SUPPLEMENTAL INFORMATION.

EXTENDED EXPERIMENTAL PROCEDURES.

Q-RT-PCR analysis of gene expression.

Reverse transcription was carried out using a qScript cDNA synthesis kit (Quanta Biosciences). Quantitative PCR was carried out with reference to a standard curve using SYBR Green PCR master mix (ABI / Life Technologies) on a 7900HT sequence detection system (ABI / Life Technologies) and normalized to 18S rRNA signals. Primers used are described below. Primers used for Q-RT-PCR were as follows (5' to 3'): 18S-fwd, GCCGCTAGAGGTGAAATTCTTG; 18S-rev, CTTTCGCTCTGGTCCGTCTT; ALDOC-fwd, TCATCAAGCGGGCTGAGGTGAA; ALDOC-rev, TGTGCTGCTGCTCCACCATCTT; ANKRD37-fwd, TGTGTTGCCGTGCTCAGACAGA; ANKRD37-rev, ACCCACGTGACATCAGCACTTC; CDK19-fwd, GCCACGGCTAGGGCCT; CDK19-rev, GCGAGAACTGGAGTGCTGATAA; CDK8-fwd, GGGATCTCTATGTCGGCATGTAG; CDK8-rev, AAATGACGTTTGGATGCTTAAGC; CITED2-fwd, CCACTACATGCCGGATTTGC; CITED2-rev, TCTCGGAAGTGCTGGTTTGTC; CXCR4-fwd, TTCTTCGCCTGTTGGCTGCCTT; CXCR4-rev, AAGAAAGCTAGGGCCTCGGTGA; DUSP1-fwd, AGGCAGACATCAGCTCCTGGTT; DUSP1-rev, TGGCAGTGGACAAACACCCTTC; DUSP5-fwd, TTATGACCAGGGTGGCCCAGTT; DUSP5-rev, GAGGAACTCGCACTTGGATGCA; MAGI1-fwd, TTTGCGGTGCCCAAAACCGA; MAGI1-rev, TGCGCTTCTCTTCTGTCAGCGA; MXD1-fwd, AGCTGGAGAAGCTGGGCATTGA; MXD1-rev, TGCTCTCCACGTCAACGTCGAT; NR4A1-fwd, TTCAAAACCCAAGCAGCCCCCA; NR4A1-rev, ACCAGCTCCTGGAACTTGGAGT; P4HA1-fwd, AGCAAAACCAAGGCTGAGCCGA; P4HA1-rev, TTCATAGCCAGAGAGCCAGGCA; PER1-fwd, ATCCCCACCCAGCAGTTCCATT; PER1-rev, TGACTGTTCACTGCTGCAGCCA; PFKFB4-fwd, TGTGTGCATGACCAACTGCCCA; PFKFB4-rev, ACTGGCCAACATTGAACTCCCG; STC2-fwd, ACTTGCTGCTGCACGAACCCTA; STC2-rev, TCACACTGAACCTGCACGCTGT; TMEM45A-fwd, TGGCTTTAACTGGCATGGCTGG; TMEM45A-rev, CCACACCCAACAGCCCAAAGAA; UHMK1-fwd, ATGGTTTCCGCAAAGAGAGGGC; UHMK1-rev, AGCAACAGACAGCGTGATGGCA; VGF-fwd, CCTCTCCACCTCTCGCGTCGT; VGF-rev, GGTGCTGCCCCTAACCCGTTG; ZFP36-fwd, TGACTTCAGCGCTCCCACTCT; ZFP36-rev, TCAGCGACAGGAGGCTCTCGTA.

Tissue Panel RNA analysis

For analysis of CDK8 and CDK19 mRNA expression in normal human tissues, the FirstChoice Human Total RNA Survey Panel (AM600) and Pancreas Total RNA (AM7954) were purchased from ABI / Ambion. Three replicates of cDNA synthesized from these RNA pools were subjected to Q-RT-PCR analysis. Results are shown in Figure S1B as the mean of three replicates relative to the adipose pool and normalized to 18S rRNA, with error bars representing standard error of the mean.

Western blotting

Protein sample preparation, quantitation and Western blot analyses were carried out as described previously (Henry et al., 2012). Detection was by enhanced chemiluminescence and images were captured on standard x-ray film or using an ImageQuant LAS4000 digital camera system (GE Healthcare). Antibodies used are described below.

Antibodies used: AFF4: Bethyl A302-539A; CDK8: Santa Cruz sc -1521; CDK19: Sigma HPA007053; CCNC: Santa Cruz sc-1061; MED12: Bethyl A300-774A; MED13: Bethyl A301-278A; MED13L: Bethyl A302-421A; HIF1A: BD Transduction 610959; HIF2A: Millipore MAB3472; Nucleolin: Santa Cruz sc-8031; α-tubulin: Sigma T9026; p300: Santa Cruz sc-584; MED1: Santa Cruz sc-5334; MED14: Abcam Ab72141; MED15: gift from Dylan Taatjes (Meyer et al., 2008); MED23: Santa Cruz sc-5376; MED26: Santa Cruz sc-48776.

HaloTag Pull-Down Assay

HCT116 cells (1.2×10^7) were plated in 150 mm dishes and grown to 70-80% confluence (approximately 24 hr). Cells were then transfected with 30 µg of plasmid DNA using Fugene HD Transfection Reagent (Promega), according to manufacturer's protocol. DNA clones expressing N-Terminal HaloTag fusions of human CDK8 (NM_001260.1) and CDK19 (NM_015076.3) were obtained from Kazusa DNA Research Institute (Kisarazu, Japan). Twenty-four hours post-transfection, cells were harvested and lysed in mammalian lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, and 0.1% sodium deoxycholate) supplemented with Protease Inhibitor cocktail (Promega), 10 mM KCl, and 1.5 mM $MgCl_2$ for 10 min on ice. Lysate was then homogenized with a syringe and centrifuged at 14,000x g for 5 min to pellet cellular

debris. Clarified lysate was incubated with HaloLink Resin (Promega) that had been pre-equilibrated in resin wash buffer (TBS and 0.05% IGEPAL CA-640 (Sigma)) for 15 min at 22^{o}C with rotation. Complex-bound resin was then washed 5 times with resin wash buffer, supplemented with 10 mM KCl and 1.5 mM $MgCl_2$. Protein interactors were eluted with SDS elution buffer (50 mM Tris-HCl, pH 7.5, and 1% SDS) for 30 min at 24^{o}C.

shRNA-mediated knockdown of CDK8 and CDK19.

