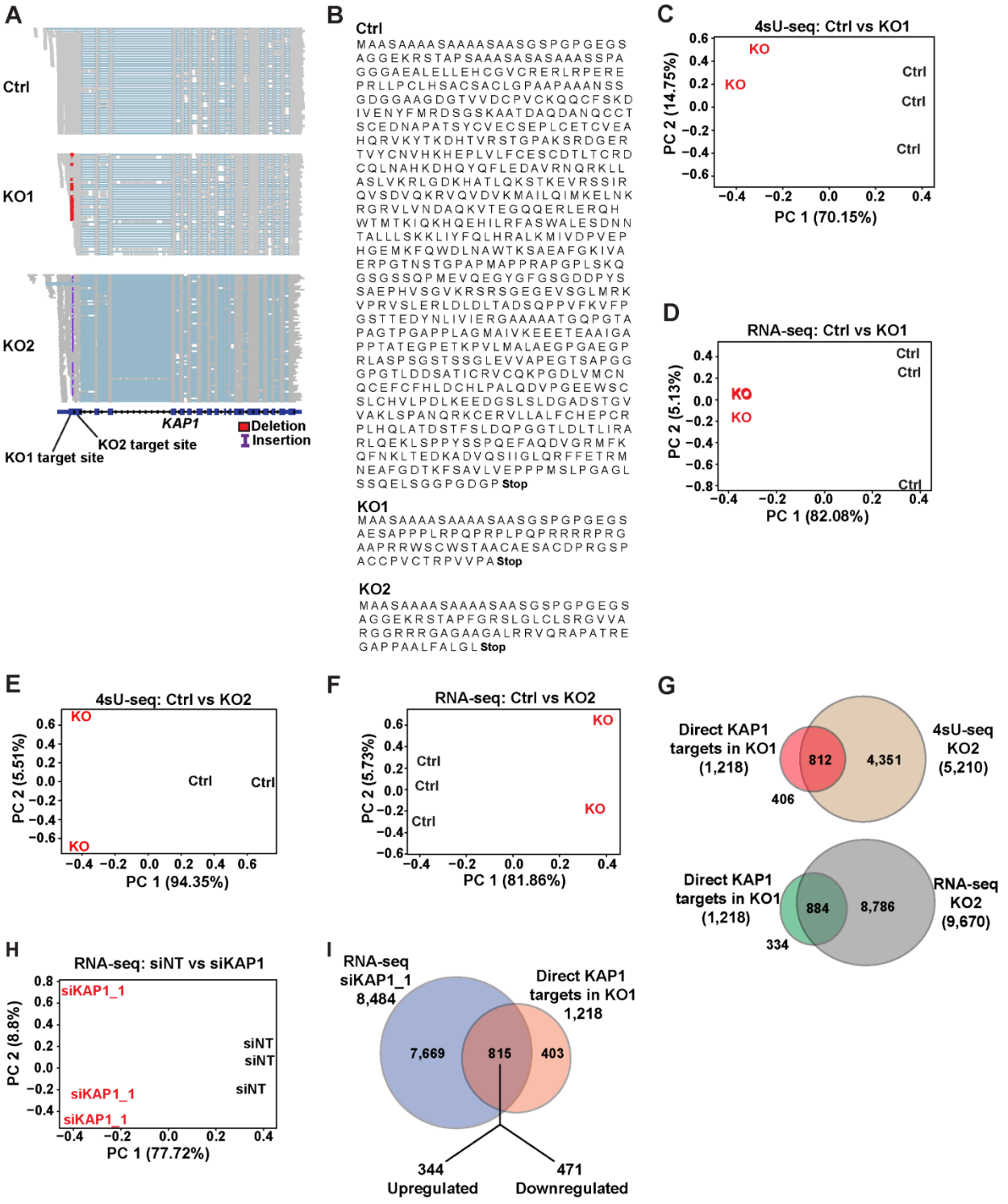

Figure S1. Verification of KAP1 KO, Sequencing Statistics, and Cross-validation of KAP1 Target Genes in Multiple Systems. Related to Figure 1

(A) Alignment files from RNA-seq of Ctrl, KO1, and KO2 cell lines. *KAP1* deletion and insertion are indicated by red and purple boxes, respectively.

(B) Genetic alterations resulting from CRISPR-Cas9 generate early stop codons in both the KO1 and KO2 cell lines.

(C–F) Biological replicates for 4sU-seq and RNA-seq experiments are highly correlated. PCA plots of Ctrl vs KO1 samples for (C) 4sU-seq and (D) RNA-seq, and Ctrl vs KO2 samples for (E) 4sU-seq and (F) RNA-seq.

(G) Overlap of direct KAP1 target genes identified in **Figure 1H** with differentially expressed genes identified by 4sU-seq (**Top**) and RNA-seq (**Bottom**) in the KO2 cell line. Note that the KO2 analysis differs from that presented in **Figure 1** because the KO2 did not contain ERCC spike-ins for normalization (see **STAR Methods**).

(H) PCA plot of siNT vs siKAP1_1 RNA-seq.

(I) Overlap of direct KAP1 target genes identified in **Figure 1H** with differentially expressed genes identified by RNA-seq after KAP1 depletion with siKAP1_1.

