Online Methods

Plasmid construction

Plasmid containing human CDK11 (GenBank Accesion number: AAC72077) was obtained from Dr. J. Lahti lab (Memphis, Tennessee). CDK11 cDNA was sub-cloned into HindIII and XhoI restriction sites of doxycycline inducible pcDNA5/FRT/TO plasmid (Thermo Fisher Scientific) in frame with 5'flag or 5'myc tag. CDK11 deletion mutants were prepared using QuikChange II XL Site-Directed Mutagenesis Kit (Agilent, #200522) according to the manufacturer´s protocol. Primers for the mutagenesis were designed to flank the region to be deleted.

Complete list of plasmids used in the study is in the **Supplementary Table 8**

Cell Culture

Human colorectal HCT116 Flp-in cell line (gift from Dr. B. G. Wouters, Toronto, Canada), [61] 293 Flp-in cell line (Thermo Fisher Scientific, R75007) and HCT116 cell line were all maintained in DMEM/high glucose media supplemented with L-glutamine, sodium pyruvate and 5% FBS at 37°C and 5% CO_2. Cell lines were not tested for mycoplasma.

To prepare cell lines stably expressing CDK11 proteins, 293 Flp-in or HCT116 Flp-in cells were transfected with corresponding CDK11 plasmids. Resistant colonies were selected with 100 µg/ml (293 Flp-in) or 175 µg/ml (HCT116 Flp-in) of hygromycin (Thermo Fisher Scientific, 10687-010) and two weeks later individual clones were expanded. Expression of transgene was induced with 1 µg/ml of doxycycline (Sigma, D3072) and verified by western blotting. Transgenic CDK11 protein expression levels were always lower or equal when compared to the endogenous CDK11 expression.

Complete list of cell lines used in the study is in the **Supplementary Table 8**.

Cell synchronization

HCT116 cells were synchronized with a double thymidine block method. Briefly, cells were blocked with 2 mM thymidine (Sigma, T1895) for 16 h, washed twice with PBS, released into fresh media for 8 h and blocked again with 2 mM thymidine for 16 h. Finally, cells were released into fresh media. G_1/S, S and G_2/M cells were collected at 0 h, 2 h and 8 h after the release from the second thymidine block, respectively. Progression into the different cell cycle phases was verified by flow cytometry (propidium iodide staining). Briefly, cells were trypsinized, washed twice with PBS, fixed with 70% ethanol and stored at -20°C. At the day of measurement the cells were washed twice with PBS, resuspended in Vindal buffer (10 mM Tris-Cl, pH 8, 1 mM NaCl, 0.1% TritonX-100), treated with RNase A (200 µg/ml) and stained with propidium iodide (50 µg/ml) at 37°C for 30 min. Cell cycle profiles (propidium iodide area versus count) were measured with BD FACSVerse and analysed with BD FACSuite software.

siRNA-mediated knockdown

Cells were usually plated at 30% confluency 6-10 h before transfection. Cells were transfected with siRNA at a final concentration of 10 nM (20 nM for FLASH) using Lipofectamine RNAiMax (Thermo Fisher Scientific, 13778-150) according to the manufacturer´s instructions. Briefly, to transfect one well in 6-well plate, we mixed 2.5 µl of siRNA (10 µM stock solution) diluted into 250 µl of Opti-MEM (Thermo Fisher Scientific, 31985-070) together with 5 µl of Lipofectamine diluted into 250 µl of Opti-MEM. After 15 min the mixture was added dropwise into the cultured cells with 2 ml of media. If larger plates were used for transfections, the amount of reagents was scaled up proportionally. Control samples were transfected with non-targeting control siRNA-A (Santa Cruz, sc-37007). The level of protein depletion was always verified by western blotting with appropriate antibodies.

The list of siRNAs used in this study is specified in **Supplementary Table 9.**

Reverse transcription qPCR (RT-qPCR)

RNA was isolated with Tri-Reagent (MRC, #TR118) according to the manufacturer´s instructions. RNA analysed by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) was first treated with 1 µl of DNase (Sigma, AMPD1). Usually 1µg of total RNA was then reverse transcribed using 200 U SuperScript II RT (Thermo Fisher Scientific, 18064-014), 1 µl of either random hexamers (IDTDNA, 50 µM stock) or 5'Phos oligo$(dT)_{20}$ (IDTDNA, 50 µM stock). Resulting cDNA was further diluted with water (40x) and 5 µl of diluted cDNA served as a template for each qPCR reaction using SYBR Green JumpStart TaqReadyMix (Sigma, S4438) with the following parameters: 95°C for 2 min followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. All primer sequences used in this study are specified in **Supplementary Table 10**. The untreated sample (or siRNA-A treated sample) served as a reference and the PPIA mRNA was used for normalization of cDNA synthesis unless stated otherwise in the text. qPCR were performed in triplicate for each biological replicate and error bars represent standard error of the mean of three biological replicates (unless stated otherwise in the text).