Short-hairpin RNA (shRNA) sequences targeting the CDK8 (shCDK8 #1) and CDK19 mRNAs for knockdown, were designed using the pSicoOligomaker 1.5 software (http://web.mit.edu/jacks-lab/protocols/pSico.html) and cloned into the pLL3.7 lentiviral expression vector. A second shRNA targeting CDK8 (shCDK8 #2, TRCN0000196702) and a non-targeting control (SHC0002) in the pLKO.1 vector was purchased from a Sigma Mission shRNA library maintained by the Functional Genomics Facility at CU Boulder. Sequences used were as follows (5' to 3'):

shCDK8 #1,
TGGATGATAAAGACTATGCTTTCAAGAGAAGCATAGTCTTTATCATCCTTTTTTC;
shCDK19,
TGTAGCTAAGTCTACCTTAATTCAAGAGATTAAGGTAGACTTAGCTACTTTTTTC;
shCDK8 #2,
CCGGGCTATTGATATTTGGGCTATACTCGAGTATAGCCCAAATATCAATAGCTTTTTTG.

Lentiviral particles were produced in HEK293FT cells by co-transfection of the shRNA vector and lentiviral helper plasmids. Viral supernatant collected 48 hr post-transfection and 0.45 μM-filtered was used for transduction of HCT116 cells with the addition of 8 ng/μl polybrene. Selection and maintenance of stable transductants was carried out with G418 (100 μg/ml, Sigma) or puromycin (10 μg/ml, Sigma). Knockdown efficiency was confirmed by Western blotting and Q-RT-PCR.

Microarray analysis.

Total RNA from HCT116 cells harvested using an RNeasy kit (Qiagen) was used for gene expression analysis on Affymetrix HuGene 1.0 ST arrays following the manufacturer's instructions. Differential gene expression was determined with Partek

software using one-way ANOVA. Heatmaps show log-2 fold-change values calculated from means relative to untreated shControl cells (ranked as indicated in Figure 1).

Chromatin immunoprecipitation.

HCT116 cells were subjected to normoxia or hypoxia for 24 hr. Media was removed and the cells cross-linked by the addition of 1% formaldehyde in PBS for 15 min, followed by the addition of glycine to 0.125 M to quench the formaldehyde. Plates were then washed twice with cold PBS to remove all traces of formaldehyde. Cross-linked cells were harvested by scraping directly into RIPA buffer (150 mM NaCl, 50 mM Tris pH 8, 5 mM EDTA, 1% IGEPAL, 0.5% sodium deoxycholate, 0.1% SDS, protease/phosphatase/HDAC inhibitors) and snap-frozen. Thawed whole-cell lysates were sonicated to give DNA fragments of ~200 bp and centrifuged at 12,000 x g to remove insoluble material. The supernatant was quantified using a BCA protein assay kit (Pierce) and diluted to 1 mg/ml with RIPA buffer.

For immunoprecipitation, 1 mg of ChIP extract was first pre-cleared by incubation with RIPA-washed Protein-G sepharose beads (GE Healthcare) at 4°C for 2 hr. Pre-cleared supernatants were then mixed with Protein-G sepharose beads that had been blocked for 2 hr with 1 mg/ml bovine serum albumin (Sigma) and 0.3 mg/ml salmon sperm DNA (Invitrogen) and immunoprecipitated by incubating overnight at 4°C with the desired antibody. Each ChIP was then washed (5 min each wash) twice with RIPA buffer, four times with IP wash buffer (100 mM Tris pH 8.5, 0.5 M LiCl, 1% IGEPAL, 1% sodium deoxycholate), twice more with RIPA buffer, twice with TE (10 mM Tris pH 8, 1 mM EDTA), and finally re-suspended in 100 μl TE. Immunocomplexes were then eluted by the addition of two volumes of elution buffer (70 mM Tris pH 8, 1 mM EDTA, 1.5% SDS) and incubated at 65°C for 10 min. To reverse cross-linking, NaCl was added to a final concentration of 200 mM, followed by incubation at 65°C for 5 hr. To digest remaining protein, 20 μg proteinase K was added and incubated at 45°C for 30 min. Immunoprecipitated DNA was then purified by phenol/chloroform extraction, ethanol precipitated, and finally dissolved in 0.1x TE. Input DNA was prepared essentially in the same manner, omitting the immunoprecipitation steps.

Antibodies used for ChIP were as follows: AFF4: gift from Ali Shilatifard (Lin et al., 2011); BRD4: Bethyl A301-985A; CDK7: Bethyl A300-405A; CDK8: Santa Cruz sc - 1521; CDK9: Santa Cruz sc-8338; H3K9ac: Upstate/Millipore 06-942; H4 pan-acetyl:

Upstate/Millipore 06-866; HIF1A: BD Transduction 610959; MED1: Santa Cruz sc-5334-X; Total RNAPII: Santa Cruz sc-9001X; MED26: Santa Cruz sc-48776; RNAPII S5P (H14): Covance MMS134R; RNAPII S2P (H5): Covance MMS129R; SPT5: Santa Cruz sc-28678.

Primers used for ChIP-Q-PCR were as follows (5' to 3'):

ANKRD37; -2011-fwd, CACACACTGCCCCCAGACTGA; -2011-rev, TGCTAAGGAGTCCAGGAAGGGAAGA; -982-fwd, CCCTTGGGTAACTAAGTATAACAACATAAA; -982-rev, CCTCTAAAGATAAGTGGTCTTTTCATTCT; -25-fwd, TCCACAGCTGGCCAATCGGC; -25-rev, CGGCCGCACGTACACAAACA; +627-fwd, GCTGACCTCAACCAGCAGGTAACT; +627-rev, GGGGACGGCTGTACACAGCA; +1114-fwd, ACCAAAAGTCTCCCTGCAGCAATTG; +1114-rev, GGAAATTAGAATGCTTGTGGACATAGCTCC; +2042-fwd, AAGTGACTAAGACTTCTTATTTCTGAAAACAAACCC; +2042-rev, TTGTTAATATTCATTCTCAGTTCCGGTTTTGGG; +3436-fwd, TGGCTTTACCATATGTTGTGTCTAATCTCCTT; +3436-rev, TGGATCTTCACTTGGAAGAAAGTTTTTCGTC; +4498-fwd, CTTGCAAGGAGGCTGAGACTCTGAA; +4498-rev, GGAGGGAGTCAGACCGCTGC.

