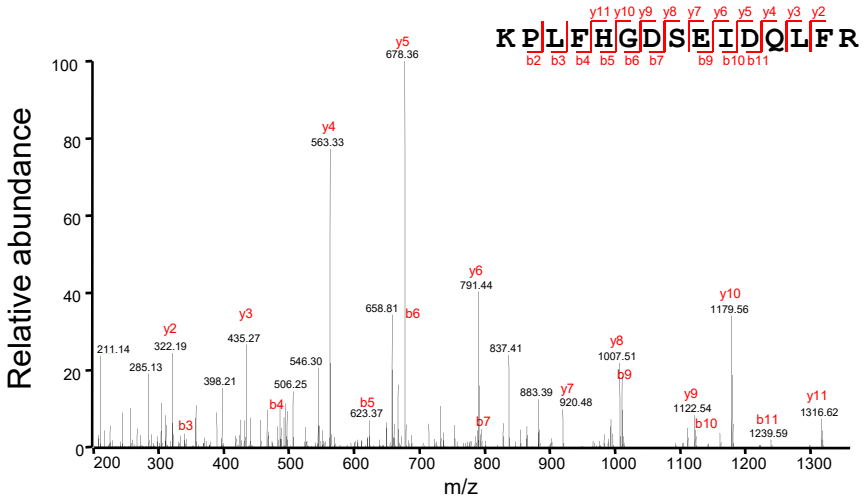


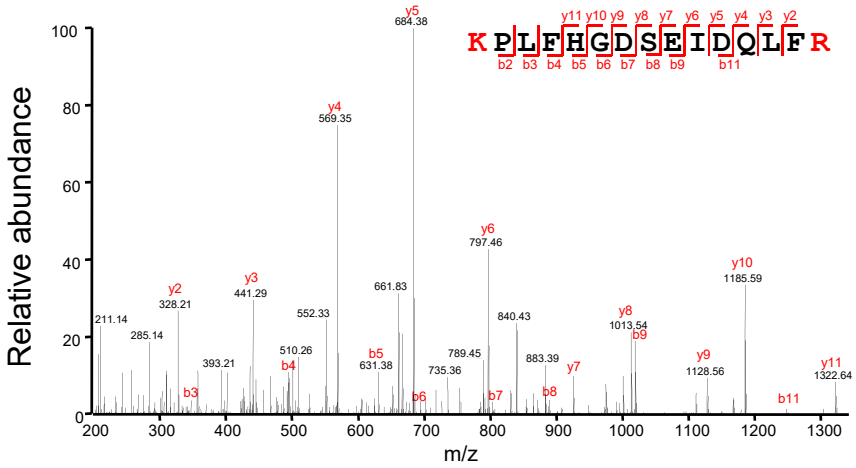
Supplementary Figure S8

A

CDK1 KPLFHGDSEIDQLFR Light 601.3126