CDK11 dimerization assay

293 cells were co-transfected by PEI transfection reagent with 7 µg of M-CDK11 plasmids and 7 µg of plasmid expressing indicated F-CDK11 proteins. Media was changed 3 h after transfection. Cells were harvested 48 h after transfection, washed twice with PBS and lysed in HEPES buffer (20 mM HEPES-KOH, pH 7.9, 150 mM KCl, 0.2% NP-40, 15% glycerol, 1 mM DTT and protease inhibitors, Sigma, P8340). Equal amounts of clarified extracts (10,000g for 10 min) were incubated with 15 µl of packed flag agarose M2 affinity beads (Sigma, A2220) rotating for 2 h at 4°C. Samples were then washed three-times with HEPES buffer (rotating for 5 min at 4°C during each wash). After the last wash the remaining buffer was carefully removed and immunocomplexes were eluted by boiling in 50 µl of 3xLaemmli

sample buffer. Following SDS-PAGE, immunoblotting was performed using FLAG (Sigma, F3165), MYC (Sigma, M4439) and FUS (Santa Cruz, sc47711) antibodies.

iCLIP-seq

iCLIP was performed as previously described [62] with only minor modifications described below. Briefly, F-CDK11 293 Flp-in cells as well as F-CDK11 (226-783) 293 Flp-in cells were plated onto 150 cm^2 plates to reach 75% confluency at the day of crosslinking. CDK11 was induced with 1 µg/ml of doxycycline 24 h before crosslinking. We used two methods to crosslink RNA and proteins: either by UV-C (254 nm, 200 mJ/cm^2) or by UV-A (365 nm, 200 mJ/cm^2) after 100 µM final concentration of 4-thiouridine (Sigma, T4509) was added to the cells 6-8 h prior to the crosslinking. Cells from one 150 cm^2 plate were used per one immunoprecipitation and two technical replicates were performed for each condition, both of them were mixed together after reverse transcription step. Composition of all buffers was the same as described in [62]. Each cell pellet (originally from one 150 cm^2 plate) was lysed in 1 ml of lysis buffer and the lysate was homogenized by passing three-times through an insulin syringe (B.BROWN, Omnican U-100, 32G). Lysate was treated with 4 U/ml Turbo DNase (Thermo Fisher Scientific, AM2238), 12 U/ml RNase I (Thermo Fisher Scientific, AM2295) shaking at 1100 rpm and 37°C for 3 min. Clarified extracts (21000g for 30 min) were incubated for 2 h with 2 µg of flag antibody (Sigma, F1804) pre-bound to 50 µl of protein G Dynabeads. After series of stringent washes, adenylated L3 RNA adapter was ligated to the 3'end of crosslinked RNAs. Crosslinked protein-RNA complexes were resolved by SDS-PAGE (NuPAGE 4-12% Bis-Tris Protein Gel, Thermo Fisher Scientific, NP0322) and transferred to nitrocellulose membrane. The region of the membrane containing the radioactively labelled crosslinked protein–RNA complexes was excised, RNA was isolated and reverse transcribed to cDNA (technical replicates were mixed together after this step). cDNA was size-selected using urea denaturing gel electrophoresis and three fractions running

between 70-85 nt (L-low), 85-120 nt (M-medium) and 120-200 nt (H-high) were isolated. Each fraction was independently circularized by single-stranded DNA ligase, annealed to an oligonucleotide complementary to the restriction site and cut between the two adapter regions by BamHI. After final PCR amplification using P3 and P5 Solexa primers all three fractions were pooled together in ratio 1:5:5 (L:M:H). Multiplexed libraries were sequenced as 50bp single-end reads on Illumina sequencer (EMBL, Heidelberg).

Chromatin immunoprecipitation (ChIP-qPCR)

ChIP was performed with antibodies indicated in the **Supplementary Table 11**. Briefly, 20 µl of protein G Dynabeads (Thermo Fisher Scientific, 10009D) per one immunoprecipitation were pre-blocked with 0.2 mg/ml BSA (Thermo Fisher Scientific, AM2616) and 0.2 mg/ml salmon sperm DNA (Thermo Fisher Scientific, 15632-011) for 4 h, washed 3 times with RIPA buffer (50 mM Tris-Cl, pH 8, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, supplemented with protease inhibitors, Sigma, P8340), followed by the incubation with specific antibody for at least 4 h at 4°C. HCT116 cells were plated onto 150cm^2 plates to reach 75% confluency at the day of experiment. Cells were crosslinked with 1% formaldehyde for 10 min, reaction was quenched with glycine (final concentration 125 mM) for 5 min. Cells were washed twice with ice-cold PBS, scraped, and pelleted. Each 20 µl packed cell pellet was lysed in 650 µl of RIPA buffer and sonicated 20 x 7s (amp 0.85) using 5/64 probe (QSonica Q55A). Clarified extracts (13,000g for 10 min) were precleared with protein G Dynabeads (Thermo Fisher Scientific, 10009D) rotating for 2-4 h at 4°C and then incubated overnight with antibody pre-bound to protein G Dynabeads. We used 600 µl of clarified extract to immunoprecipitate CDK11, CPSF100, FLASH and P-Thr4 and 300 µl of clarified extract for RNAPII, P-Ser5 and P-Ser2. 10% of clarified extract was saved and used as input DNA. Next day beads were washed sequentially with low salt buffer (20 mM Tris-Cl, pH 8, 150 mM NaCl, 2 mM EDTA, 1% TritonX-100, 0.1% SDS), high salt buffer