STC2; -2023-fwd, CCCCTCCCCCAAAACCTGCC; -2023-rev, GGTTGACGCCTGGAGCCCAT; -1033-fwd, ACAGGTGACCAGATGCCTTCTTA; -1033-rev, CTGCTTATGGGTACAGCTGGGTA; -54-fwd, GACCGAGAGCAGTTCCTGTCC; -54-rev, CGCCGCTTGGAGCG; +2040-fwd, CTGGGGGCACTGCTTTCGGA; +2040-rev, ACGGTTCGGGTCGTGTCACC; +6083-fwd, CTCTCTCCCTTCCCCGGCCT; +6083-rev, CGCAGAGCGTGGGCCTTACA; +8002-fwd, GGGCTCACAGCTCCCCACAT; +8002-rev, CCAGAGGCACCTGAAGGAAGGG; +14619-fwd, TCGAACCAAGCAAAAGGCTTCACG; +14619-rev, GCAAGCTCACGTGGTGCCAA; +16723-fwd, GCCACCATTGAACATCACGCCT; +16723-rev, CGAATGGGAAACGGAACCAGCTG.

CXCR4; -1995-fwd, ACATCGTGCAGGGAGGAGTTTTGA; -1995-rev, TCCTGGACCGTAAGCTGCACC; +432-fwd, CGGCTTGCACGCTGTTTGCA; +432-rev, ACCGGTGGTCTGAGTCCCGA; +1908-fwd, CGCCTGGGAGTGGCCTCTTT; +1908-rev, GCTAACTCCTCTGCCCCGCC; +3718-fwd, GGCACTTATAACCAAAGCCCAAAGTGG; +3718-rev,

AGGTGCTGAAATCAACCCACTCCT; +4711-fwd, AGCAAGCTCCCTGTTGCTCACA; +4711-rev, GCCTGGGGCTTGCACAATGG.

MAGI1; -2019-fwd, CACCAAGCAAAGCCATGGAA; -2019-rev, AGGAGGGATGTTGATGCTTCT; -205-fwd, CCAGGGCACTGAAAGAAAAGG; -205-rev, ATGGGTCCTAAAACCGACCT; +2-fwd, AATAGAATGAACTGTAACAAAACAAGCC; +2-rev, AGCAGGGCGGGAGGTAA; +684577-fwd, GGAGGGAAAAGTCTGTGAATGC; +684577-rev, TCCTCAGCCTTACTACAGCTC; +685652-fwd, AACAGCACAGCACAGTATAGC; +685652-rev, CACACCAGAAGTGATGAAGCTC.

UHMK1; -3449-fwd, AGTCCTGAGAACAAGCTCCC; -3449-rev, CCATCACCCCTGCTTTGTTG; -30-fwd, GGGGGAGAAATGGGTCCTTC; -30-rev, GAGGCTTCATCTGCATGACAA; +7157-fwd, AACGGTCTGGGGACAGATAC; +7157, GTCAGCATGGCAAAATCACCT; +32505-fwd, AGGAGTTGCTTTTTAATTTCAACTTTTT; +32505, ATGGGTGGTTAAAGCTACATGG; +33720-fwd, GAGTCACCAGTGTGCTAGGT; +33720, GCAGGAGACAGGGATCTTCT.

ChIP-seq library preparation and Illumina sequencing

Twenty nanograms of ChIP-enriched DNA or input DNA (prepared as above) were used for generation of Illumina sequencing libraries using a modified version of the Illumina ChIP-seq protocol. Briefly, DNA fragments were end-repaired using the End-It DNA End-Repair Kit (Epicentre) and then a single “A” base was added using Klenow fragment (New England Biolabs). The fragments were ligated to Illumina Indexed adaptors (TruSeq DNA Sample Prep Kit) using T4 DNA ligase (New England Biolabs). The ligated products were size-selected for 350-450 bp fragments on a 2% agarose gel to remove the unligated adaptors, and were subjected to 18 PCR cycles (Illumina TruSeq primers). PCR product was purified on a 2% agarose gel to retain fragments between 350-450bp. Libraries were quantified using a Qubit fluorometer (Invitrogen) and by quantitative PCR (Kapa Biosystems). Two barcoded libraries were pooled and sequenced to 50bp in a single lane on an Illumina HiSeq2000 using standard procedures for cluster amplification and sequencing by synthesis (instrument: HCS 1.5.15.1 - RTA 1.13.48, Pipeline: Illumina CASAVA 1.8.2).

ChIP-seq data analysis and visualization

Raw 50-nucleotide ChIP-seq reads were subjected to quality assessment (97% bases ≥ Q30) using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and adapter sequences (~13%) were filtered out using the FASTX toolkit

(http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads were then aligned to the reference human genome (GRCh37/hg19) using Bowtie 0.12.7 (Langmead et al., 2009) with zero-mismatches and discarding non-unique alignments (~80% uniquely aligned). Enriched CDK8 ChIP peak regions were determined using MACS 2.0.9 (Zhang et al., 2008) with both ChIP and control (input) samples, $p<0.05$, keeping at most two duplicate reads, with an estimated shift-size of 225bp. High-confidence hypoxia CDK8 peaks were found by taking only those peaks that overlapped by ≥ 150-nucleotides between two biological replicates using BEDtools and Galaxy (Goecks et al., 2010; Quinlan and Hall, 2010). CDK8 hypoxia peaks were associated with known genes by using GREAT with a regulatory region for each gene defined as 5 kb up stream to 1 kb downstream of canonical transcription start site with a maximum non-overlapping extension of 200 kb (McLean et al., 2010). The TSS heat map profiles and dot plots were generated with seqMINER 1.3.3, after random downsampling of reads to match the count for the lowest sample. Metagenes were created by taking the mean number of reads per 50 bp bin across all regions as indicated. UCSC Genome browser views show CDK8-enriched tags extended to the MACS2-estimated ChIP DNA fragment size.

Cloning and expression of GST-HIF1A-TAD bait proteins

The HIF1A TAD domains (2X-TAD: residues 523-826; N-TAD: residues 523-586; C-TAD: residues 776-826) were amplified by PCR with Phusion polymerase (Thermo Fisher) from pFN21A containing HIF1A cDNA using the following primers to incorporate BamHI and NotI sites at each end (5' to 3'):
HIF1A-2xTAD-fwd, CGTCACGGATCCGTGGATAGTGATATGGTC; HIF1A-2xTAD-rev, CAGTTGGCGGCCGCTCAGTTAACTTGATCCAA; HIF1A-N-TADrev, CAGTTGGCGGCCGCTAATGGTGACAACTGATC; HIF1A-C-TAD-fwd, CGTCACGGATCCTCTGATTTAGCATGTAGA.
Following agarose gel-purification using a Qiaex kit (Qiagen), the fragments were digested with BamHI and NotI to allow ligation into pGEX-4T-3 (GE Healthcare) in-frame with GST. Appropriate clones were verified by sequencing before transfer into E. coli strain BL21 for expression and purification. The pGEX-4T-3 GST-VP16 (residues 411-490) expression plasmid was a gift from the Taatjes lab (Taatjes and Tjian, 2004).
The GST fusion proteins were expressed by induction of 500 ml BL21 cultures in LB broth with 1 mM IPTG. Bacterial cell pellets were collected at 2 hr post-induction and lysed by sonication in cold Harvest/Extraction (H/E) buffer (50 mM Tris pH 8, 0.5 M

NaCl, 0.5 mM EDTA, 10% glycerol, 0.5% IGEPAL, 1 mM DTT, protease inhibitors). Insoluble material was removed by ultra-centrifugation and the supernatants containing GST fusion proteins retained for binding to glutathione-sepharose beads.