Heavy 605.9793

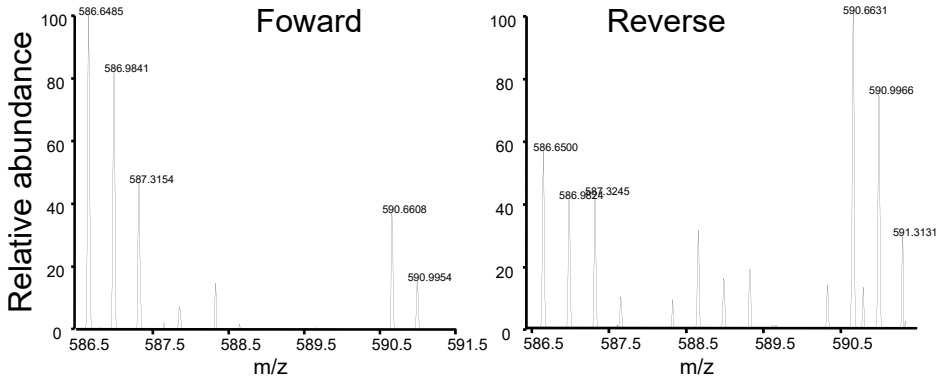


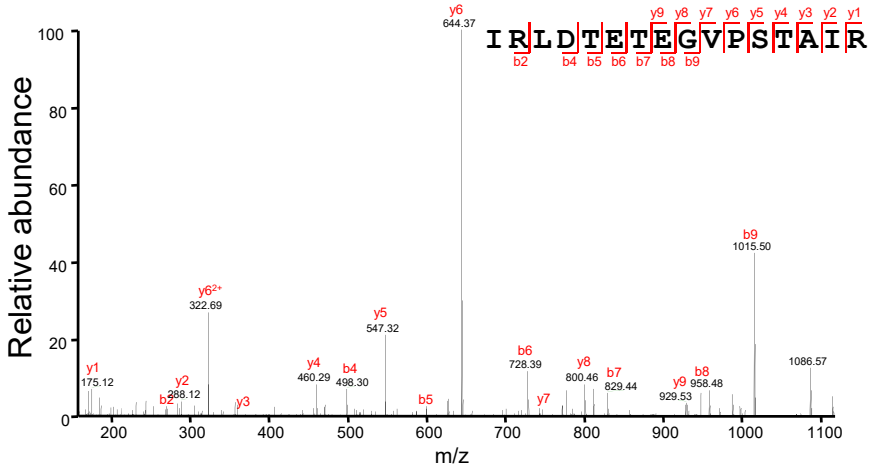


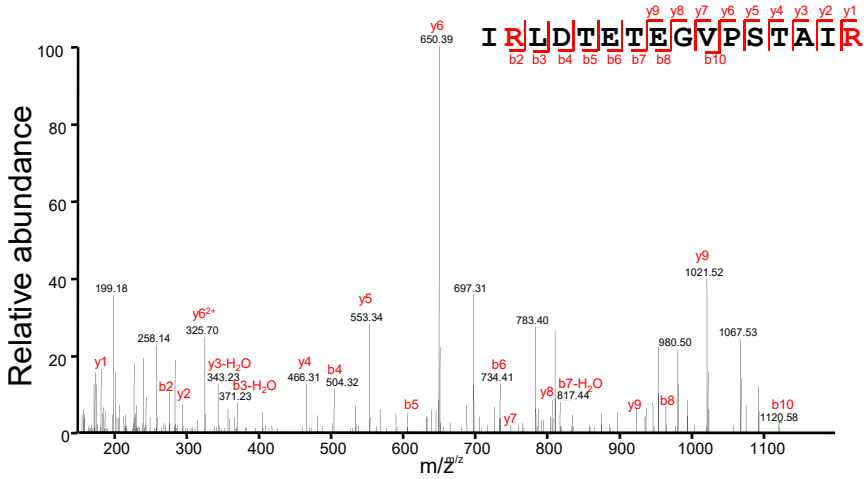