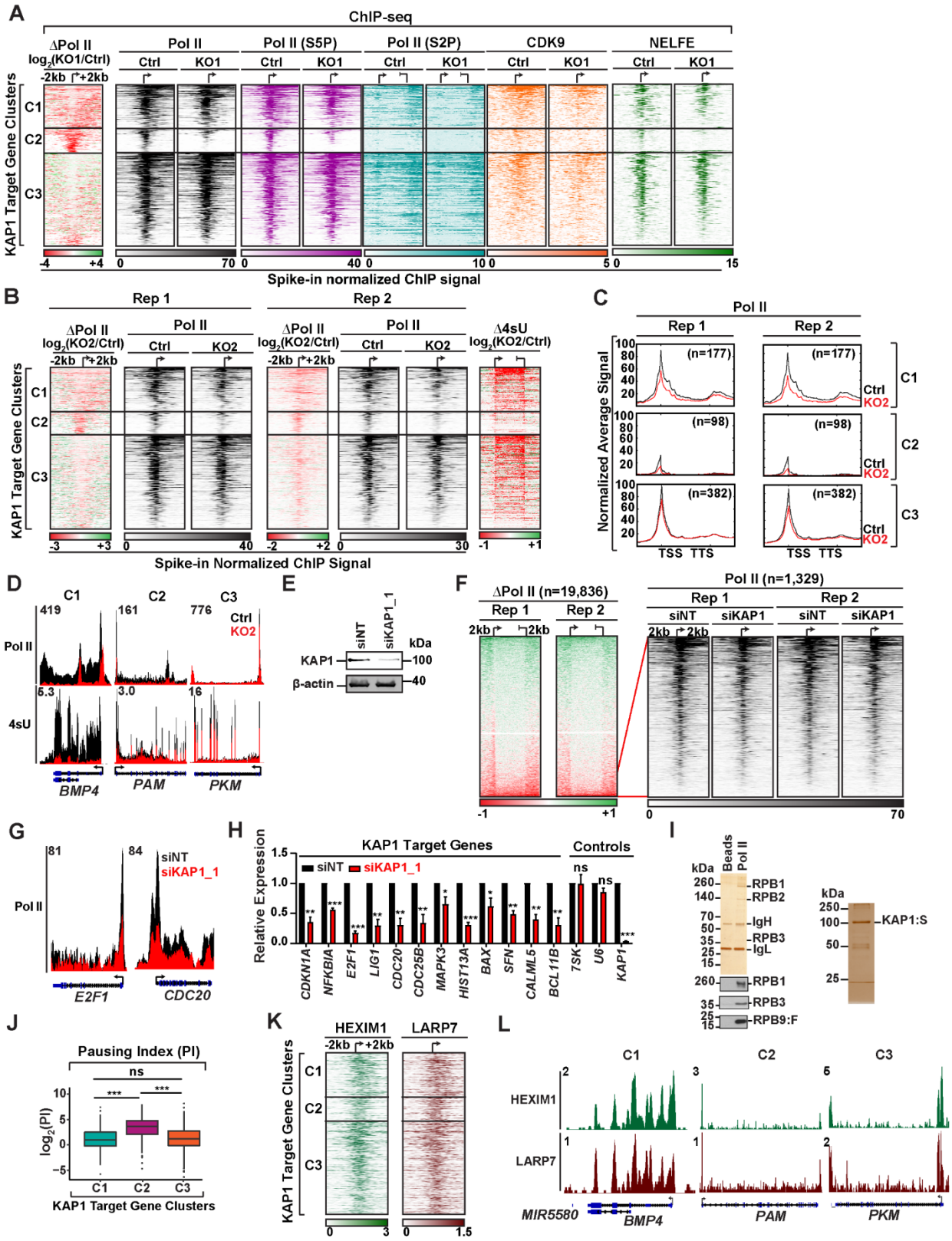

Figure S2. KAP1 Regulates Pol II Promoter Levels Across Multiple Cell Line Clones and Cancer Cell Types. Related to Figure 2

(A) Heatmaps of the second replicate of Pol II, phosphorylated Pol II, CDK9, and NELFE ChIP-seq in Ctrl and KAP1 KO1 cell lines. ChIP-seq signal was normalized to *Drosophila* spike-ins.

(B) Heatmaps of Pol II ChIP signal and changes in 4sU-seq signal (Δ4sU) in the KAP1 KO2 cell line. ChIP-seq signal was normalized to *Drosophila* spike-ins.

(C) Metagene plots (± 2 kb flanking regions) of Pol II in Ctrl vs KO2 cell lines. ChIP-seq signal was normalized to *Drosophila* spike-ins.

(D) Pol II ChIP-seq signal tracks of representative genes from each gene cluster in Ctrl and KO2 cell lines.

(E) Western blot depicting KAP1 KD efficiency in MCF7 breast cancer cells.

(F) Left; ΔPol II heatmaps from Pol II ChIP-seq in MCF7 breast cancer cells of all human protein coding genes sorted by decreasing ΔPol II signal throughout the entire transcriptional unit. Right; Pol II heatmaps sorted by decreasing Pol II signal surrounding the TSS (± 2 kb) of genes that showed decreased Pol II promoter and gene body signal upon KAP1 depletion (siKAP1) relative to Control (siNT). Data of replicates 1 (Rep 1) and 2 (Rep 2) is shown. ChIP-seq signal was normalized to *Drosophila* spike-ins.

(G) Pol II ChIP-seq signal tracks of two genes from panel (F) are shown.

(H) RT-qPCR validation of genes showing decreased Pol II promoter and gene body occupancy after RNAi-mediated KAP1 silencing in MCF7 breast cancer cells.

(I) Silver stains of purified FLAG-tagged core Pol II complex and STREP-tagged KAP1.