The bait protein-affinity resins were prepared by incubating reduced glutathione-sepharose resin equilibrated in H/E buffer with the cleared lysates at 4°C for 1 hr. The resins were then washed five times with high-salt buffer (1 M NaCl, 0.5% CHAPS, 50 mM Tris pH 8, 0.5 mM EDTA, 10% glycerol, 0.5% IGEPAL, 1 mM DTT, protease inhibitors), twice with H/E buffer, twice with 0.15 M KCl HEGN buffer (0.15 M KCl, 20 mM HEPES, 0.1 mM EDTA, 0.02% IGEPAL, 1 mM DTT, protease inhibitors), and stored as a ~50/50 slurry at 4°C. Purification of GST-fusion proteins was verified by SDS-PAGE and Coomassie-Blue staining.

GST-HIF1A TAD affinity purification assay

Affinity purification of proteins binding to GST-fusion baits, or GST-alone, was carried out by incubating ammonium sulfate-precipitated nuclear extracts, prepared from HeLa cells as described previously (Knuesel et al., 2009), in Buffer D-20 (20 mM HEPES, 20% glycerol, 20 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, pH 7.9) at 8 mg/ml total protein concentration in 1 ml with the affinity resins at 4°C for 3 hr. The resins were then washed five times with 1 ml 0.5 M KCl HEGN (0.5 M KCl, 20 mM HEPES, 0.1 mM EDTA, 0.02% IGEPAL, 1 mM DTT, protease inhibitors) and once with 0.15 M KCl HEGN. After removal of the supernatant, proteins were eluted from the resin by re-suspending in 100 μl of fresh GSH elution buffer (0.15 M KCl, 80 mM, 0.1 mM EDTA, 10% glycerol, 0.02% IGEPAL, 1 mM DTT, protease inhibitors, 30 mM glutathione, adjusted to pH 7) and incubating with mixing at 4°C for 45 min, followed by a second elution step. Combined elutions were 0.45 μm-filtered to remove any remaining resin and analyzed by Western blotting.

GRO-seq library preparation and Illumina sequencing.

Nuclear run-on and sequencing library preparation were performed essentially as described in Wang et al. (2011) for a total of three biological replicates.

HCT116 cells were plated at a concentration of 1 x 10^7 on 15 cm plates in McCoy's 5A medium and allowed to grow 24 hr before harvesting. Cells were washed three times with ice cold PBS and then treated with 10 ml per plate of ice-cold lysis buffer (10 mM Tris-Cl pH 7.4, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.5% IGEPAL, 10% glycerol, 1 mM DTT,

1x Protease Inhibitor Cocktail (Roche 11 836 153 001), 4U / ml SUPERase-In RNase inhibitor) and collected by scraping. Cells were then centrifuged at 1000x g for 7 min at 4ºC and re-suspended in 1.5 ml lysis buffer by pipetting 20-30 times. A further 8.5 ml of lysis buffer was added and the suspension centrifuged at 1000x g for 7 min at 4ºC to collect nuclei. Nuclei were washed in 1 ml lysis buffer and re-suspended in 500 µl freezing buffer (50 mM Tris pH 8.3, 40% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA, 4U / ml SUPERase-In), pelleted again (2000x g for 2 min at 4ºC), and finally re-suspended in 100 µl freezing buffer. To determine concentration, nuclei were counted and diluted with freezing buffer before 100 µl aliquots of 5 x 10^6 nuclei were snap-frozen in liquid Nitrogen and stored at -80ºC. Nuclear run-on was performed by adding 100 µl aliquots of nuclei to 100 µl reaction buffer (10 mM Tris pH 8.0, 5 mM $MgCl_2$, 1 mM DTT, 300 mM KCl, 20 units SUPERase-In, 1% Sarkosyl, 500 µM each of ATP, GTP, CTP and Bromo-UTP) and incubating for 5 min at 30 °C. Reactions were stopped by adding 1 ml Trizol to the reaction and vortexing to homogeneity. Samples were split in half and another 500 µl Trizol added to each half. To isolate RNA, 220 µl chloroform was added to each half sample and samples were centrifuged at 12,000x g for 15 min. The aqueous phase was transferred to a new tube and 22.5 µl 5M NaCl added. Samples were extracted twice with phenol-chloroform (pH 4.5), then once with chloroform. Finally, RNA was co-precipitated with 1 µl glyco-blue (Ambion) by adding three volumes of ice cold ethanol and incubating at -20°C for at least 20 min. Pellets were washed with 70% ethanol, air-dried 2 min and dissolved in 20 µl DEPC-treated water. RNA was base-hydrolyzed with 5 µl 1M NaOH on ice for 30 min (creating an average fragment size of ~150 bp). Reactions were neutralized with 25 µl 1M Tris-Cl pH6.8 and passed through a BioRad P-30 column as per the manufacturer's protocol. Samples were then DNAse treated in 1x RQ1 DNase buffer with 3 µl RQ1 DNaseI (Promega) at 37ºC for 10 min and again passed through BioRad P-30 columns. To remove 5' phosphates, 8.5 µl 10x phosphatase buffer, 5 µl of antarctic phosphatase (New England Biolabs) and 1 µl of SUPERase-In was added to each RNA sample followed by incubation for 1 hr at 37 °C. Samples were passed through a third BioRad P-30 column and the final volume of each RNA solution was brought up to 100 µl with DEPC-treated H_2O and 1 µl 500 mM EDTA. To prepare beads for immunopurification of Br-UTP-labeled nascent RNA, 60 µl anti-BrdU agarose beads (Santa Cruz Biotech) per sample were washed in 500 µl blocking buffer (0.5x SSPE, 1 mM EDTA, 0.05% Tween-20, 0.1% PVP, and 1 mg/ml BSA), incubated in 500 µl blocking buffer for 1 hr at 4 °C, washed twice for 5