Supplementary Figure S9

A CDK2 IRLDTETEGVPSTAIR Light 586.6513 Heavy 590.6513

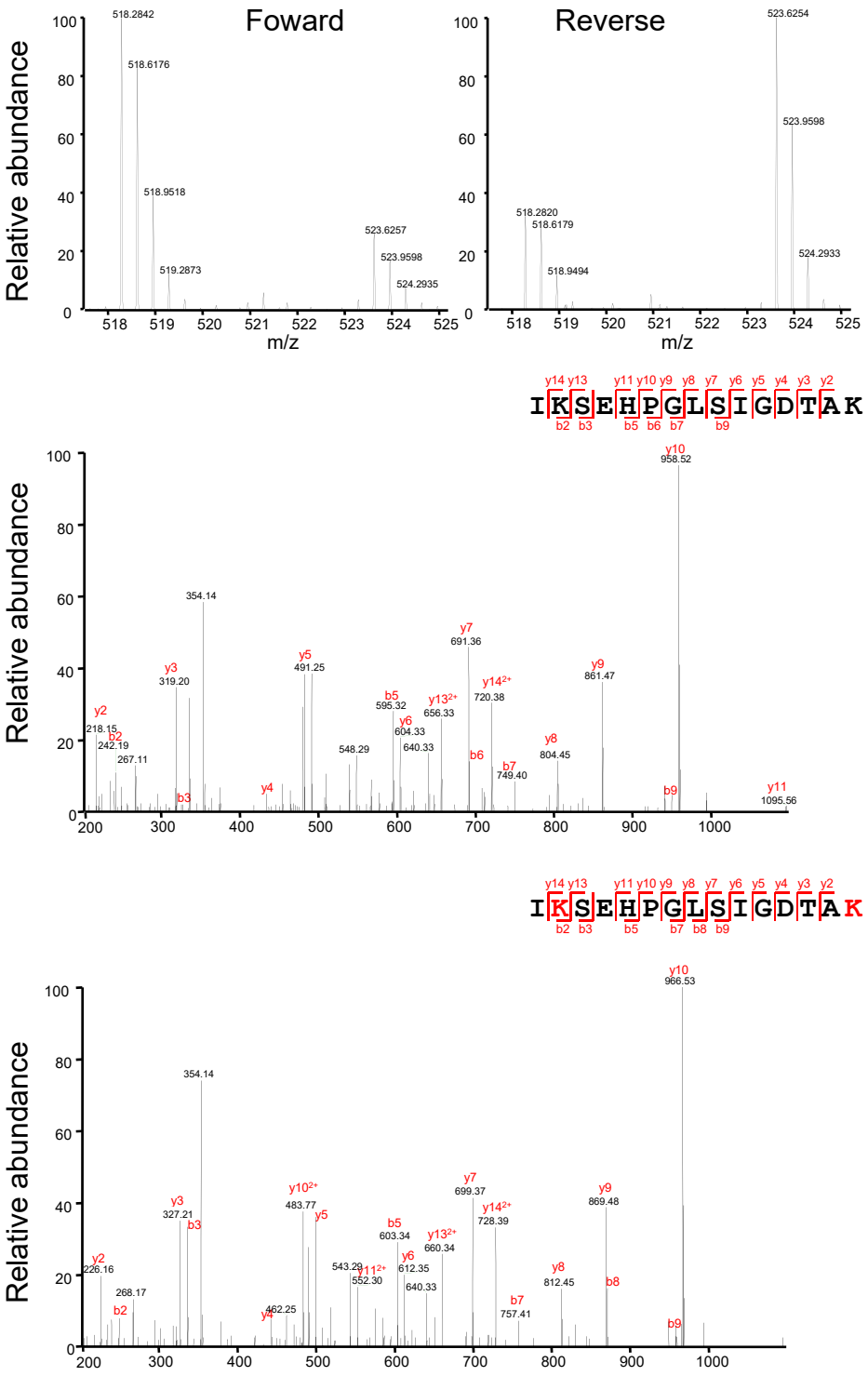




