CDK11 is required for transcription of replication-dependent histone genes

Pavla Gajdušková[1,4], Igor Ruiz de Los Mozos[2,3,4], Michal Rájecký[1], Milan Hluchý[1], Jernej Ule[2,3], Dalibor Blazek[1,5]

[1]Central European Institute of Technology (CEITEC), Masaryk University, 62500 Brno, Czech Republic

[2]The Francis Crick Institute, Midland Rd, London, NW1 1AT, UK

[3]Department of Neuromuscular Disease, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, UK

[4]These authors contributed equally to this publication

[5]Corresponding author

Contact: Dalibor Blazek, PhD. E-mail: dalibor.blazek@ceitec.muni.cz

Abstract

Replication-dependent histones (RDH) are required for packaging of newly synthetized DNA into nucleosomes during S-phase when their expression is highly upregulated. However, the mechanisms of this upregulation in metazoan cells remain poorly understood. Using iCLIP and ChIP-seq, we found that human cyclin-dependent kinase 11 (CDK11) associates with RNA and chromatin of RDH genes primarily in the S-phase. Moreover, its N-terminal region binds FLASH, RDH-specific 3′end processing factor, which keeps the kinase on the chromatin. CDK11 phosphorylates serine 2 (Ser2) of the C-terminal domain (CTD) of RNA polymerase II (RNAPII), which is initiated at the middle of RDH genes and is required for further RNAPII elongation and 3′end processing. CDK11 depletion leads to decreased number of cells in S-phase, likely due to the function of CDK11 in RDH gene expression.

Thus, the reliance of RDH expression on CDK11 could explain why CDK11 is essential for growth of many cancers.

Introduction

Transcription of protein-coding genes is mediated by RNA polymerase II (RNAPII) in several stages including initiation, elongation and termination [1-3]. RNAPII contains an unstructured C-terminal domain (CTD) with a series of evolutionarily conserved heptapeptide (YSPTSPS) repeats, where the individual serines (Ser), threonine (Thr), and tyrosine (Tyr) can each be phosphorylated to regulate various RNAPII functions [4-6]. Several kinases phosphorylate serine in position 2 (P-Ser2) [6,7]. This modification promotes RNAPII elongation and is necessary for coupling transcription with co-transcriptional processes, such as 3′end processing [8-10].

Replication-dependent histone (RDH) proteins are required for packaging of newly synthetized DNA into nucleosomes before each cell division. Thus RDH genes have distinct regulation (and structure) from the rest of protein coding genes; they are expressed predominantly in S-phase and are short and intron-less. In humans there are approximately 80 genes localized in 2 genomic clusters. Their transcripts are the only cellular non-polyadenylated mRNAs, carrying instead a conserved stem loop (SL) at their 3′end [11]. Expression of RDH genes is highly regulated by specific transcription and processing factors, including FLASH and SLBP proteins [12]. The ongoing transcription is linked with cascade recruitment of mRNA processing factors that form a platform to position the histone cleavage complex (HCC) at the 3′end of the RDH genes [11]. The HCC cleaves the pre-mRNA 5 nucleotides after the SL, in a single processing step typical for the intron-less RDH transcripts [13]. Inefficient 3′end processing leads to transcriptional read-through and accumulation of small quantities of misprocessed and polyadenylated RDH transcripts (read-through RNAPII uses cryptic polyA sites) [13,14]. Notably, depletion of transcription elongation factors or slow elongation by mutant RNAPII results in production of small amounts of RDH

polyadenylated transcripts suggesting a link between transcriptional elongation and optimal 3´end processing [15-17].

As with other protein coding genes, the CTD of RNAPII participates in transcription and 3´end processing of RDH genes. Earlier studies suggested that CDK9-dependent P-Ser2 and P-Thr4 regulate RDH-specific 3´end processing without affecting their transcription [15,18,19]. However, genome-wide analyses of Thr4/Ala CTD mutants demonstrated that P-Thr4 is needed for the global regulation of transcriptional elongation independently of CDK9 [20]. Thus it remains unclear if or how any CTD-modifying enzyme (kinase) regulates RDH-specific transcription.