min each in binding buffer (0.5x SSPE, 1 mM EDTA, 0.05% Tween-20) and finally re-suspended in 400 µl binding buffer. Total RNA samples (100 µl) were heated to 65 °C for 5 min, chilled on ice and then incubated with blocked anti-BrdU beads in 400 µl binding buffer for 1 hr at room temperature with rocking. Beads were washed (3 min with rocking) once in binding buffer, once in low salt buffer (0.2x SSPE, 1 mM EDTA, 0.05% Tween-20), once in high salt buffer (0.5% SSPE, 1 mM EDTA, 0.05% Tween-20, 150 mM NaCl) and twice in TET buffer (TE pH 7.4, 0.05% Tween-20). Nascent RNA was eluted four times with 125 µl elution buffer (5 mM Tris pH 7.5, 300 mM NaCl, 20 mM DTT, 1 mM EDTA, 0.1% SDS). Pooled RNA elutions were then extracted once with phenol/chloroform (pH 4.5), once with chloroform and precipitated by the addition of 1 µl glyco-blue, 300 mM NaCl, three volumes of ethanol at 20ºC for at least 20 min. The precipitated RNA was washed with 70% ethanol and re-suspended in 50 µl PNK reactions (45 µl DEPC-treated water, 5.2 µl T4 PNK buffer, 1 µl SUPERase-In, 1 µl T4 PNK, New England Biolabs), incubated at 37ºC for 1 hr and RNA was recovered as above. Poly-A tails were added to PNK-treated RNA fragments (in 5 µl water) by adding 0.8 µl poly-A polymerase buffer, 0.8 µl 10 mM ATP, 0.5 µl SUPERase-In and 0.75 µl poly-A polymerase (New England Biolabs) and incubating 10 min at 37ºC. Reactions were stopped by adding by adding 8 µl 500 mM EDTA, 12 µl 5M NaCl and 184 µl DEPC-water. The RNA was then recovered by phenol/chloroform extraction and ethanol precipitation and subjected to a second round of immunopurification with anti-BrdU bead binding and washes as above.

Reverse transcription was performed as follows: RNA fragments, in 8 µl water, were added to reactions with 1 µl dNTPs (10mM each), 2.5 µl 12.5uM oNTI223HIseq primer (5′-pGATCGTCGGACTGTAGAACTCT;CCTTGGCACCCGAGAATTCCATTTTTTTTTTTTTTTTTTTTVN-3’) where p indicates 5′ phosphorylation, ‘;’ indicates a furan modification that introduces a stable abasic site and VN indicates degenerate nucleotides. This mix was heated for 3 min at 75°C, chilled briefly on ice, followed by the addition of 0.5 µl SuperRase-In, 3.75 µl 0.1 M DTT, 2.5 µl 25 mM $MgCl_2$, 5 µl 5x reverse transcription buffer, and 2 µl Superscript III reverse transcriptase (Life Technologies) and incubation at 48°C for 30 min. Excess oNTI223HIseq primer was removed by adding 3.2 µl 10X Exonuclease I buffer and 4 µl Exonuclease I (Fermentas) and incubating at 37°C for 1 hr. RNA was removed by adding 1.8 µl 1 M NaOH and incubating for 20 min at 98°C. The reaction was then neutralized with 2 µl 1 M HCl and cDNA was recovered by

phenol/chloroform (pH 8) extraction and recovered by precipitation with 300 mM NaCl and three volumes of ethanol. cDNA was dissolved in 8 µl of water and added to 20 µl FLB (80% Formamide, 10 mM EDTA, 1 mg/ml Xylene Cyanol, 1 mg/ml Bromophenol Blue) and loaded on an 8% polyacrylamide TBE-urea gel. cDNA fragments of 200-650 nucleotides were excised and eluted from shattered gel slices by soaking overnight in 1x TE with 150 mM NaCl and 0.1% Tween-20. Eluates were passed through 0.22 µm spin X columns (Costar) at 10,000 rpm for 2 min at room temperature. Samples were snap frozen in liquid nitrogen and volume was reduced to <500 µl in a speed-vac and cDNA was recovered by phenol/chloroform extraction and ethanol precipitation as above. Circularization was carried out by dissolving cDNAs in 8 µl water with 1 µl CircLigase buffer, 0.5 µl mM ATP, 0.5 µl 50 mM $MnCl_2$ and 0.5 µl CircLigase (Epicentre) and incubating at 60ºC for 1 hr, followed by heat inactivation at 80ºC for 20 min. To each reaction, 3.8 µl of 4x re-linearization supplement (100 mM KCl, 2 mM DTT) and 1.5 µl ApeI (New England Biolabs) was added and the reactions incubated at 37°C for 1hr. Re-linearized cDNA fragments were recovered by ethanol precipitation as above. To amplify single-stranded DNA fragments with adapter sequences on each end, each sample was dissolved in 15 µl water and added to 10 µl 5x Phusion HF buffer, 0.5 µl 25 µM HighSeq fwd primer (5'-AATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGA-3'), 0.5 µl 25 µM HighSeq rev primer index 1 (5'-CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-3'), 2.5 µl 10 mM dNTPs, 1 µl Phusion DNA polymerase (Finnzymes) and 20 µl water. PCR amplification was performed as follows: initial denaturation at 98ºC for 3 min; 15 cycles of 98ºC for 80 sec, 60ºC for 90 sec, 72ºC for 30 sec; followed by a final extension at 65ºC for 10 min. PCR product was recovered by phenol/chloroform extraction and ethanol precipitation as above. The DNA was then run on a 6% native gel (1x TBE, 6% acrylamide-19:1). Products in the size range of 250-650 bp were excised and eluted from gel slices and recovered by precipitation as above. Samples were quantified using an Agilent Bioanalyzer and the three replicate GRO-seq libraries were sequenced to 50 bp in individual lanes on an Illumina HiSeq 2000 using standard procedures for cluster amplification and sequencing by synthesis.

GRO-seq data analysis and visualization.

Unless otherwise noted all data processing was carried out using custom Python scripts (Python version 2.6). The reads from three biological replicates were combined into a single file comprising 425,379,448 total reads which were mapped to the reference human genome (GRCh37/hg19) using Bowtie 0.12.7, allowing for at most 2 mismatches (Langmead et al., 2009). Using this strategy 309,772,394 (73%) of the reads mapped to the genome. The resulting SAM file was then processed by SAMtools (version 0.1.16) to create a sorted BAM file.

Genome mappability. To calculate the mappability of each position in the genome, FASTQ file with all possible 50-mers from both strands of the human genome was created from FASTA file of the human genome (GRCh37/hg19) obtained from UCSC. The reads were given perfect fastq scores and mapped to the human genome exactly as above for GRO-seq samples. BEDtools genomeCoverageBed (v2.12.0)(Quinlan and Hall, 2010) was used strand specifically to find all positions with no reads mapping uniquely to them. These positions were removed from all calculations described below as described in Core and Lis (2008).