(J) C1 and C3 genes are lowly paused compared to highly paused C2 genes. Boxplot representation of the Pausing Index (PI) of genes from all three clusters in the HCT116 Ctrl cell line. Statistical significance was determined using Wilcoxon rank-sum test.

(K) The 7SK snRNP complex (LARP7 and HEXIM1 subunits) occupies the promoter-proximal region of genes from each KAP1 target gene cluster. Heatmaps of LARP7 and HEXIM ChIP-seq signals in parental HCT116 cells from (McNamara et al., 2016). Target genes in each cluster are sorted by decreasing Pol II levels surrounding the TSS (± 2 kb).

(L) 7SK snRNP (LARP7 and HEXIM1 subunits) ChIP-seq signal tracks of representative genes from each cluster.

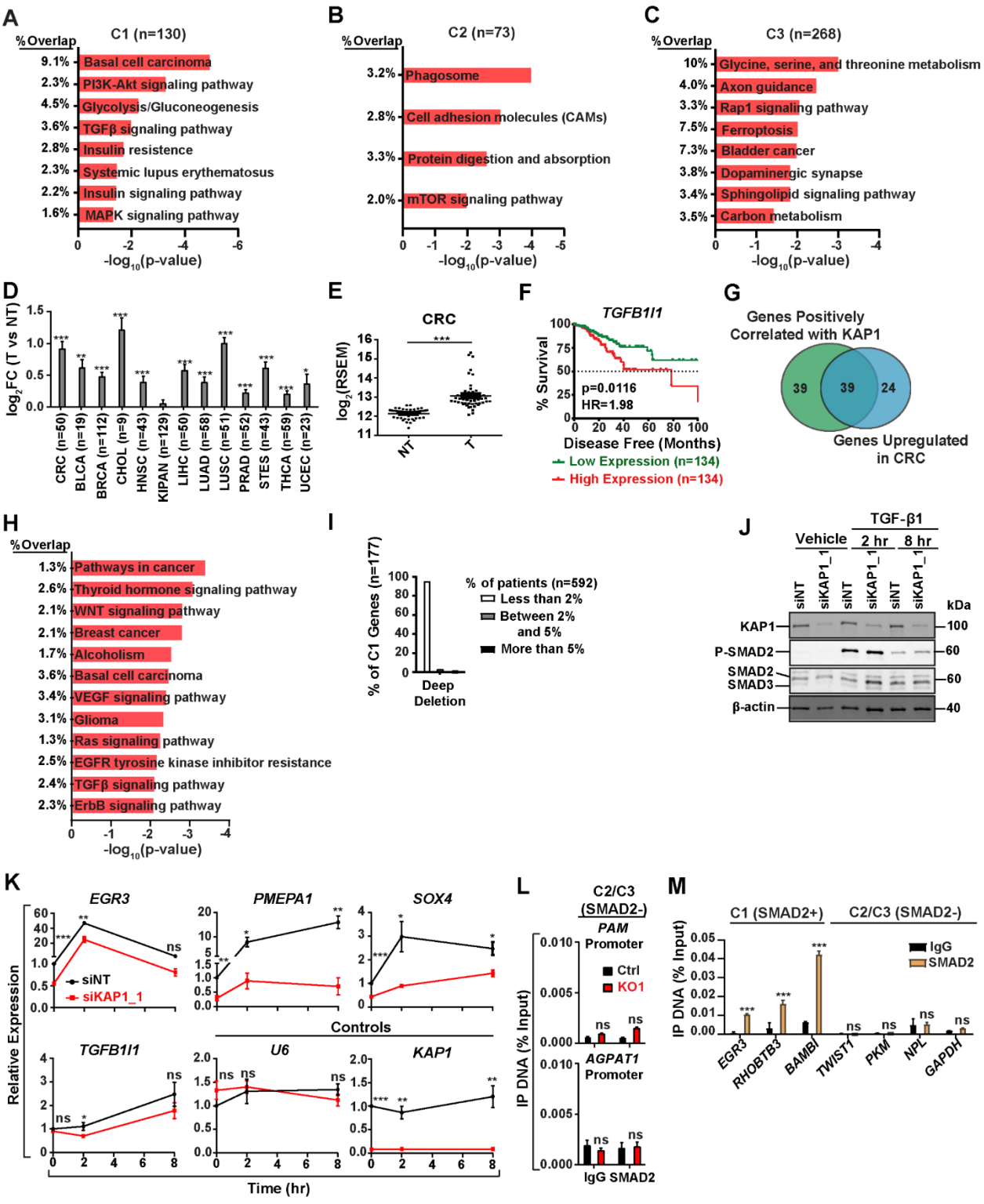

Figure S3. KAP1 Regulates Genes Associated with Poor Patient Prognosis and is Necessary for Expression of TGF-β Induced Target Genes in Primary Human Colon Epithelial Cells. Related to Figure 3

(A-C) KEGG pathway analysis of (A) C1, (B) C2, and (C) C3 KAP1 target genes that were also differentially expressed upon acute KAP1 silencing with siKAP1_1.

(D) KAP1 is overexpressed in several cancer tumors (T) relative to non-tumor (NT). Differential expression of KAP1 was analyzed in colon adenocarcinoma/rectal adenocarcinoma (CRC), Bladder Urothelial Carcinoma (BLCA), Breast Invasive Carcinoma (BRCA), Cholangiocarcinoma (CHOL), Head and Neck Squamous Cell Carcinoma (HNSC), Pan-Kidney Cancer (KIPAN), Liver Hepatocellular Carcinoma (LIHC), Lung Adenocarcinoma (LUAD), Lung Squamous Cell Carcinoma (LUSC), Prostate Adenocarcinoma (PRAD), Stomach and Esophageal Carcinoma (STES), Thyroid Carcinoma (THCA), Uterine Corpus Endometrial Carcinoma (UCEC) using TCGA RNA-seq data. Fold change for each matched sample of NT and T adjacent tissue was calculated (mean $\log_2$(fold change) ± SEM T vs NT). Number of patients for each tumor is indicated in brackets. Statistical significance was determined using paired *t*-test.