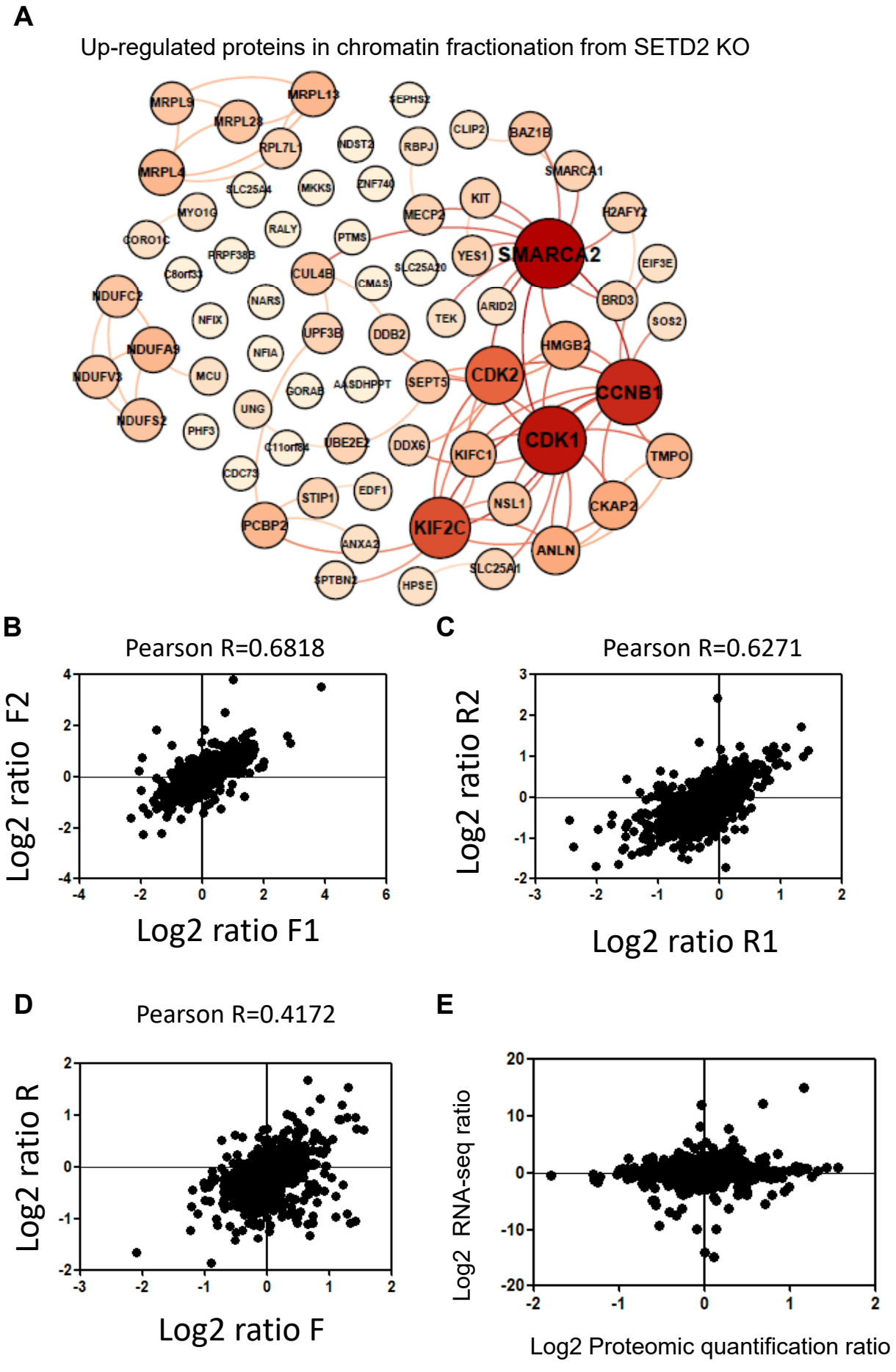


Supplementary Figure S10

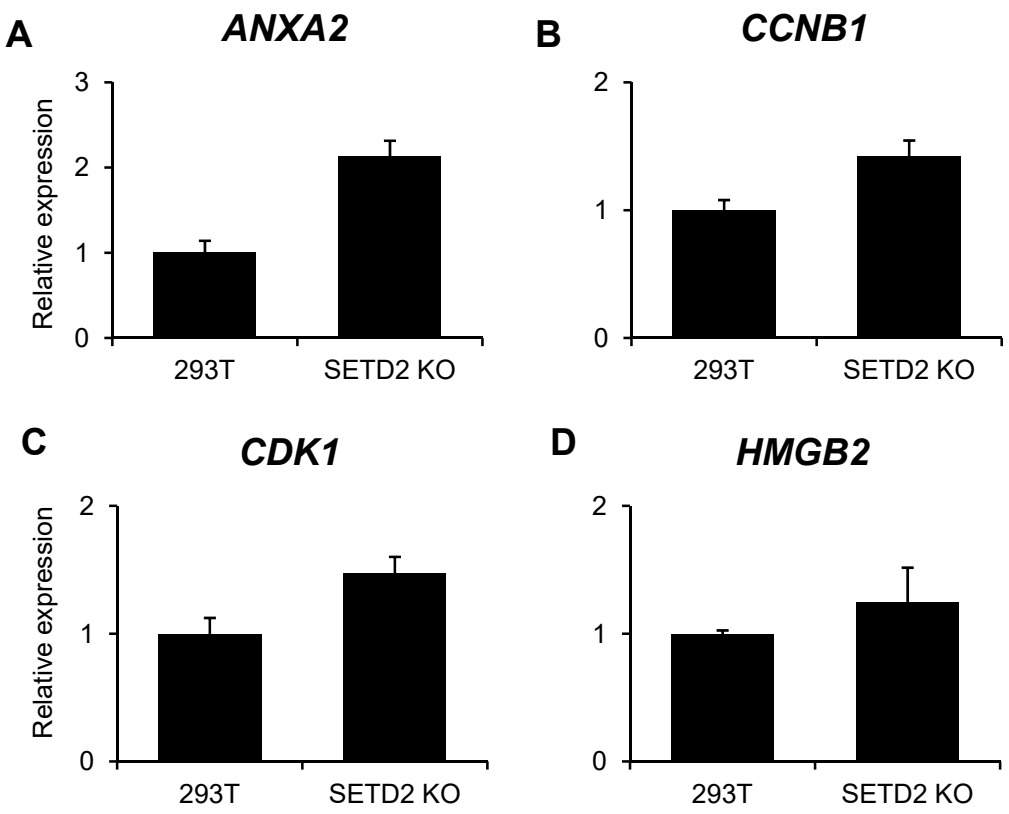
A HMGB2 IKSEHPGLSIGDTAK Light 518.2825 Heavy 523.6159