Gene activity and pausing calculations. Calculation of gene activities and pausing indices were carried out as in Core and Lis (2008). Gene annotations (GRCh37/hg19) were downloaded from UCSC as a BED file and given unique names. The lengths of the promoter and gene regions were adjusted to account for mappability of the genome as calculated above. The number of reads in each gene body (from +1kb to the end of the annotation and excluding genes less than 3kb) and in promoter proximal regions (defined as 50 bp region within 2 kb of the annotated transcription start site containing highest read density after mappability correction) were counted by BEDtools genomeCoverageBed (v2.12.0). A script to calculate pausing indices (defined as promoter read density / gene body read density), gene activity (defined as the number of coding strand reads / number of mappable bases in the gene body region +1 kb to end), and promoter activity (defined as the number of reads in 50 bp window / number of mappable bases) was written and run in Python 2.6. Fisher's exact test was used to identified paused genes as those having significantly higher read density in their promoter proximal regions as compared to a uniform density of reads in the gene body ($p \leq 0.01$) and was carried out using the Python module fisher 0.1.4 downloaded from https://pypi.python.org/pypi/fisher/.

Creation of heatmaps and genome browser snaphots. Density profiles for heatmaps and metagenes in Figure 5 were generated using seqMINER 1.3.3 and a list of 'unique'

RefSeq gene regions generated by taking as the TSS the 5’ coordinate with the highest GROseq density and the 3’ coordinate that which gave the longest region in order to be as inclusive as possible without counting genomic regions more than once. The sorted BAM files mentioned above were used to make a Bedgraph by running genomeCoverageBed –bg on each strand separately (v2.12.0). The negative strand values were assigned negative values in the BedGraph. The BedGraph values were then divided by the number of million mapped (309,772,394) reads to give values of reads per million per bp (rpm/bp) for each position in the genome. The two files (strands) were concatenated back together and igvtools sort and igvtools tile was used to create a TDF file for visualization using the IGV browser (IGVtools 1.5.10, IGV version 2.0.34)(Thorvaldsdottir et al., 2012).

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. CDK8 and CDK19 are co-expressed across a variety of tissues and cell lines, related to Figure 1.

(A) Amino acid primary sequence alignment of CDK8 and CDK19. **(B)** Expression of CDK8 and CDK19 mRNA across a panel of normal human tissues relative to the adipose RNA pool and normalized to 18S rRNA. Values shown are means of three cDNA replicates with SEM. **(C)** Western blot showing detection of CDK8 and CDK19 protein across cell lines of different origins: H1299 (non-small cell lung carcinoma); HCT116 (colorectal carcinoma); IMR90 (primary lung fibroblast); A549 (lung adenocarcinoma); U2OS (osteosarcoma); HEK293 (embryonic kidney); HeLa (cervical carcinoma); SJSA (osteosarcoma); H226 (squamous cell carcinoma); BV173 (chronic myelogenous leukemia). **(D)** Western blot showing efficacy of CDK8 and CDK19 stable shRNA knockdown in HCT116 cells.

Figure S2. CDK8 knockdown and CDK8 ChIP-seq, related to Figure 2.

(A) Western blot showing levels of CDK8, HIF1A, HIF2A and nucleolin loading control, for control and shCDK8 #1 HCT116 cells in normoxia or after 24 h hypoxia. **(B)** Relative expression of hypoxia-inducible genes as measured by Q-RT-PCR for control and CDK8 knockdown (shCDK8 #1) HCT116 cells in normoxia to show the effect of shCDK8 on basal expression levels. Expression values were normalized to 18S rRNA and are expressed relative to the control normoxia value. Error bars represent standard error of the mean (SEM) from three independent biological replicates. **(C)** Western blot showing levels of CDK8, HIF1A and nucleolin loading control, for control and shCDK8 #2 HCT116 cells in normoxia or after 24 h hypoxia. **(D)** Relative expression of hypoxia-inducible genes as measured by Q-RT-PCR for control and CDK8 knockdown (shCDK8 #2) HCT116 cells in normoxia or after 24 h hypoxia (1% O_2). Expression values were normalized to 18S rRNA and are expressed relative to the control normoxia value. Error bars represent standard error of the mean (SEM) from three independent biological replicates. **(E)** Annotation of CDK8 ChIP-seq peaks by position relative to genomic features. **(F)** Box and whisker plots (5^{th}-95^{th} percentiles) of CDK8 ChIP-seq reads (reads per million mapped per bp) in the region $\pm$1 kb centered on transcription start sites for RefSeq genes (blue) and hypoxia-inducible genes (red) in normoxia and after 24 h hypoxia (1% O_2). Differences in the means are significant (two-tailed t test with Welch's correction) between RefSeq genes and hypoxia-inducible genes in normoxia ($p <$

0.0001), after hypoxia ($p < 0.0001$), and between normoxia and hypoxia for RefSeq genes ($p < 0.0001$). **(G-H)** Dot plots comparing CDK8 enrichment in normoxia (x axis) and hypoxia (y axis) at all unique RefSeq TSS and 3' end regions (blue), and at TSS and 3' end regions of hypoxia-inducible genes (red). The grey lines represent a 1:1 relationship between normoxia and hypoxia enrichment values (see Experimental Procedures for details). See also Table S2.

Figure S3. Expression of HIF1A target genes in shCDK8 cells, ChIP analysis of *STC2* in HCT116 cells and of *ANKRD37* in U2OS cells, related to Figure 3.
(A) Expression of HIF1A target genes *ANKRD37* and *STC2* as measured by Q-RT-PCR for indicated HCT116 cells in normoxia or after 24 h hypoxia (1% O_2). Expression values were normalized to 18S rRNA and are expressed relative to shControl normoxia. Error bars represent standard error of the mean (SEM) from three independent biological replicates. **(B)** Quantitative ChIP analysis of CDK8, total RNAPII, serine-5 and serine-2-phosphorylated RNAPII CTD (S5P, S2P), HIF1A, H3K9ac and H4ac histone acetylation, the CDK9 subunit of P-TEFb, the histone reader BRD4, the SEC subunit AFF4, the core mediator subunits MED1 and the TFIIH subunit CDK7 at the *STC2* locus in control and CDK8 knockdown (shCDK8 #1) HCT116 cells in normoxia or after 24 h hypoxia (1% O_2). Values are plotted as a percentage of the maximum signal for that locus. Error bars represent standard error of the mean (SEM) from three independent biological replicates. Grey area represents the transcribed region. **(C)** Expression of hypoxia-inducible genes *ANKRD37* and *ALDOC* in U2OS (osteosarcoma), SW480 (colorectal adenocarcinoma), DLD-1 (colorectal adenocarcinoma) and A549 (lung adenocarcinoma) cells as measured by Q-RT-PCR for control and CDK8 knockdown (shCDK8 #1) in normoxia or after 24 h hypoxia (1% O_2). Expression values were normalized to 18S rRNA and are expressed relative to the control normoxia value. Error bars represent standard error of the mean (SEM) from three independent biological replicates. **(D)** Western blot showing levels of CDK8, HIF1A, and nucleolin loading control, for control and shCDK8 #1 U2OS cells in normoxia or after 24 h hypoxia. **(E)** Quantitative ChIP analysis of CDK8, total RNAPII, serine-5 and serine-2-phosphorylated RNAPII CTD (S5P, S2P), HIF1A, H3K9ac and H4ac histone acetylation and the CDK9 subunit of P-TEFb in control and CDK8 knockdown (shCDK8 #1) U2OS cells in normoxia or after 24 h hypoxia (1% O_2). Values are plotted as a percentage of the maximum signal for that

locus. Error bars represent standard error of the mean (SEM) from three independent biological replicates. Grey area represents the transcribed region.