CDK11 (cyclin-dependent kinase 11) acts in complex with cyclins L1 and L2 (CYCL1 and CYCL2) [21] and is expressed as two protein isoforms, $CDK11^{p110}$ and $CDK11^{p58}$ [22]. $CDK11^{p58}$ is weakly expressed only in G2/M-phase of the cell cycle [23,24]. In contrast, abundantly and cell cycle-independently expressed $CDK11^{p110}$ differs from $CDK11^{p58}$ in the presence of 380 amino acid long N-terminal region which carries many charged amino acids and has an unknown function [25]. $CDK11^{p110}$ is ubiquitously expressed in all tissues and the $CDK11^{p110}$ null mouse is lethal at an early stage of development indicating an important role for $CDK11^{p110}$ in the adult as well as during development [26]. $CDK11^{p110}$ (from here on CDK11) is believed to play a role in RNAPII-directed transcription and co-transcriptional mRNA-processing [27-29]. However, its genome-wide function in regulating the human transcriptome is unknown. Notably, numerous recent studies identified CDK11 as a candidate essential gene for growth of several cancers [30-35]. Therefore, understanding the molecular mechanism(s) of CDK11-dependent gene expression would be of significant clinical interest.

In this study we find that CDK11 specifically regulates the expression of RDH genes. It binds to RDH RNAs and FLASH and associates with chromatin of RDH genes in a cell cycle-dependent manner. We further demonstrate that CDK11 can phosphorylate Ser2 in the

CTD of RNAPII positioned on the RDH genes to specifically control their transcriptional elongation and recruitment of 3´end processing factors.

Results

CDK11 binds chromatin of RDH genes and promotes their transcription.

To understand the role of CDK11 in human gene expression, we performed RNA-seq analyses from nuclear extracts of HCT116 cells treated with either control or CDK11 siRNA. CDK11 depletion resulted in down-regulation of 1131 genes (log_2FoldChange<-1, p-adj<0.01) (**Fig. 1a, Supplementary Table 1**), with enrichment in gene ontology (GO) terms for nucleosome and chromatin organization (**Extended Data Fig. 1a, b**), indicating a role for CDK11 in regulating histone gene expression. Strikingly, 93% of expressed RDH genes (**Fig. 1a**) were significantly downregulated (**Fig. 1b**), as confirmed by RT-qPCR for seven genes (**Extended Data Fig. 1c, d**). Nuclear run-on assays demonstrated a decrease in nascent mRNA of selected RDH genes in CDK11 knockdown cells (**Extended Data Fig. 1e**), indicating a transcriptional role of CDK11.

Next, we performed chromatin immunoprecipitation (ChIP-seq) to identify 393 peaks of CDK11 occupancy across the genome, which were enriched in GO terms for nucleosome and chromatin functions (**Extended Data Fig. 1f**). The peaks were present in 31 RDH genes, or 71% of expressed RDH genes (see **Extended Data Fig. 1g**), but not in the remaining down-regulated genes (**Fig. 1c, Supplementary Table 2**). We confirmed specificity of signal with ChIP-qPCR on selected RDH genes from cells treated with control and CDK11 siRNAs (**Extended Data Fig. 1h**). These results indicate that CDK11 is recruited to the chromatin of RDH genes to participate in their transcription.

FLASH recruits CDK11 to the RDH genes.