(20 mM Tris-Cl, pH 8, 500 mM NaCl, 2 mM EDTA, 1% TritonX-100, 0.1% SDS), LiCl buffer (20 mM Tris-Cl, pH 8, 250 mM LiCl, 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate) and twice with TE buffer (10 mM Tris-Cl, pH 8, 1 mM EDTA). Bound complexes were eluted with 500 µl of elution buffer (1% SDS and 0.1 M $NaHCO_3$). To reverse formaldehyde crosslinks both immunoprecipitated and input DNAs were incubated at 65°C for at least 4 h and subsequently treated with proteinase K at 42°C for 2 h (10 µg/ml, Sigma P5568) with 2 µl of GlycoBlue added (Thermo Fisher Scientific, AM9516). After phenol:chloroform extraction (Sigma, P3803) both immunoprecipitated DNA and input DNAs were dissolved in 200 µl water and 5 µl of DNA served as a template for each qPCR reaction. Enrichment of specific gene sequences was measured by qPCR (Agilent AriaMx Real-time PCR System) using SYBR Green JumpStart TaqReadyMix (Sigma, S4438) with following parameters: 95°C for 2 min followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. ChIP enrichment of specific target was always determined based on amplification efficiency and Ct value, and calculated relative to the amount of input material. All primer sequences used in this study are specified in the **Supplementary Table 10**. qPCR was performed in triplicate for each biological replicate and error bars represent standard error of the mean of three biological replicates (unless stated otherwise in the text).

Chromatin immunoprecipitation coupled with sequencing (ChIP-seq)

The salmon sperm DNA blocking was omitted for all ChIP-seq experiments, otherwise the same protocol as for ChIP-qPCR was used. For one ChIP-seq experiment (CDK11, RNAPII, P-Ser2) we usually performed 3 technical replicates, dissolved each replicate in 17 µl of water and pooled them together to get at least 2.5 ng of immunoprecipitated DNA before library preparation (measured by Qubit). ChIP-seq libraries were generated using the KAPA Biosystems Hyper Prep Kit (KK8502) and NEBNext Multiplex Oligos for Illumina (Index

Primers Set 1 and Set 2 (NEB E7335S, E7500S). Libraries were sequenced (50-bp single-end reads) using an Illumina HiSeq 2500 (VBCF Vienna).

RNA-seq

siRNA mediated-CDK11 knockdown (Sigma, SASI_WI_00000026) was performed in HCT116 cells growing in 6-well plate using Lipofectamine RNAiMax (Thermo Fisher Scientific, 13778-150). Cells from one well were washed twice with ice-cold PBS 36h post-transfection, scraped, pelleted at 500g for 3min and treated for 5 min with 150 µl of cytoplasmic lysis buffer (10 mM Tris-Cl, pH 8, 0.32 M Sucrose, 3 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.5% TritonX-100, supplemented with 40 U/ml RNase inhibitor, Roche, 3335402001). Cytoplasmic RNA present in the supernatant was removed by centrifugation (500g for 3 min). Nuclear pellet was treated again with 90 µl of cytoplasmic lysis buffer and supernatant was completely removed after centrifugation (500g for 3 min). Nuclear RNA was isolated from the remaining nuclear pellet using Tri-Reagent (MRC, #TR118). 500 ng of RNA was treated with 1µl of DNase (Sigma, AMPD1). Ribosomal RNA was depleted using NEBNext rRNA Depletion Kit (E6310S). Sequencing libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB, E7760) and sequenced on Illumina HiSeq 2500 (50bp single-end).

UV-RIP

UV crosslinking experiments were performed in HCT116 Flp-in cells stably expressing F-CDK11 constructs. One 150cm^2 plate at 75% confluency was used for each immunoprecipitation.F-CDK11 expression was induced with 1µg/ml of doxycycline 24 h before crosslinking with UV-C (254nm, 200 mJ/cm^2). Cell pellets were lysed in RIPA buffer (50 mM Tris-Cl, pH 8, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, supplemented with protease inhibitors, Sigma, P8340 and 40 U/ml RNase inhibitor, Roche, 3335402001). 850 µl of clarified extract (21,000g for 30 min) were

incubated for 2-5 h with antibodies (see **Supplementary Table 11**) pre-bound to 20 µl of protein G Dynabeads. 10% of clarified extract was saved and used as input RNA. Beads were then washed sequentially with low salt buffer (20 mM Tris-Cl, pH 8, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), high salt buffer (20 mM Tris-Cl, pH 8, 500 mM NaCl, 2 mM EDTA, 1% TritonX-100, 0.1% SDS), LiCl buffer (20 mM Tris-Cl, pH 8, 250 mM LiCl, 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate) and twice with TE buffer (10 mM Tris-Cl , pH 8, 1 mM EDTA). Bound complexes were eluted with 300 µl elution buffer (1% SDS and 0.1 M $NaHCO_3$). Immunoprecipitated and input RNAs were then purified by treatment with proteinase K at 42°C for 1 h (10 µg/ml, Sigma P5568), followed by phenol:chloroform extraction (RNA phenol, Sigma, 77619). Immunoprecipitated RNA as well as input RNA were dissolved in 8 µl of water and treated with 1µl of DNase (Sigma, AMPD1), reverse transcribed using 200 U of SuperScript III RT (Thermo Fisher Scientific, 18080-044) and 1 µl random hexamers (IDTDNA, 50 µM stock). cDNA was further diluted with water (20x) and 5 µl of diluted cDNA served as template for each qPCR reaction using SYBR Green JumpStart TaqReadyMix (Sigma, S4438) with the following parameters: 95°C for 2 min followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. Enrichment of specific target was always determined based on amplification efficiency and Ct value, and calculated relative to the amount of input material. qPCR was performed in triplicate for each biological replicate and error bars represent standard error of the mean of three biological replicates (unless stated otherwise in the text).