(E) Scatterplot representation of *KAP1* expression levels in NT and T of CRC patient samples. Differential expression of *KAP1* was analyzed in CRC patients of 50 matched samples of T and NT adjacent tissue. Dots represent expression value of each patient and error bars represent mean ± SEM.

(F) The association of C1 genes (e.g., *TGFB1I1*) expression level (low or high) with disease-free survival of selected CRC patients. Plotted is the Kaplan-Meier survival analysis and *P*-value Cox-regression/Hazard Ratio.

(G) Expression of 39 C1 genes is upregulated in matched samples of T vs NT (n = 50) and correlates with *KAP1* tumor expression levels (n = 626) in CRC patients ($P<0.05$ by Pearson analysis). Differential expression of genes upregulated in CRC was determined as in **Figure S3D**. Statistical significance for genes upregulated in CRC was determined using paired *t*-test. $P<0.05$ was considered statistically significant.

(H) KEGG pathway analysis identified functional annotations for the 39 C1 genes in panel (G). Statistical significance was determined using paired *t*-test. $P< 0.01$.

(I) C1 genes deep deletion events in 592 CRC patients (TCGA) were analyzed. Histogram plots show the percentage of genes that are deleted in less than 2%, between 2 to 5%, or more than 5% of CRC patient samples.

(J) Western blot verification of efficient RNAi-mediated KAP1 silencing and SMAD2 phosphorylation (P-SMAD2) in HCEC cells transfected with siNT and siKAP1 and treated with TGF-β1 for 2 hr, 8 hr, or vehicle (DMSO) control.

(K) Expression of TGF-β–inducible/SMAD2 target genes, a control gene not induced by TGF-β (*U6*), and *KAP1* after TGF-β1 treatment in siNT and siKAP1 HCEC cells (mean expression relative to vehicle treatment ± SEM; normalized to *7SK*; n = 3).

(L) ChIP-qPCR analysis of SMAD2 in Ctrl vs KO1 HCT116 cell lines at C2/C3 (SMAD2-) gene promoters (mean ± SEM; n = 3).

(M) SMAD2 ChIP analysis, and IgG as negative control, in parental HCT116 cells followed by qPCR of C1 (SMAD2+) and C2/C3 (SMAD2-) gene promoters (mean ± SEM; n = 3).