To understand how CDK11 is specifically recruited to the chromatin of RDH genes, we tested whether CDK11 interacts with any known RDH-specific factors. For instance, the serine/threonine-rich FLASH protein associates only with the chromatin of RDH genes to regulate RDH-specific transcription and 3´end processing [36,37]. Notably, immunoprecipitation of endogenous FLASH resulted in a specific pulldown of CDK11 protein but not of CDK12[38] (**Fig. 2a**). Reciprocal immunoprecipitation of endogenous CDK11 from a cell line expressing flag-tagged FLASH (F-FLASH) showed an interaction between the proteins (**Extended Data Fig. 2a**), and F-FLASH also immunoprecipitated endogenous CDK11 (**Extended Data Fig. 2b**), further confirming the result. To find whether interaction between FLASH and CDK11 is direct, we expressed his-tagged fragments of FLASH (**Fig. 2b**) in *E. coli* and performed GST pulldown assay with GST-CDK11 purified from insect cells. The N- and C-terminal fragments of FLASH showed strong binding to CDK11, indicating the direct interaction between both proteins (**Fig. 2c**). Moreover, we found a strong overlap between the CDK11 and FLASH[36] ChIP-seq occupancies solely on RDH genes (**Fig. 2d, Extended Data Fig. 2c**), indicating that the two proteins interact when present on chromatin. To test if CDK11 recruitment depends on its interaction with FLASH we depleted FLASH from cells and measured CDK11 occupancy on RDH genes by ChIP-qPCR. This resulted in lower recruitment of CDK11 to the RDH genes **(Fig. 2e)** without affecting CDK11 protein levels **(Extended Data Fig. 2d)**. Notably, the FLASH occupancy on the RDH genes was decreased comparably to the CDK11 occupancy **(Extended Data Fig. 2e)**. Altogether, these results show that interaction with FLASH is needed for CDK11 recruitment to the RDH genes.

CDK11 is recruited to RDH genes predominantly in S-phase.

Transcription of RDH genes occurs mostly in S-phase [39]. To understand if abundance and associations of FLASH and CDK11 with chromatin are cell cycle-dependent, we synchronized the cells by double thymidine treatment, which was confirmed by expression of

cell cycle markers (**Extended Data Fig. 3a**), RDH transcripts (**Extended Data Fig. 3b**) and by flow cytometry (**Extended Data Fig. 3c**). The abundance and phosphorylation of FLASH, as evident by its slower mobility on the gel (**Fig. 3a**), and its occupancy on RDH genes (**Fig. 3b**) were highest in S-phase. Strikingly, CDK11 was required for the phosphorylation of FLASH in S-phase (**Fig. 3a, c, d**). In agreement, F-CDK11 can *in vitro* phosphorylate the N-terminal and central fragments of FLASH protein purified from bacteria (**Fig. 3e**). Notably, long treatments with CDK11 siRNA led to a strong decrease of FLASH protein levels (**Extended Data Fig. 3d**), whereas depletion of FLASH did not affect CDK11 protein levels (**Extended Data Fig. 3e**). Protein levels of CDK11 did not change during the cell cycle (**Extended Data Fig. 3a, Fig. 3a**), but it is enriched on RDH transcripts and chromatin in S-phase, as evident through F-CDK11 IP followed by RT-qPCR and ChIP-seq, respectively (**Fig. 3f, g**). For example, change is seen in *HIST1H4E* and *HIST1H1C* RDH genes (**Extended Data Fig. 3f**), but not on the control non-canonical histone *H3F3A* mRNA or on the non-RDH down-regulated genes identified from the RNA-seq experiment (**Fig. 3f, g**). Cell cycle analyses of CDK11-depleted cells manifested decreased numbers of cells in S- and their accumulation in G1-phase (**Fig. 3h**). The phenotype can result from deficient expression of RDH genes [40]. Collectively, our findings demonstrate that interaction with FLASH ensures that CDK11 is recruited to RDH genes specifically in S-phase, CDK11 also phosphorylates and maintains protein levels of FLASH and depletion of CDK11 leads to accumulation of cells in G1-phase at the expense of S-phase.