Chromatin association assay

HCT116 cells were plated onto 150 cm^2 plate to reach 75% confluency at the day of experiment. Cells were washed twice with ice-cold PBS, scraped and treated for 5 min with 500 µl of buffer I (10 mM Tris-Cl, pH 8, 0.32 M Sucrose, 3 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.5% TritonX-100, supplemented with protease inhibitors). Cytoplasmic fraction

(supernatant) was removed by centrifugation (500g for 3 min). Nuclei were washed with 1 ml of buffer I, and after the last wash with 1ml of buffer I without TritonX-100 the remaining buffer was carefully removed. Nuclear pellet was lysed in 500 µl of nuclear lysis buffer (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, protease inhibitors Sigma, P8340). Lysate was equally distributed into two tubes and samples were taken for western blot analysis (nuclear soluble 1). One tube was treated with 5 U of RNase A and 200 U of RNase T1 (Thermo Fisher Scientific, AM2286); the other tube was treated with the same volume of the RNase storage buffer (10 mM HEPES pH 7.5, 20 mM NaCl, 0.1% Triton X-100, 1 mM EDTA, 50% glycerol) for 30 min at RT. Nuclear soluble fractions were separated by centrifugation (1,700g for 5 min) and samples were taken for western blot analysis (nuclear soluble 2). Insoluble chromatin pellet was washed twice with 1 ml of nuclear lysis buffer followed by centrifugation (10,000g for 5 min). The remaining buffer was carefully removed (without disturbing the pellet), solubilized in 100 µl of Laemmli buffer, followed by sonication (10 x 1s pulses). Nuclear soluble and nuclear insoluble (chromatin) fractions were analysed by SDS-PAGE and immunoblotting using RNAPII (Santa Cruz, sc-899), CDK11 (rabbit or rat serum), CDK9 (Santa Cruz, sc-484), FUS (Santa Cruz, sc-47711), ACTIN (Sigma, A3853), FLASH (Abcam, ab8420), FLAG (Sigma, F3165) and histone H3 (Abcam, ab1791) or histone H2A (Abcam, ab18255) antibodies.

***IVKA* with phospho-specific antibodies**

HCT116 cells (50% confluency) grown in DMEM (Sigma, D6429) were transfected on 150cm^2 plate using 48 µg of polyethylenimine (Polyscience, 24765) and 12 µg of expression vectors containing flag-tagged (F) F-EV, F-CDK9, F-CDK11, F-CDK11KD or F-CDK12. F-CDK11 and F-CDK11KD were co-transfected with 12 µg of Xpress-tagged-Cyclin L1 (X-CycL1). F-CDK12 was co-transfected with 12 µg of Xpress-tagged-Cyclin K (X-CycK). Growing medium was replaced with the fresh one 3 hours after transfection. Cells were lysed

in 1 ml of lysis buffer (20 mM HEPES-KOH, pH 7.9, 15% glycerol, 0.2% Igepal CA-630, 300 mM KCl, 0.2 mM EDTA, 1 mM dithiothreitol, 1 µl/ml Protease Inhibitor Cocktail (Sigma)) 68 hours after transfection. Flag-tagged kinases were immunoprecipitated using 20 µl of Anti-FLAG M2 Affinity Gel (Sigma), washed 3 times with lysis buffer containing 500 mM KCl and once in 20 mM HEPES-KOH, pH 7.9, 15% glycerol, 150 mM KCl, 1 mM dithiothreitol. Kinases were eluted from beads using 40 µl of 20 mM HEPES-KOH, pH 7.9, 150 mM KCl, 1 mM dithiothreitol and 0.238 mg/ml 3xFLAG Peptide (Sigma, F4799). Eluates were stored on ice until use. Kinase reaction consisted of 12 µl of eluted kinase and 48 µl of kinase buffer (20 mM HEPES-KOH, pH 7.9, 2 mM dithiothreitol, 1.67 mM ATP and 400 ng of human GST-CTD per reaction). Reaction was run for 1 hour at 30°C with shaking at 300 rpm. Reaction was terminated by addition of 3× Laemmli buffer and heating to 95°C for 3 min. Samples were resolved on SDS-PAGE followed by western blotting to a nitrocellulose membrane. The membrane was blocked for 1 hour in 5% low-fat milk in PBS-0.05% Tween 20 (PBS-T) and incubated overnight at 4°C in 5% low-fat milk in PBS-T with phospho-specific antibodies – phospho-Ser2 (clone3E10, Active Motif, 61083) diluted 1:500 or phospho-Ser5 (clone 3E8, Active Motif, 61085) diluted 1:4000. Membranes were washed 3×10 min in PBS-T and incubated for 1 hour in 5% low-fat milk in PBS-T with goat anti-rat IgG-HRP (Santa Cruz, sc-2032) diluted 1:3000. Membranes were washed 3×10 min in PBS-T and visualized using Western Blotting Luminol Reagent (Santa Cruz, sc-2048) and UltraCruz Autoradiography film (Santa Cruz, sc-201696) or Amersham Hyperfilm ECL (GE Healthcare, 28906836).