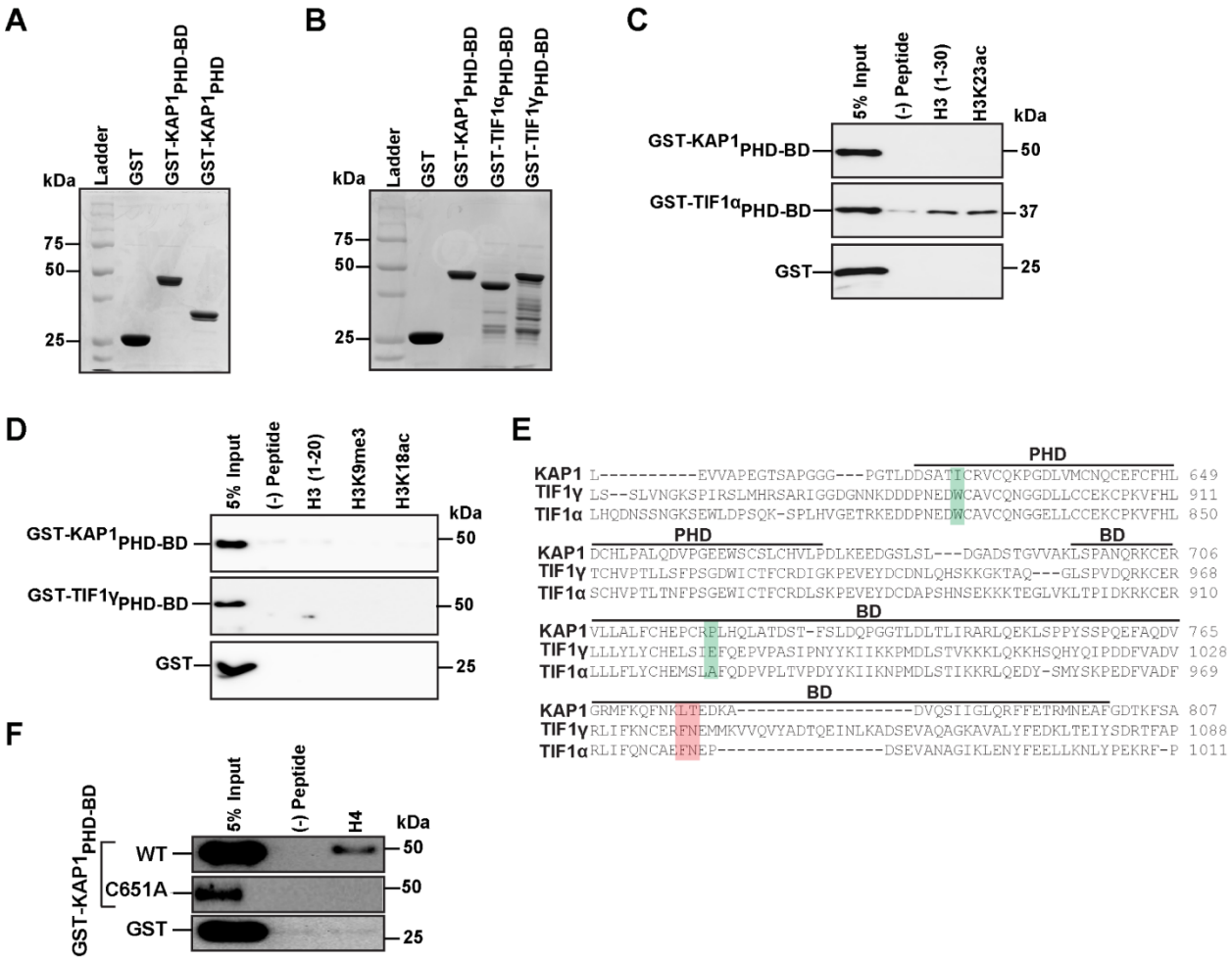

Figure S4. The KAP1 PHD-BD Domain Does Not Interact with the Preferred Histone Peptide Substrates of Other TIF1 Family Members. Related to Figure 4

(A) Coomassie staining of recombinant GST and GST-tagged KAP1 PHD-BD and PHD domains.

(B) Coomassie staining of recombinant GST and GST-tagged KAP1, TIF1α, and TIF1γ PHD-BD domains.

(C) The KAP1 PHD-BD cassette does not bind histone peptides recognized by TIF1α. *In vitro* peptide binding assays between recombinant GST-tagged KAP1 or TIF1α PHD-BD domains and the histone peptides recognized by TIF1α. Western blots probed with anti-GST.

(D) The KAP1 PHD-BD cassette does not bind histone peptides recognized by TIF1γ. *In vitro* peptide binding assays between recombinant GST-tagged KAP1 or TIF1γ PHD-BD domains and the histone peptides recognized by TIF1γ. Western blots probed with anti-GST.

(E) Sequence alignment of KAP1, TIF1γ, and TIF1α PHD-BD domains. Residues highlighted in green are critical for TIF1γ's interaction with H3K9me3 and H3K18ac marks, while residues highlighted in red are critical for TIF1α's interaction with H3K23ac.

(F) *In vitro* peptide binding assay between the SUMO deficient C651A KAP1 PHD-BD domain mutant and the H4 tail. Western blots probed with anti-GST.

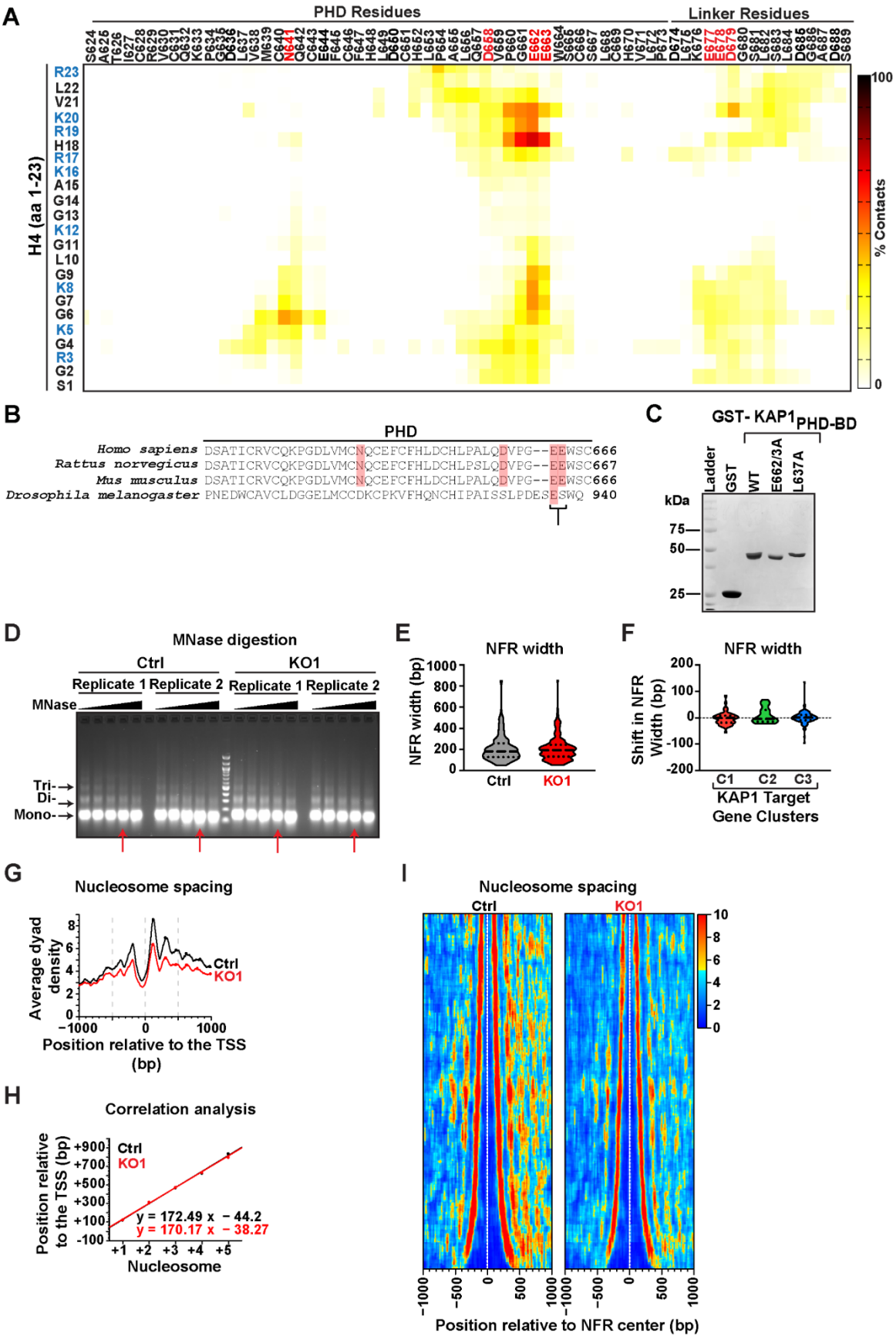
A
PHD Residues
Linker Residues
H4 (aa 1-23)
% Contacts
100
0
B
PHD
Homo sapiens DSATICRVCQKPGDLVMCNQCEEFCFHLDCHLPALQDVPG--EEWSC666
Rattus norvegicus DSATICRVCQKPGDLVMCNQCEEFCFHLDCHLPSLQDVPG--EEWSC667
Mus musculus DSATICRVCQKPGDLVMCNQCEEFCFHLDCHLPALQDVPG--EEWSC666
Drosophila melanogaster PNEDWCAVCLDGGELMCCDKCPKVFHQNCHIPAISSLPDESESWQ 940
C
GST- KAP1PHD-BD
Ladder
GST
WT
E662/3A
L637A
kDa
75
50
25
D
MNase digestion
Ctrl
KO1
Replicate 1
Replicate 2
Replicate 1
Replicate 2
MNase
Tri-
Di-
Mono-
E
NFR width
NFR width (bp)
1000
800
600
400
200
0
Ctrl
KO1
F
NFR width
Shift in NFR Width (bp)
200
100
0
-100
-200
C1
C2
C3
KAP1 Target Gene Clusters
G
Nucleosome spacing
Average dyad density
8
6
4
2
0
Ctrl
KO1
−1000
0
1000
Position relative to the TSS (bp)
H
Correlation analysis
Position relative to the TSS (bp)
+900
+700
+500
+300
+100
-100
Ctrl
KO1
y = 172.49 x − 44.2
y = 170.17 x − 38.27
+1
+2
+3
+4
+5
Nucleosome
I
Nucleosome spacing
Ctrl
KO1
10
8
6
4
2
0
−1000
−500
0
500
1000
Position relative to NFR center (bp)