RNA promotes CDK11 recruitment to the FLASH-containing RDH chromatin

The N-terminal region (corresponding to amino acids 1-220) of human CDK11 is highly conserved, suggesting an important biological function (**Extended Data Fig. 4a**). It is rich in arginines and lysines, reminiscent of the intrinsically disordered regions that are common in non-canonical RNA-binding proteins [41] (**Fig. 4a**). Moreover, CDK11 was identified as a

candidate RNA-binding protein in two proteome-wide screens [41,42]. To examine the potential role of RNA binding in CDK11 functions, we performed individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) (**Extended Data Fig. 4b**) [43] with an anti-Flag antibody from 293 cell lines stably expressing Flag-tagged CDK11 (F-CDK11), its N-terminal deletion mutant (F-CDK11$_{226-783}$), or empty plasmid vector (F-EV) (**Extended Data Fig. 4b, c**). Two biological replicates of libraries crosslinked with 4-thiouridine (4SU) + 365nm UV or 254nm were prepared from cells carrying F-CDK11 and F-CDK11$_{226-783}$, with no-antibody or no-UV as negative controls (**Extended Data Fig. 4d, Supplementary Table 3**). At least 5-15x more cDNAs were obtained from full length F-CDK11 compared to F-CDK11$_{226-783}$ (FDR<0.05) (**Supplementary Table 3**), indicating that RNA interaction is mediated primarily by the conserved N-terminal region. The unique cDNA counts of four replicates of F-CDK11 iCLIP revealed high correlation (R^2=0.64-0.84) (**Extended Data Fig. 4d**), and therefore we combined the replicates for further analyses. Largest proportion of binding was observed on noncoding RNAs (especially snRNAs) and 3' UTRs of mRNAs (**Extended Data Fig. 4e**).

We analysed the density of iCLIP significant crosslink sites (iCount, FDR < 0.05) in mRNAs, which identified 371 mRNAs with highest density (CLIP crosslink density > 0.01, **Supplementary Table 4**) enriched in GO terms for nucleosome and chromatin organization (**Extended Data Fig. 5a**). Notably, 30 RDH mRNAs were among the 100 most bound transcripts (**Supplementary Table 4**). A metaplot of summarised F-CDK11 crosslinking, and examples of *HIST1H3B* and *HIST1H1E* mRNAs, show that F-CDK11 binds primarily at the 3'ends of RDH genes, just upstream of the conserved SL sequence (**Fig. 4b, c, Extended Data Fig. 5b**). Binding was strongly diminished in the F-CDK11$_{226-783}$ mutant and absent in the uncrosslinked F-CDK11. In contrast, other abundant protein-coding mRNAs, including cell cycle-independent non-canonical histones, either do not bind CDK11 or have much

weaker binding as compared to RNA-seq (**Extended Data Fig. 5c, d, e**), excluding scenario of false positive binding of highly expressed genes, such as RDH genes, in iCLIP assays [44]. A comparison of CDK11 iCLIP to eCLIP data from ~200 proteins in the ENCODE database [44] indicates that CDK11 likely binds nascent mRNAs, suggesting it interacts with RDH transcripts during transcription (data not shown). The specificity of CDK11 enrichment on RDH transcripts and the importance of the N-terminal region was further validated with UV-RNA immunoprecipitation (UV-RIP) followed by RT-qPCR, using F-CDK11$_{226\text{-}783}$, CDK11 knockdown, mock IP (no Ab) and the non-canonical histone *H3F3A* mRNA as controls (**Extended Data Fig. 5f-i**). We constructed myc-tagged variants to demonstrate that the residual association of F-CDK11$_{226\text{-}783}$ with RDH mRNA (**Extended Data Fig. 5h**) most likely results from its interaction with full length CDK11 (**Extended Data Fig. 5j**). We could not identify any strongly enriched unique sequence motif at CDK11-binding sites (data not shown), a situation common for non-canonical RNA-binding proteins.