Nuclear Run-On

Nuclear run-ons were performed by using combination of published protocols [63,64]. HCT116 cells (60% confluency) grown on 150cm^2 plate in DMEM (Sigma, D6429) were transfected with 12.5 nM CDK11 siRNA (Sigma, SASI_WI_00000026) using Lipofectamine RNAiMax

(Thermo Fisher Scientific, 13778-150). After 65 hours, cells were harvested on ice, washed twice in ice-cold PBS and pelleted by 400g/4°C/4 min centrifugations. Nuclei were isolated by two washes in 1 ml of 10 mM Tris, pH 8, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% Igepal CA-630 with 300g/4°C/4 min centrifugations (first wash was followed by 5min incubation on ice). Nuclei were resuspended in 40 µl of 50 mM Tris, pH 8, 0.1 mM EDTA, 5 mM $MgCl_2$, 40% glycerol and kept on ice until run-on reaction. Nuclear run-on reaction was carried out by the addition of 50 µl of 2× transcription reaction mix (20 mM Tris, pH 8, 5 mM $MgCl_2$, 300 mM KCl, 4 mM dithiothreitol, 1 mM ATP, 1 mM GTP, 1 mM UTP, 0.6 mM CTP (Roche, 11277057001), 0.4 mM biotin-11-CTP (Thermo Fisher, 19519016), 50 U RNaseOUT Inhibitor (Thermo Fisher, 100000840)) to resuspended nuclei. Reaction was stopped after 30 min at 30°C by addition of 150 µl of nuclease-free water and 750 µl of TriReagent LS (Sigma, T3934). RNA was isolated according to the manufacturer's protocol. Dynabeads MyOne Streptavidin C1 (Thermo Fisher, 65001) were washed once in 100 mM NaOH, 50 mM NaCl, twice in 100 mM NaCl and stored in binding buffer (10 mM Tris, pH 8, 300 mM NaCl, 0.1% Triton X-100) until use. Washed beads were incubated with 8 µg of isolated RNA and 1µl of RNase OUT Inhibitor (Thermo Fisher, 10777019) in binding buffer for 30 min at RT. Nascent RNA captured on beads was washed twice in 50 mM Tris, pH 8, 2 M NaCl, 0.5% Triton X-100, twice in binding buffer and once in 5 mM Tris, pH 8, 0.1% Triton X-100. All washing buffers contained 1 µl of RNase OUT Inhibitor per 5 ml. Beads were re-suspended in 300 µl of TriReagent (Sigma, T9424), vortexed, vortexed again after addition of 60 µl of chloroform and centrifuged 14000×g/4°C/4 min. Aqueous phase (180 µl) was transferred to new tube, 300 µl of TriReagent was added to leftover organic phase and extraction was repeated. Aqueous phases were pooled, mixed with 360 µl of chloroform and centrifuged 14,000g/4°C/4 min. Aqueous phase was transferred to the new tube, mixed with 1 µl of GlycoBlue (Thermo Fisher, AM9516) and 3 volumes of 99.8% ethanol, and after 10

min at RT centrifuged 14,000g/4°C/20 min. The pellet was washed with 75% ethanol, centrifuged 14,000g/4°C/5 min, dried and dissolved in 5 µl of nuclease-free water (Sigma, W4502). DNase (Sigma, AMPD1) treatment was performed according to manufacturer's protocol. 2 µl of RNA sample were used for synthesis of cDNA for RT-qPCR. Another 2 µl were processed as no-reverse-transcriptase control. Superscript II (Thermo Fisher, 18064014) was used according to the manufacturer's protocol for random primers to synthetize cDNA (RNaseOUT inhibitor was added according to optional step of the protocol). Resulting cDNA was 10× diluted and 5 µl were added to qPCR reaction consisting of 0.11 µl of 10 µM forward primer, 0.11 µl of 10 µM reverse primer, 0.28 µl of MilliQ water and 5.5 µl of 2× SYBR Green JumpStart Taq ReadyMix (Sigma, S4438). Measurement was performed on an Aria Mx instrument (Agilent) using following set-up: 94°C for 2 min followed by 45 cycles of 94°C/15 sec, 55°C/30 sec, 72°C/30 sec. At the end of the experiment, melting temperatures of PCR products were measured from 90°C to 55°C and back to 90°C in 0.5°C intervals. Data were analysed by $2^{-\Delta\Delta Cq}$ method [65]. *PPIA* gene was used as a normalizer and data were presented as fold change over untreated control. qPCR was performed in triplicate for each biological replicate and error bars represent standard deviation of three biological replicates.

Primers used in the nuclear-run on are in the **Supplementary Table 10**

RNase Protection Assay

Genomic DNA from 21cm^2 50% confluent dish of HCT116 cells was isolated by Quick-gDNA MicroPrep kit (Zymo Gene, D3020). Template for anti-sense HIST1H1C RNA probe synthesis [66] was created by PCR using 50 ng of genomic DNA, Q5 Hot Start High-Fidelity DNA Polymerase (NEB, M0493L) and primers listed in the **Supplementary Table 10**. PCR program was as follows: 98°C/30 sec denaturation followed by 35 cycles of 98°C/10 sec, 63°C/10 sec, 72°C/20 sec. Final extension 72°C/2 min. PCR product was purified on a 1.5%

agarose gel and extracted using QIAquick Gel Extraction Kit (Qiagen, 28706). Anti-sense RNA probe with 40% Us labelled with biotin was synthetized using MAXIscript T3 Transcription Kit (Thermo Fisher, AM1316) and biotin-16-UTP (Roche, 11388908910).