Figure S5. Identification of KAP1:H4 Interacting Residues and Examination of Nucleosome Positioning at Downregulated Genes. Related to Figure 5

(A) Heatmap highlighting hotspots of pairwise contacts in the production run of the MD simulation.

(B) Sequence alignment of KAP1 PHD domains from various species. Residues highlighted in red are exact matches between species. The arrow denotes the position of the key E662 residue in human KAP1 and its conservation across various species.

(C) Coomassie staining of purified GST, GST-tagged WT KAP1 and point mutants.

(D) MNase-digested nucleosomal DNA profiles of HCT116 Ctrl and KO1 cell lines for two replicates. Red arrows indicate samples used for library preparation. The position of the Mono-, Di-, and Tri-nucleosomes is indicated with arrows.

(E) Violin plots showing distribution of Nucleosome Free Region (NFR) width in Ctrl and KO1 cell lines for downregulated KAP1 target genes. The thick dash line represents the median width and the two dotted lines represent quartiles.

(F) NFR widths were calculated for each gene cluster and the differences (KO1/Ctrl) were plotted as a violin plot [differences were non-significant (P=0.2866 between the three clusters by the Kruskal-Wallis test)].

(G) Metagene analysis showing the distribution of normalized nucleosome dyads relative to the TSS of downregulated KAP1 target genes from all three clusters.

(H) Linear fit of the nucleosome dyads in Ctrl and KO1 cell lines near the 5'-end of downregulated genes. The slope represents the average spacing between the indicated nucleosomes.

(I) Heatmap representation of nucleosome dyads relative to the NFR center of the downregulated genes. Genes were ranked order based on increasing NFR width. The white long dash line denotes the center of the NFR.

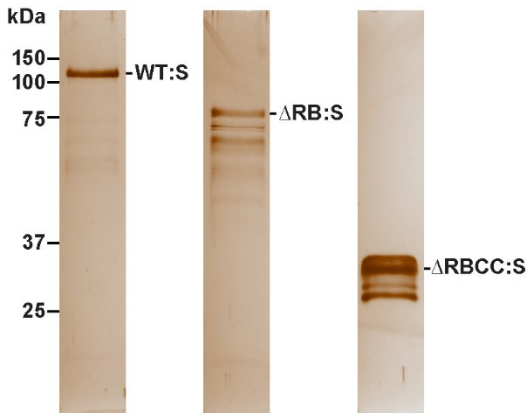

Figure S6. Purification of KAP1 Deletion Constructs. Related to Figure 6

Silver stains of purified STREP-tagged WT KAP1 and deletion constructs (ΔRB and ΔRBCC).