To understand if RNA binding contributes to the association of CDK11 with chromatin, we performed subnuclear fractionation [45], which was then either left untreated or incubated with RNases before further fractionating it into nucleoplasmic (soluble2) and chromatin (nuclear insoluble) fractions (**Extended Data Fig. 6a**). Optimal fractionation was verified by the presence of phosphorylated RNAPII and histone 3 (H3) only in the chromatin fractions (**Fig. 4d**). CDK11 was found in both nuclear soluble (1&2) and chromatin fractions (**Fig. 4d**), in agreement with a previous study [21]. Importantly, RNase treatment disengaged CDK11 from the chromatin fraction, in contrast to the proteins CDK9 and FUS, which were disengaged from the soluble2 fraction (**Fig. 4d**) [45]. In agreement with the reliance of the CDK11-chromatin interaction on RNA (**Fig. 4d**), the interaction between CDK11 and FLASH was partly RNase-sensitive (**Extended Data Fig. 6b**) even though chromatin association of FLASH was not dependent on RNA (**Extended Data Fig. 6c**). In concordance, CDK11 binds

224 FLASH via its N-terminal RNA-binding region **(Extended Data Fig. 6d)**. Moreover,
225 RNAPII transcription inhibition with either Amanitin or Triptolide led to considerable
226 dissociation of CDK11 from chromatin (**Fig. 4e**) without affecting CDK11 proteins levels
227 (**Extended Data Fig. 6e**). Thus, both RNA and active transcription are essential to bring
228 CDK11 to the FLASH-containing RDH chromatin.

229 **CDK11 promotes elongation of RDH genes.**

230 CDK11 can phosphorylate the CTD of RNAPII *in vitro* [46], and Ser2 in the CTD during HIV
231 transcription [47]. We used an *in vitro* kinase assay (*IVKA*) to verify that CDK11
232 phosphorylates GST-CTD, albeit its activity was weaker compared to the canonical and well
233 characterized CDK9 [48] (**Fig. 5a**). Next, we used P-Ser2 and P-Ser5 phospho-specific
234 antibodies to find that CDK11 phosphorylated both Ser2 and Ser5, whereas the negative
235 controls F-EV and CDK11 kinase dead (CDK11 KD) led to no phosphorylation (**Fig. 5b**).
236 CDK9 primarily phosphorylated Ser5, and CDK12 phosphorylated both Ser2 and Ser5,
237 which agrees with expectations (**Fig. 5b**) [49]. P-Ser2 is associated with elongating RNAPII [4]
238 and P-Ser2 ChIP-seq signal accumulates at the 3´ends of all genes (**Extended Data Fig. 7a**),
239 including RDH genes (**Fig. 5c**), resembling CDK11 iCLIP and ChIP-seq profiles on RDH
240 genes (**Fig. 4c, Extended Data Fig. 1g**, respectively). CDK11 knockdown led to a collapse
241 of the P-Ser2 signal on RDH genes (**Fig. 5c-e, Extended Data Fig. 7b, Supplementary**
242 **Table** 5), with a much lower (~30%) decrease on highly expressed genes, and little effect on
243 all other genes (**Extended Data Fig. 7a**). Notably, P-Ser2 ChIP-seq signal starts
244 accumulating close to the middle of RDH gene bodies (**Fig. 5c)**, which coincides with the
245 peak of CDK11 ChIP-seq signal (**Fig. 1c,** 5**d, f**), suggesting that a "transition" point in
246 transcriptional elongation is located approximately in the middle of RDH genes (**Fig. 5c**).
247 CDK11 depletion led to the decline in P-Ser2 occupancy (**Fig. 5c**) from this "transition" point
248 onward (**Fig. 5c, d, f**) and to the decrease of RNAPII occupancy specifically on RDH genes

(**Fig. 5g, Supplementary Table 6**). However, the decrease in total RNAPII levels was smaller than in P-Ser2 levels (**Fig. 5c-e, Extended Data Fig. 7b, c**) and no significant changes were seen in P-Ser5 or P-Thr4 signal on selected RDH genes (**Extended Data Fig. 7d-g**) [18]. This indicates that CDK11 is required primarily for the onset of Ser2 phosphorylation at the "transition" point in RDH genes, which is essential for their productive elongation (**Fig. 5c**).