HCT116 cells in 21cm^2 dish and at 40% confluency were transfected by corresponding siRNA (see **Supplementary Table 9**) using Lipofectamine RNAiMax (Thermo Fisher Scientific, 13778-150) and were grown for another 65 h. Two 21cm^2 dishes were transfected for each condition. RNA was isolated with Trizol (Thermo Fisher, 15596026). RNase protection assay was performed using RPA III Ribonuclease Protection Assay Kit (Thermo Fisher, AM1415) according to the manufacturer's protocol. Briefly, 20 µg of isolated RNA was mixed with 4 µl of anti-sense probe and precipitated using ammonium acetate and ethanol. As a control 5 µg of yeast RNA from the kit was processed in the same way as other samples. RNA sample was re-suspended in 10 µl of hybridization buffer from the kit, heated to 95°C and kept at 42°C overnight in a PCR machine. Samples were then incubated 30 min at 37°C in an RNase Digestion Buffer from the kit mixed with RNase T1 in ratio 150:1. Reaction was stopped and precipitated using reagents from the kit and resulting RNA was dissolved in nuclease-free water (Sigma, W4502). Samples were resolved on a denaturing 6% polyacrylamide/7 M urea gel. Gel and Hybond-N+ hybridization membrane (Sigma, GERPN203B) were soaked in 0.5× Tris-Borate-EDTA buffer (TBE, Sigma, T4415) for 10 min before wet blotting procedure. Wet blotting was done in a western blot apparatus using 0.5× TBE and 400mA for 2 hour. After transfer, the membrane was briefly washed in 1× TBE and RNA was cross-linked to the membrane by 3 min exposure to 302-nm UV light from transilluminator. Membrane was washed twice for 5 min in 0.5% SDS in PBS, twice for 5 min in 0.5% SDS, 0.5% BSA in PBS and once for 30 min in 0.5% SDS, 0.5% BSA in PBS. Membrane was labelled with HRP by 30-min treatment in 0.5% SDS, 0.5% BSA in PBS containing 1:10000 diluted Pierce High Sensitivity Streptavidin-HRP (Thermo Fisher,

21130). The membrane was washed once for 15 min in 0.5% SDS, 0.5% BSA in PBS and three times for 15 min in 0.5% SDS in PBS. Finally, the membrane was briefly washed in PBS and visualized using Western Blotting Luminol Reagent (Santa Cruz, sc-2048) and UltraCruz Autoradiography film (Santa Cruz, sc-201696) or Amersham Hyperfilm ECL (GE Healthcare, 28906836).

***IVKA* using [γ- ^{32}P]ATP**

293 cells were seeded onto 150 cm^2 plates at 60% confluence and were transfected with 15 µg of expression vector containing F-CDK9, or co-transfected with 15 µg of DNA in total with 10 µg F-CDK11 + 5 µg of F-CycL1α and 10 µg of F-CDK11KD + 5 µg of F-CycL1α using 45 µl of PEI per transfection (pH = 7.0, 24765, Polysciences Inc). After 48 h, cells were lysed in lysis buffer (20mM HEPES-KOH (pH = 7.9), 15 % glycerol, 0.2 % NP-40, 300 mM KCl, 0.2 mM EDTA, 1 mM DTT, protease inhibitor cocktail (1 µl/ml, P8340, Sigma-Aldrich)), flag-tagged proteins were precipitated from cleared lysates with 20 µl of Flag agarose M2 affinity gel (A2220, Sigma-Aldrich). Beads with bound proteins were washed three times with high salt wash buffer (20mM HEPES-KOH, pH 7.9, 15% glycerol, 0.2% NP-40, 750 mM KCl, 0.2 mM EDTA, 1 mM DTT, protease inhibitor cocktail (1 µl/ml, Sigma-Aldrich)), once with detergent-free buffer (20mM HEPES-KOH, pH 7.9, 15 % glycerol, 150 mM KCl, 1 mM DTT) and 3xFlag tagged proteins were eluted with 45 µl of Flag elution buffer (20mM HEPES-KOH, pH 7.9, 150 mM KCl, 1 mM DTT, 3X FLAG Peptide (0.2 mg/ml, F4799, Sigma-Aldrich). Each kinase reaction (total volume 60 µl) contained 12 µl of eluted 3xFlag tagged kinase and 1 µg of substrate (human full length GST-CTD (gift from Stefl lab, CEITEC), FLASH A (amino acids 1-571), FLASH B (490-919), FLASH C (920-1489), FLASH D (1490-1982) or BSA (K41-001, GE Life Sciences)) in kinase buffer (20 mM HEPES-KOH, pH 7.9, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mM ATP, 0.5 µl [γ- ^{32}P]ATP (0.185 MBq (FP-301, Hartmann Analytic))). Kinase reactions were incubated for

1 h at 30°C. Reactions were stopped by adding 15 µl of 4x NuPAGE loading buffer (NP0007, Thermo Fisher Scientific) and boiling at 80°C for 5 minutes. Phosphoproteins were resolved by SDS-PAGE and detected by autoradiography. For plasmids and antibodies used see **Supplementary Table 8 and 11**, respectively.