Table S6. DNA Oligonucleotides used in this study. Related to STAR Methods and Figures 1–6

Target	Primer Sequence (5'-3')	Assay
gRNA: *KAP1* KO1	Fwd: CACCGGGAGCGCTTTTCGCCGCCAG Rev: AAACCTGGCGGCGAAAAGCGCTCCC	CRISPR-Cas9
gRNA: *KAP1* KO2	Fwd: CACCGAAAGCGCTCCACCGCCCCTT Rev: AAACAAGGGGCGGTGGAGCGCTTTC	CRISPR-Cas9
Primer: *RPL19* Gene Body	Fwd: ATCGATCGCCACATGTATCA Rev: GCGTGCTTCCTTGGTCTTAG	RT-qPCR
Primer: *U6*	Fwd: CTCGCTTCGGCAGCACATATAC Rev: GGAACGCTTCACGAATTTGCGTG	RT-qPCR
Primer: *BMP4* Gene Body	Fwd: CCAGACTGAAGCCGGTAAAG Rev: TGATACCTGAGACGGGGAAG	RT-qPCR
Primer: *SMAD2* Gene Body	Fwd: GATGGCTTTCTCAAGCTCATC Rev: GCCGCCAGTTGTGAAGAG	RT-qPCR
Primer: *TGFB1I1* Gene body	Fwd: GGTATGCAAGCCTCG Rev: TAAGTTCCTGAAGCAACC	RT-qPCR
Primer: *PDGFC* Gene Body	Fwd: GGGTCTTCAAGCCCAAATCT Rev: CAGCCCAAGGTTTCCTCATA	RT-qPCR
Primer: *EGR3* Gene Body	Fwd: TTGAGGGTGAGCGGC Rev: CATGATTCCTGACTACAACCT	RT-qPCR
Primer: *ID3* Gene Body	Fwd: ACATCGCATTGTTACAGAAAGTCACC Rev: GGCAGAGCTGGTCTTCTGGT	RT-qPCR
Primer: *PAM* Gene Body	Fwd: TGCAACCAGGCAGTGACCAG Rev: GTGAACTGGCCTGGCAGAGG	RT-qPCR
Primer: *BAMBI* Gene Body	Fwd: TGATGCTGCCCACTG Rev: TATGGTGGTGCCAGA	RT-qPCR
Primer: *TWIST1* Gene Body	Fwd: CTGCCCTCGGACAAGCTGAG Rev: TAGTGGGACGCGGACATGGA	RT-qPCR
Primer: *ZNFHX2* Gene Body	Fwd: TTCGGTCCTGCCTACCACCA Rev: GTGCCTGCTGTGGAGGTGTT	RT-qPCR
Primer: *AGPAT1* Gene Body	Fwd: TGGGCGACCTCAGACATGACA Rev: GGCCGCTGTGTGCCC	RT-qPCR
Primer: *SOX4* Gene Body	Fwd: AAGATCATGGAGCAGTCGCC Rev: CGCCTCTCGAATGAAAGGGA	RT-qPCR
Primer: *PMEPA1* Gene Body	Fwd: TGCAAACGCTCTTTGTTCCAG Rev: GATGAAGGACCGTGCAGACA	RT-qPCR
Primer: *CDK9* Gene Body	Fwd: GTGTTCGACTTCTGCGAGCATGAC Rev: CTATGCAGGATCTTGTTTCTGTGG	qRT-PCR
Primer: *7SK*	Fwd: GGATGTGAGGCGATCTGGC Rev: AAAAGAAAGGCAGACTGCCAC	RT-qPCR
Primer: *KAP1* Gene Body	Fwd: CAGGATGCGAACCAGTGCT Rev: CTTGGTGTACTTCACCCGCT	RT-qPCR
Primer: *CDKN1A* Gene Body	Fwd: CTGGAGACTCTCAGGGTCGAAA Rev: GATTAGGGCTTCCTCTTGGAGAA	RT-qPCR
Primer: *NFKBIA* Gene Body	Fwd: TCCTGAGCTCCGAGACTTTC Rev: GTAGTTGGTAGCCTTCAGG	RT-qPCR
Primer: *E2F1* Gene Body	Fwd: TCAGCACCTCGGCAGCCC Rev: AGAAGTCACGCTATGAGACCT	RT-qPCR