CDK11 promotes 3´end processing of RDH genes.

Slow RNAPII elongation disrupts RDH mRNA processing [17] and Ser2-phosphorylated CTD serves as a binding platform for factors involved in 3´end formation and processing [10]. Indeed, following CDK11 depletion, the occupancy of CPSF100, a component of the HCC [13], (**Fig. 6a**) decreased on all tested RDH genes to a similar extent as P-Ser2, and more than RNAPII (**Fig. 6a-e**). We also observed an increase by a factor of 3-5 in read-through (uncleaved) RDH transcripts (**Fig. 6f, Extended Data Fig. 8a, b, Supplementary Table 7**). No increase in read-through transcript was seen in non-RDH bound genes (*MYC, MAZ*) and one RDH gene *HIST1H1C*, which contains a cryptic polyA signal immediately downstream of the SL [50] that becomes increasingly used upon CDK11 knockdown **(Extended Data Fig. 8b)**. This phenomenon of increased cryptic polyA use was additionally observed in *HIST1H2AC, HIST1H2BD* and *HIST1H4E* genes, and could be induced also by depletion or inhibition of CDK9 [15], CDK7 [51], ARS2 [52] and SLBP [53], which contribute to transcription and mRNA processing of RDH genes (**Extended Data Fig. 8c-g, Supplementary Table 7**). The small changes in read-through and/or use of cryptic polyadenylation sites are thus a likely result of defective 3´end processing upon CDK11 knockdown. We conclude that the CDK11-dependent phosphorylation of Ser2 is required for efficient elongation and 3' end processing of RDH genes.

Discussion

Our study reveals that CDK11 interacts with FLASH, a factor which has been known to be present only at RDH genes[36]. We show that FLASH promotes selective recruitment of CDK11 to RDH genes predominantly in the S-phase. CDK11 occupies coding regions of RDH genes; the binding is strongest at the "transition" point close to the middle of the RDH genes, where it coincides with accumulation of P-Ser2 (**Fig. 5c, d**), which in turn promotes efficient elongation and 3´end processing of RDH transcripts (summary of genome-wide data, working model and iCLIP- and ChIP-seq data on example RDH genes are presented in **Fig. 7a**, **b** and **Extended Data Fig. 9a-f**, respectively). CDK11 occupancy on RDH genes does not overlap with promoter-paused RNAPII **(Fig. 1c, 5c)** hence CDK11 likely does not mediate RNAPII transition to early elongation, the step regulated by CDK9 on most protein-coding genes[48]. CDK11 phosphorylates GST-CTD *in vitro*, but less efficiently than CDK9 or CDK12 (**Fig. 5a** and data not shown), indicating that it might phosphorylate just a subset of CTD repeats, which could ensure an RDH-specific function of CDK11 in RNAPII-mediated transcription. CDK11 also phosphorylates FLASH, perhaps regulating FLASH stability however the exact *in vivo* function(s) of the phosphorylation(s) remains to be determined. We find that the arginine-rich N-terminus of CDK11 contacts RNA, we used iCLIP to identify its binding to RDH transcripts, particularly strong at their 3´ends, and we show that such RNA binding helps to maintain CDK11 on chromatin (**Fig. 7a, b, Extended Data Fig. 9a-f**).

CDK11 homolog is absent from yeast *Saccharomyces cerevisiae*, while the CDK11 homolog in *Schizosaccharomyces pombe* contains only the kinase domain without the N-terminus [54]. CDK11 is an essential gene in metazoans [26], but not in *S. pombe* [54,55], possibly because yeast species transcribe RDH genes only in the S-phase, and all RDH mRNAs are polyadenylated [56]. Thus, the role of CDK11 in promoting S-phase specific RDH

transcriptional elongation and processing appears to have evolved only in metazoans. We conclude that the RNA binding capacity contributes to maintaining metazoan CDK11 close to chromatin, where it further achieves specificity for RDH genes through direct interactions with FLASH.