Phosphatase treatment

HCT116 cells were synchronized by a double thymidine block. G_1/S and S cells were collected at 0 h and 2 h after the release from the second thymidine block, respectively. Cells were washed twice with ice-cold PBS, scraped and lysed in EDTA-free RIPA buffer (50 mM Tris-Cl, pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, supplemented with protease inhibitors, Sigma, P8340, and phosphatase inhibitor cocktail 3, Sigma P0044). During cell lysis extracts were sonicated (5 x 1s, amp 0.30, using 5/64 probe, QSonica Q55A). Protein extracts were clarified by centrifugation at 10,000g for 10 min, 4°C. 30 µl of each cell extract was mixed with either 10 µl of alkaline phosphatase (Thermo Fisher Scientific, EF0651) or 10 µl of RIPA buffer and incubated for 15 min at 37°C. Reactions were ended by mixing with 20 µl of Laemmli buffer and boiled for 2 min. Samples were resolved by SDS-PAGE on 5% acrylamide gels.

Immunoprecipitation

HCT116 cells were plated onto 150 cm2 plate to reach 70% confluency at the day of experiment. Cells were washed twice with ice-cold PBS, scraped and lysed in 1ml of triton containing lysis buffer (20 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% Triton X-100, supplemented with protease inhibitors, Sigma, P8340) for 20 min. During cell lysis extracts were sonicated (5 x 1s, amp 0.30, using 5/64 probe, QSonica Q55A). Protein extracts were clarified by centrifugation at 10,000g for 10 min, 4°C. When performing RNase treatment before FLASH immunoprecipitation, clarified extracts were equally distributed into two tubes, one was treated with 10 µl of RNase cocktail (Thermo Fisher Scientific, AM2286) for

30 min at RT. Extracts were than incubated with 1.5 µg of primary FLASH antibody, rotating for 3 h at 4°C. Subsequently, 15 µl of packed protein G Sepharose (GE Life Sciences, 17-0618-01) was washed three-times with lysis buffer, added to each immunoprecipitation and rotated for 1 h at 4°C. Samples were then washed three-times with lysis buffer (rotating for 5 min at 4°C during each wash). After the last wash all the remaining buffer was carefully removed and proteins were eluted using 25 µl of 3x Laemmli buffer and boiled for 2 min.

For immunoprecipitations with transfected F-FLASH plasmid or with stable F-CDK11 cell lines, protein G Dynabeads were used. 20 µl of the Dynabeads per one immunoprecipitation were washed three times with lysis buffer (20 mM Hepes, pH 7.4, 200 mM NaCl, 0.1% Triton X-100, supplemented with protease inhibitors, Sigma, P8340). Dynabeads were than incubated with specific antibody (FLASH Abcam, ab133899, Flag, Sigma,F1804) for at least 4 h at 4°C. Clarified cell extracts (10,000g for 10 min) were than rotated with antibody coated Dynabeads for 3 hours at 4°C and subsequently washed three times with lysis buffer. Proteins were eluted by adding 25 µl of 3x Laemmli buffer and boiled for 2 min.

GST pulldown experiment

The 6xHis-FLASH protein fragments (amino acids 1-571, 490-919, 920-1489, 1490-1982) were expressed in BL21 (DE3) pLysS Escherichia coli strain using pET28b 3':6xHis FLASH 1-419, pET28b 3':6xHis FLASH 490-919, pET28b 3':6xHis FLASH 920-1489, pET28b 3':6xHis FLASH 1490-1982, respectively. Cultures were grown in 150 ml of LB media (supplemented with 30 µg/ml kanamycin and 25 µg/ml chloramphenicol) at 37°C with vigorous shaking to an OD600 of 0.5. Cultures were induced for 16 hours at 25°C in the presence of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (Sigma, I5502). Cells were harvested by centrifugation. Extract of cells were prepared by sonication in 5 ml of lysis buffer (50 mM NaH_2PO_4, pH 8.0, 500 mM NaCl, 10 mM imidazole, 1% Triton X-100, 0.01% NP-40, 1 mM β-mercaptoethanol, 2 mM PMSF). Lysates were clarified by centrifugation,

and the resulting supernatant was incubated with 1 ml of Ni-NTA Agarose (Qiagen, 30210) for 1 hour at 4°C. The beads were washed with 10 ml of lysis buffer and 40 ml of wash buffer (50 mM NaH_2PO_4, pH 8.0, 500 mM NaCl, 35 mM imidazole, 10% glycerol, 1% Triton X-100, 0.01% NP-40, 1 mM β-mercaptoethanol). FLASH fragments were eluted with five fractions of 500 µl of elution buffer (50 mM NaH_2PO_4, pH 8.0, 500 mM NaCl, 250 mM imidazole, 10% glycerol, 1% Triton X-100, 0.01% NP-40, 1 mM β-mercaptoethanol). Fractions with FLASH fragments were pooled together and concentrated in an Amicon Ultra-4 Centrifugal Filter Unit (Sigma, UFC801024) with PBS. Samples were stored at -80°C until use.

500 µg of His-tagged FLASH fragments FLASH A (amino acids 1-571), FLASH B (490-919), FLASH C (920-1489), FLASH D (1490-1982) were each mixed with 500 µg of GST-tagged CDK11 B (SignalChem, 23-30G) in a buffer consisting of 25 mM Tris, pH 7.5, 10 % glycerol, 0.5 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.5 % NP-40. Samples were incubated on ice for 10 min and mixed with 17 µl of 3x washed Glutathione Sepharose 4 Fast Flow GST-tagged protein purification resin (GE Healthcare, 17513201). After 30-min incubation on ice with occasional mixing the samples were centrifuged 1000g/RT/30 s, supernatant was stored and resin was washed 3x in 1 ml of the buffer. Finally, 30 µl of 3x Laemmli sample buffer was added to resin and supernatant samples and the samples were boiled at 95°C for 3 min and analysed by western blotting. Anti-GST antibody (Santa Cruz, sc-138) and anti-His-tag antibody (Sigma, 70796-3) were used to detect proteins.