Primer: *LIG1* Gene Body	Fwd: ACGCCAAGCTCCAGGC Rev: TGAGCAACTTGCTGCGCT	RT-qPCR
Primer: *CDC20* Gene Body	Fwd: GTTCTGGCCACATCCACCACC Rev: CCACACATTGACCAAGTTATC	RT-qPCR
Primer: *CDC25B* Gene Body	Fwd: TTGTCTTCAAGATGCCATGGA Rev: CCGAGCTGGGTCTCTGGG	RT-qPCR
Primer: *MAPK3* Gene Body	Fwd: TGTGGTTGAGCTGATCCA Rev: CCAAGTCCATCGACATCTGGT	RT-qPCR
Primer: *HIST13A* Gene Body	Fwd: CGTTTCCAGAGCTCCGCTGTG Rev: ATGATAGTGACGCGCTTG	RT-qPCR
Primer: *BAX* Gene Body	Fwd: TGATTGCCGCCGTGGA Rev: CAAAGTAGAAAAGGGCGACAA	RT-qPCR
Primer: *SFN* Gene Body	Fwd: CTGGGCCTGCTGGACAGC Rev: TTCTTGTCGTCACCGGTG	RT-qPCR
Primer: *CALML5* Gene Body	Fwd: CGGTTGACACGGATGGAAAC Rev: TGGAAGCTGATTTCGCCGT	RT-qPCR
Primer: *BCL11B* Gene Body	Fwd: ATCACTTCACCTCTGCGTGC Rev: ACCTGACAACTGACACTGGC	RT-qPCR
Primer: *BMP4* Promoter	Fwd: CTGCAGGCTCGAGATAGCTT Rev: GAAGATGCGAGAAGGCAGAG	ChIP-qPCR
Primer: *TGFB1I1* Promoter	Fwd: GTCCGTGGCCCCTCAC Rev: GCGGGCAGAGGCGAAA	ChIP-qPCR
Primer: *PDGFC* Promoter	Fwd: ACTGGCTGTCAACAGGTGCT Rev: TAGAGGTGTTCCGTGGAAGG	ChIP-qPCR
Primer: *PAM* Promoter	Fwd: GGGGGAGGGAGCTCAACAGA Rev: ACTCCGGAATGACAGGGGCT	ChIP-qPCR
Primer: *AGPAT1* Promoter	Fwd: TGGAGGGGAGGTGGGAGTG Rev: TAGCGGCGGCAGCAGC	ChIP-qPCR
Primer: *ID3* Promoter	Fwd: CGGTCACTTATAGAGCCTGCC Rev: TTGAATCCGCGGCTCC	ChIP-qPCR
Primer: *EGR3* Promoter	Fwd: ACGCGGCCTCAGTATTGA Rev: CTAGGAAGCGGCGGG	ChIP-qPCR
Primer: *RHOBTB3* Promoter	Fwd: GATGAGCGGATTGCGGGTGA Rev: GAACAGGAAGTGCGGGGGAC	ChIP-qPCR
Primer: *BAMBI* Promoter	Fwd: AGAGACCTGGGCTGGCG Rev: CTAGCCCCGGGTCGG	ChIP-qPCR
Primer: *TWIST1* Promoter	Fwd: TGCGCCGCTTGCGTC Rev: AAGCTGGCGGGCTGAG	ChIP-qPCR
Primer: *PKM* Promoter	Fwd: TCACCTCCGGCGCTGAC Rev: GAGGCTGAGGCAGTGGCTC	ChIP-qPCR
Primer: *NPL* Promoter	Fwd: GACGAGGCAGGGGGA Rev: CGGGTGTCACCTGAA	ChIP-qPCR
Primer: *GAPDH* Promoter	Fwd: TTTGCGTCGCCAGGTGAAGA Rev: CAGCGGCGCGAACACATC	ChIP-qPCR
Primer: *CD69* Promoter	Fwd: AATCCCACTTTCCTCCTGCT Rev: GCCGCCTACTTGCTTGACTA	ChIP-qPCR
Primer: KAP1 C651A	Fwd: GGGCCGGCAGGTGAGCGTCCAGGTGGAAAC Rev: GTTTCCACCTGGACGCTCACCTGCCGGCCC	Mutagenesis
Primer: KAP1 E662/663A	Fwd: GTGAGCAGCTCCACGCCGCCCCTGGTACATCC Rev: GGATGTACCAGGGGCGGCGTGGAGCTGCTCAC	Mutagenesis

Primer: KAP1 L637A	Fwd:GGTTGCACATAACCGCATCGCCTGGCTTCTGGCAG Rev:CTGCCAGAAGCCAGGCGATGCGGTTATGTGCAACC	Mutagenesis
Primer: KAP1 PHD-BD	Fwd: GTAGGATCCCCGGGAACCCTG Rev: GTAGAATTCTCAACCAAGTTCTCTGCT	pGEX2T BamHI/EcoRI
Primer: KAP1 PHD	Fwd: GTAGGATCCCCGGGAACCCTG Rev: GTAGAATTCTCAGTGCTCCCTGACCTG	pGEX2T BamHI/EcoRI
Primer: TIF1α PHD-BD	Fwd: CCGGGATCCCCCAATGAGGAC Rev: CCGGGATCCTTATGGATAGAGGTTCTT	pGEX2T BamHI
Primer: TIF1γ PHD-BD	Fwd: CCGGGATCCGATGATGACCCA Rev: CCGGGATCCTTATGCGAAGGTCCT	pGEX2T BamHI
Primer: KAP1ΔPHD-BD	Fwd: CCGAAGCTTATGGCGGCCTCCGCGGCGGC Rev: AATCTCGAGCTTGGCCACCAC	pcDNA4TO HindIII/XhoI
Primer: KAP1ΔBD	Fwd: CCGAAGCTTATGGCGGCCTCCGCGGCGGC Rev: AATCTCGAGCTTGGCCACCAC	pcDNA4TO HindIII/XhoI
Primer: KAP1ΔPHD	Fwd: CCGGGAACCCTGGATGACCTGAAGGAG Rev: CTCCTCCTTCAGGTCATCCAGGGTTCC	pcDNA4TO HindIII/XhoI
Primer: KAP1ΔCC	Fwd:CACCAGTACCAGTTCTTAAAGATGATTGTGGATCCC Rev:GGGATCCACAATCATCTTTAAGAACTGGTACTGGTG	pcDNA4TO HindIII/XhoI
Primer: KAP1ΔRB	Fwd:CCGAAGCTTATGCCACCTAAAAAGAAGCGTAAGGTTGAGGATGCAGTGAGGAACCAG Rev:CCGCTCGAGGGGGCCATCACCAGGGCCAC	pcDNA4TO HindIII/XhoI
Primer: KAP1ΔRBCC	Fwd:CCGAAGCTTATGCCACCTAAAAAGAAGCGTAAGGTTAAGATGATTGTGGATCCC Rev:CCGCTCGAGGGGGCCATCACCAGGGCCAC	pcDNA4TO HindIII/XhoI
Primer: SMAD2	Fwd:CGGAATTCGCCACCATGTCGTCCATCTTGCCATTCAC Rev: CCGCTCGAGTGACATGCTTGAGCAACGCAC	pcDNA4TO HindIII/XhoI