Downregulation of RDH mRNAs by knockdown of various RDH-specific transcription/3´end processing factors causes a disruption of cell cycle by accumulation of cells in either G1- or early S-phase (this study, **Fig. 3h** and [12,40,57]). Alternatively, the downregulation can be explained by the reduction of number of cells in S-phase (when RDH expression occurs). Although we cannot completely exclude a direct role of CDK11 in regulation of cell cycle progression, it´s binding to RDH chromatin and nascent transcripts and interaction with FLASH strongly suggests direct and specific function in RDH transcription. CDK11-specific inhibitor (when available) will allow to determine whether the kinase also directly regulates cell cycle progression. As P-CTD-specific antibodies have limitations in recognition of the specific epitopes [58], the inhibitor in combination with mass spectrometric analyses [59] can be also used for identification of any CDK11- or RDH-specific-P-CTD pattern.

Altogether, considering the fundamental role of RDH gene expression for cellular replication and proliferation, the mechanism identified in our study could underlie the essential role of CDK11 in many cancers [25,32,33], and could serve as a framework for developing CDK11 inhibitors with therapeutic potential. Indeed, when this paper was in revision, the first potent CDK11 inhibitor was reported, identified as the mischaracterized anticancer agent [60].

Acknowledgements

We thank all members of Blazek and Ule laboratories for discussions throughout the project and helpful comments on the manuscript. We also wish to thank Dr. Jasnovidova (Stefl lab) for the GST-CTD, Dr.Wouters for the HCT116 Flp-in cell line, Zuzana Slaba for preparation of His-FLASH constructs and proteins and Dr. Bartholomeeusen and Dr. Dettenhofer for comments on the manuscript. The work was supported by the following grants from: the Czech Science Foundation ("16-10930S"), the Masaryk University ("MUNI/E/0080/2017"), the CEITEC (Project 'CEITEC-Central-European Institute of Technology' [CZ.1.05/1.1.00/02.0068]) to D.B. and the European Research Council (617837-Translate) to J.U. The Francis Crick Institute receives its core funding from Cancer Research UK (FC001002), the UK Medical Research Council (FC001002), and the Wellcome Trust (FC001002).

Author contributions

P.G. performed most experiments except for radioactive *IVKA*, some experiments in cell cycle synchronized cells (M.H.), nuclear run-on, RNase protection assay, GST-pulldown, FLASH ChIP-qPCR and *IVKA* with P-specific antibodies (M.R.) and CDK11 and P-Ser5 ChIP-qPCR (D.B). I.R.dl.M. performed all bioinformatics analyses under supervision of J.U. and with some input from P.G. and D.B.. D.B. conceived the study, acquired funding and wrote the manuscript with support of P.G, I.R.dl.M. and J.U. All authors discussed the design of experiments, analysed the data and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Figure legends

Figure 1 CDK11 binds chromatin of RDH genes and promotes their transcription.

a, RNA-seq analysis of HCT116 cells following siRNA-mediated CDK11 knockdown. Down- and up-regulated genes ($-1>\log_2\text{FoldChange}>1$; p-adj<0.01) are shown in red and blue, respectively. Symbols of 41 down-regulated RDH and 5 most up-regulated genes are shown for n=3 biologically independent experiments.

b, RNA-seq metaplots (top) and heatmaps (bottom) of the RDH genes in control (siCTRL) and CDK11 (siCDK11) siRNA treated cells. TSS=transcription start site; SL=stem loop.

c, CDK11 ChIP-seq on RDH and 200 other down-regulated genes. CDK11 and input data are from n=4 and n=3 biologically independent experiments, respectively. TSS=transcription start site, SL/TES=stem loop/transcription end site.