Statistics and Reproducibility

Representative experiments shown in the manuscript (western blots, cell cycle profiles) were performed at least as three biologically independent experiments, unless otherwise stated in the text. Statistical comparisons of ChIP-qPCR experiments (Fig 2e, Fig 4e, Fig 6d, ED2e)

and RT-qPCR experiments (ED1d, ED8b, ED8e) were assessed with two-tailed paired t-test. Statistical significance was set to $P \leq 0.05$.

Bioinformatics is available as a Supplementary Note

Reporting summary statement

Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All next-generation sequencing source and processed data is available at NCBI-GEO (accession number GSE118051). Source data for main Figures 2, 3, 4, 5 and 6 and Extended Data Figures 1, 2, 3, 4, 5, 6, 7 and 8 are available with the paper online as Source Data.

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Supplementary Note

Bioinformatics

RNA-Seq analysis

We aligned RNA-seq data to the human genome (ENSEMB v.59 GRCh37) with STAR aligner v.2.5.2a with parameters: '-winAnchorMultimapNmax 20 -outFilterMultimapNmax 20 -twopassMode Basic' [1]. Aligned reads were quantified at gene level using HTseq with parameters: '-q --stranded=reverse -i gene_id' [2]. RNA-seq differential expressed genes (-1<log2FoldChange>1, p.adj<0.05) were assess with DeSeq2, discarding genes with less than one read [3].

ChIP-seq analysis

ChIP-seq data were aligned to human genome (ENSEMB v.59 GRCh37) using STAR v.2.5.2a defaults parameters and '--alignIntronMax 1 --alignEndsType EndToEnd' [1]. Pooled bam files, to create a single data set for each sample condition (Samtools v.0.1.19; [4]), submitted to MACS2 significant peaks, comparing no antibody input and corresponding samples using the function 'callpeak -g hs -B -q 0.05 --fe-cutoff 1.5 --broad --keep-dup all' [5]. Differential peaks comparing the control and knockdown mutant were called with MACS2 parameters: 'bdgdiff --max-gap 100 --min-len 120', normalising using sequencing deep of each library [5].

iCLIP analysis

iCLIP reads were analysed with the iCount software (https://github.com/tomazc/iCount). Briefly, reads were aligned to the human genome (ENSEMB v.59 GRCh37) using Bowtie 0.12.7 with parameters: '-v 2 -m 2 -a --best -strata' [6]. Oligonucleotides used for reverse transcription included the Unique Molecular Identifiers (UMIs), which enable to distinguish and remove the PCR duplicates [7]. Then, the genomic nucleotide preceding the mapped reads was assigned as the crosslink site. Significant crosslink sites were then

identified based on false discovery rate (FDR) < 0.05 comparing specific sites with randomised data (100 permutations) by using the iCount function '-random_perm 100 -flank 15 -fdr_th 0.05' [8].

Summary of cDNA counts within genes and genic regions were generated with iCount segments function, normalising the counts by the length of the corresponding region. The four replicate F-CDK11 samples were merged for the analyses that are presented in supplemental tables, where genes are ranked by number of significant crosslinks and normalised by gene length (only considered densities > 0.01).

Coverage tracks and metagenes

Combined coverage tracks were generated using deeptools [9]. ChIP-seq was normalised by sequencing deep and mappable genome size (Reads Per Genomic Content, RPGC) and binned per nucleotide while RNA-seq was normalised by Reads Per Kilobase per Million mapped reads (RPKM) and binned per nucleotide. Representative genomic images were displayed with the biodalliance genome browser [10]. RDH genes contain many repetitive segments where two RDH genes share an identical sequence. The average length of mapped CDK11 iCLIP reads is 31 nucleotides (stddev=155), and therefore to ensure coverage of these repetitive segments, we used the mapping settings that allow up to two multimapped positions per read for all analyses of iCLIP data. This was not applied to the rest of the sequencing data sets. Density plots and metagene heatmaps were drawn using RPGC coverages between the RDH transcriptional start site (TSS) and the stem-loop (Rfam RF0032 May-2017) using 50nt bins [9]. For the remaining genes, transcriptional termination sites (TES) were used. Read-thought analysis measure the RPKM reads per nucleotide that trespass the RDH stem-loop subtracting siCDK11 minus siCTRL RNA-seq pooled data until the next polyadenylation site. Canonical polyadenylation sites (PolyA_DB 3.2 hg19) were selected in base of the percentage of samples with detected expression and conservation in mammals

(Wang et al., 2018). RNA polymerase II CTD Ser2 phosphorylation ChIP-seq RPGC normalised tracks were calculated as the P-Ser2/RNAPII siCDK11 log2 fold ratio and binned in 10 nt [9]. CDK11 Ser2 phosphorylation normalised signal within RDH was computed between the start and end of MACS2 differential peaks per 50 nt ($p < 0.05$, supplemental table 4). Violin-plots were calculated as the RNAPII log2 RPGC siCDK11 fold change surrounding the TSS (250nt upstream and 750 downstream) and the stem-loop or TES (500nt).

Supplemental Note References

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