Supplementary Figure S11



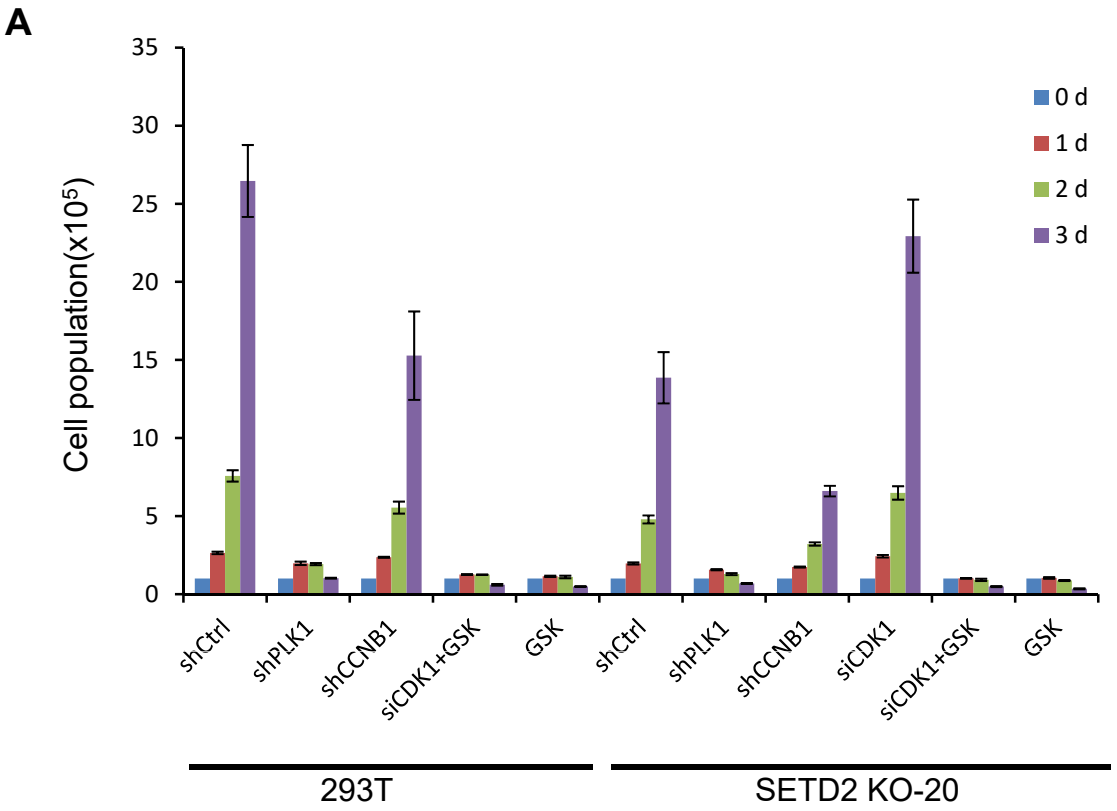
Supplementary Figure S12



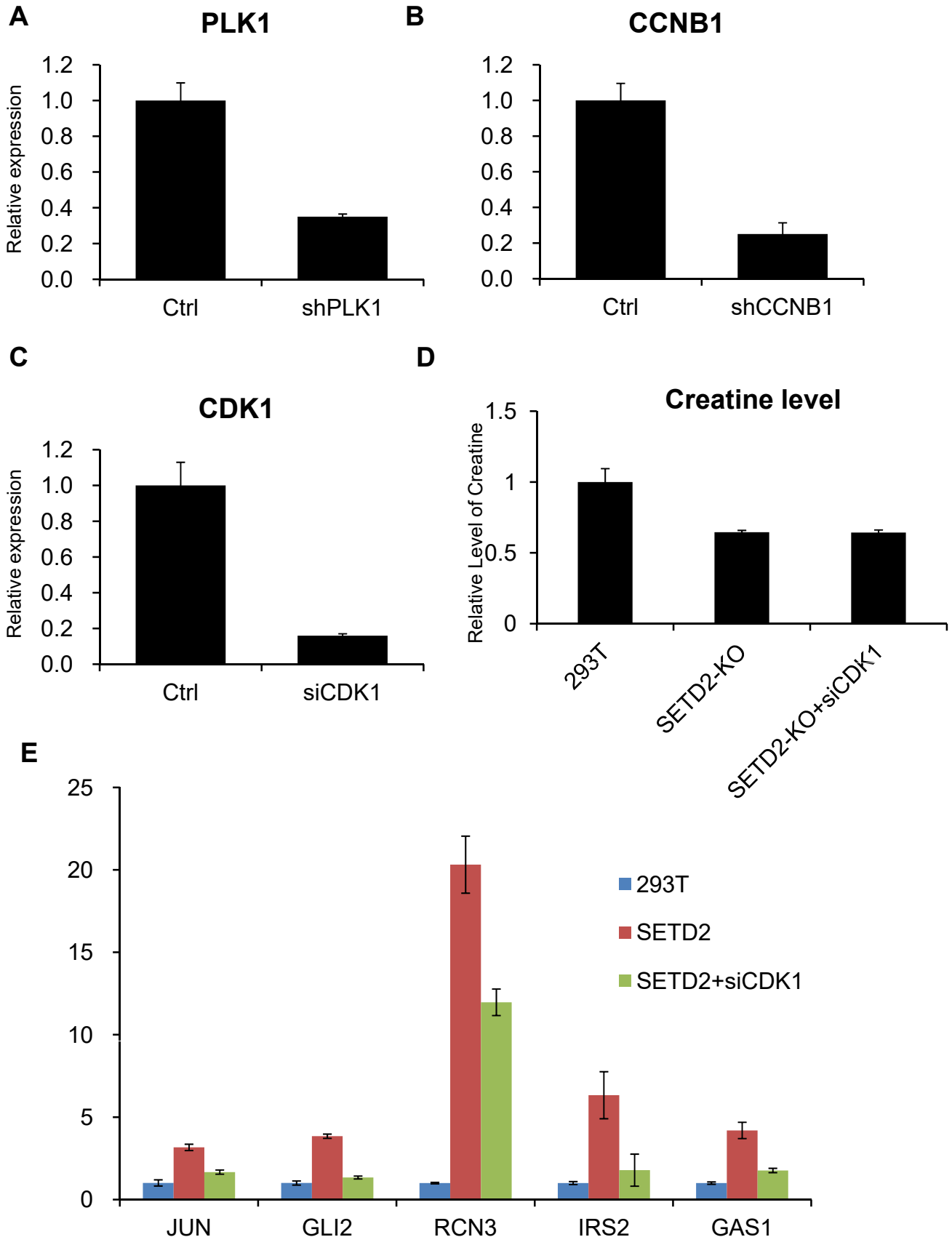
E

Name	F1	F2	1/R1	1/R2	Average ratio Mean±SD
CDK1	1.98	1.80	2.26	2.27	2.08± 0.23
CDK2	1.54	1.74	2.72	3.15	2.29±0.77
CCNB1	2.58	1.99	2.02	2.59	2.30±0.34
ANXA2	2.53	3.27	2.61	2.31	2.68± 0.41
HMGB2	1.73	1.59	2.55	2.86	2.18±0.62

Supplementary Figure S13



Supplementary Figure S14



Supplementary Figure S15