Figure S4. Expression and ChIP analysis of *CXCR4*, *MAGI1* and *UHMK1*, related to Figure 4.

(A) Relative expression of *CXCR4*, *MAGI1* and *UHKM1* as measured by Q-RT-PCR for control and CDK8 knockdown (shCDK8) HCT116 cells in normoxia or after 24 h hypoxia (1% O_2). Expression values were normalized to 18S rRNA and are expressed relative to the control normoxia value. Error bars represent standard error of the mean (SEM) from three independent biological replicates. **(B)** Quantitative ChIP analysis of CDK8, total RNAPII, serine-5 and serine-2-phosphorylated RNAPII CTD (S5P, S2P), CDK9, and MED1 and CDK9 at the *CXCR4*, *MAGI1* and *UHKM1* loci for control and CDK8 knockdown (shCDK8) HCT116 cells in normoxia or after 24 h hypoxia (1% O_2).

Table S1. Microarray data, related to Figure 1 and Figure S1.

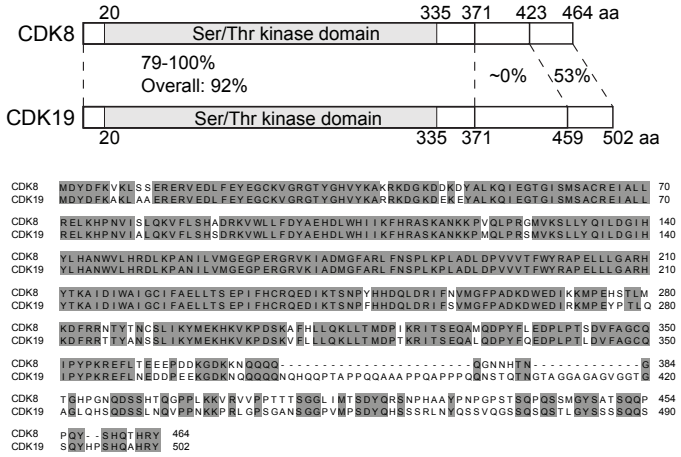
Table S2. CDK8 ChIP-seq data, related to Figure 2 and Figure S2.

Table S3. GRO-seq data, related to Figure 5.

Supplemental Figure 1

Figure S1

A



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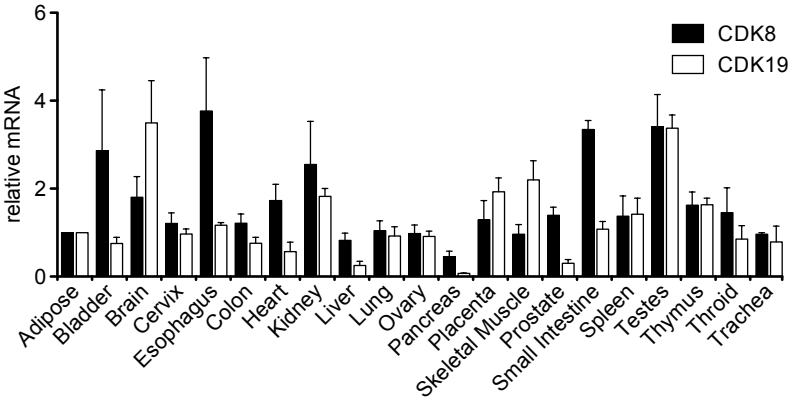

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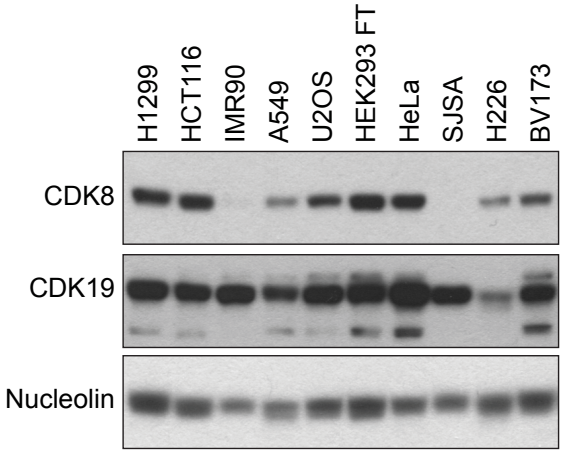

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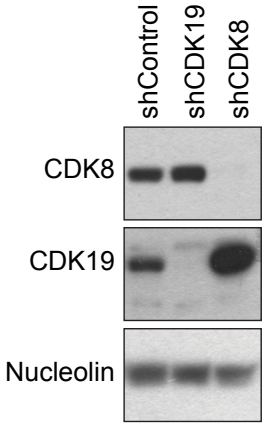

Supplemental Figure 2
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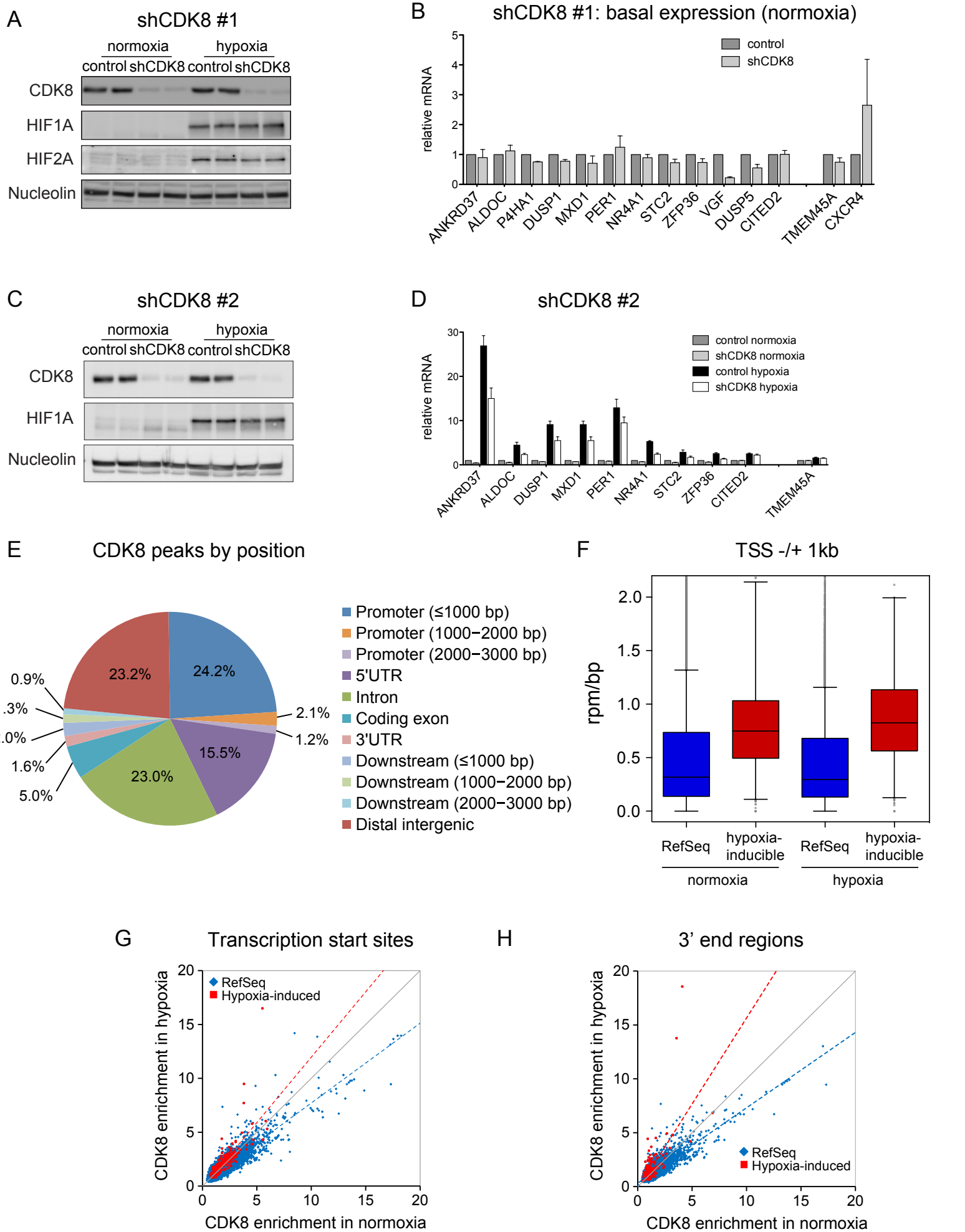
Figure S2



Supplemental Figure 3

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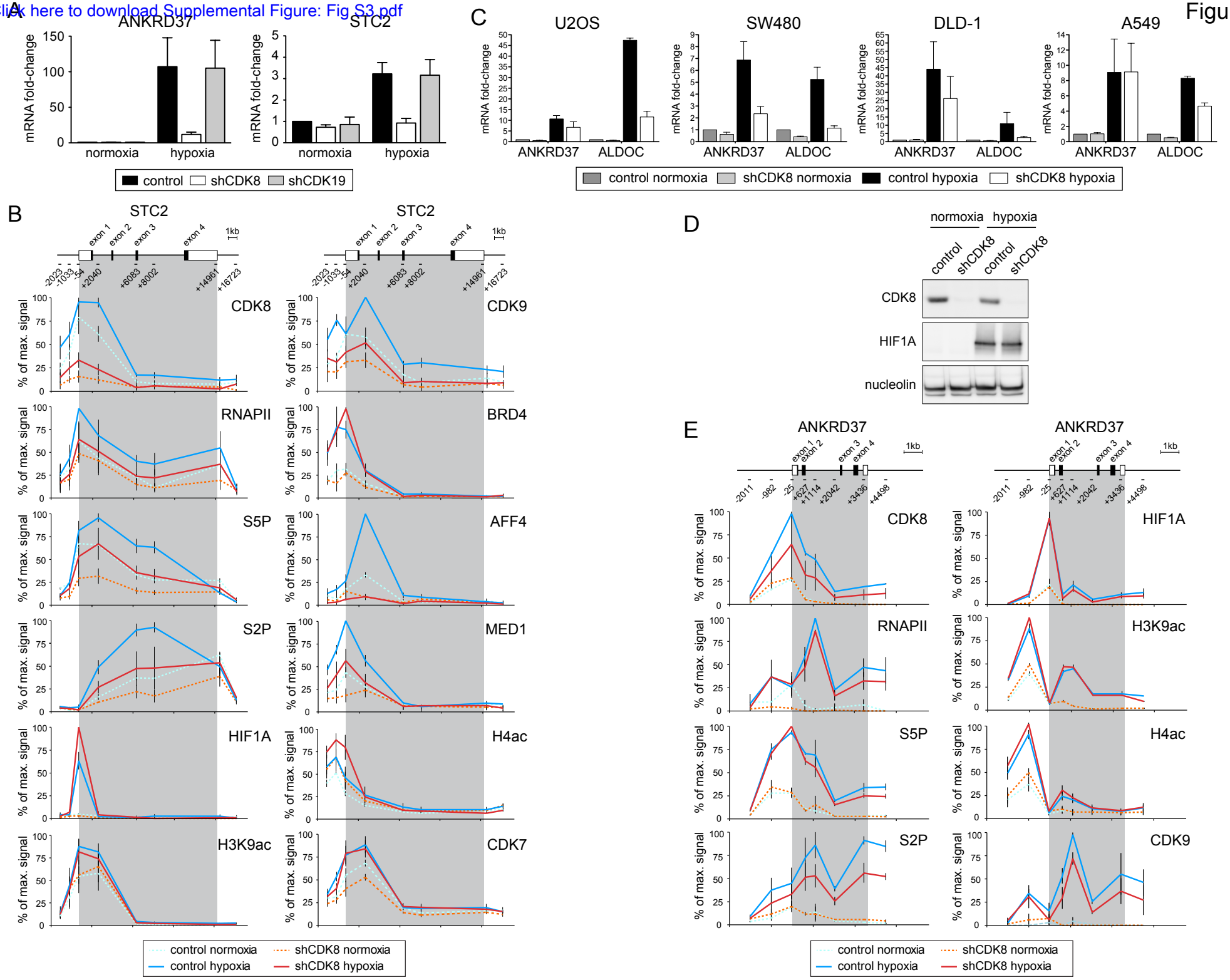
Figure S3



Supplemental Figure 4

Figure S4