Figure 2 FLASH recruits CDK11 to the RDH genes.

a, Western blot analyses of immunoprecipitates of endogenous FLASH from HCT116 cells. The blots were probed with the indicated antibodies.

b, Depiction of human FLASH protein and four his-tagged deletion mutants expressed in bacteria. Deletion mutants A and B have an overlapping region between amino acids 490-571.

c, Western blot analyses of *in vitro* binding assays of GST-CDK11 purified from insect cells and his-tagged FLASH (HIS-FLASH) deletion mutants expressed in bacteria and depicted in Figure 2b.

d, FLASH ChIP-seq in hTERT cells (GSE69149) [36] (left panel) in comparison to CDK11 ChIP-seq (middle panel) and no Ab input control (right panel) on 44 regulated (expressed) RDH (RDH with base mean expression>10), all RDH and 200 other downregulated genes.

e, Endogenous CDK11 ChIP-qPCR on indicated RDH genes or control intergenic region (Ir) in HCT116 cells treated either with control (CTRL) or FLASH siRNAs for 24 h. n=3 biologically independent experiments, error bars=SEM, *P<0.05, Student´s two-sided t-test. Source Data for graphs in panel e are available with the paper on line.

Figure 3 CDK11 is recruited to RDH genes predominantly in S-phase.

a, Western blot analyses of extracts of HCT116 cells released from double thymidine synchronization. Time points after the release and cell cycle phases are indicated. Cell cycle phase markers: CCNA2=cyclin A2, SLBP. A=asynchronous cells, 0 h=time of the release.

b, FLASH ChIP-qPCR on selected RDH genes in asynchronous and G1/S, S and G2/M synchronised HCT116 cells. FLASH ChIP-qPCR signals are normalised to the maximum signal which was set as 1. n=3 biologically independent experiments, error bars=SEM, Ir=intergenic region.

c, Western blot analyses of FLASH and phosphorylated FLASH (P-FLASH) in cell lysates of HCT116 cells treated with either control or CDK11 or FLASH siRNAs for 48 h.

d, Western blot analyses of lysates of HCT116 cells synchronized by double thymidine treatment in G1/S-phase and released 2 h into the S-phase. The lysates were treated or were not with alkaline phosphatase (AP). The phosphorylated and dephosphorylated forms of FLASH and control RNAPII and Ser2 are indicated at right by clip marks and arrows, respectively. The blots were probed with indicated antibodies. P-FLASH, P-RNAPII and F-CDK11 are phosphorylated FLASH, RNAPII and Flag-tagged CDK11, respectively.

e, *IVKA* visualized by autoradiography (upper panel). His-tagged deletion mutants of FLASH expressed in bacteria were incubated with purified CDK11 in the presence of [γ- ^{32}P] ATP. P-FLASH=phosphorylated FLASH. Western blotting of inputs of FLASH deletion mutants (lower panel).

f, Graph displays RNA immunoprecipitation (RIP) of histone transcripts with F-CDK11 from HCT116 cells synchronized in G1/S-, S- and G2/M-phases. Graph shows fold change of CDK11 binding to RDH mRNA normalized to *MAZ* mRNA binding. mRNA levels in G1/S were set as 1 for each transcript. n=3 biologically independent experiments, error bars=SEM.

g, CDK11 ChIP-seq on the RDH and 200 other down-regulated genes in either HCT116 cells asynchronous or synchronized in S- or G2/M-phases. For asynchronous and S or G2/M n=4 and 2 biologically independent experiments, respectively.

h, Histograms of cell cycle analyses of HCT116 cells transfected with control (CTRL) or CDK11 siRNA for 36 h. Percentage of cells in G0/G1-, S- and G2/M-phases are displayed.

Source data for panels b and f are available with the paper on line.

Figure 4 RNA promotes CDK11 recruitment to the RDH chromatin.

a, Schematic diagram highlighting the kinase domain and basic region of human CDK11 protein.

b, Metagene analyses of F-CDK11 and F-CDK11 (226-783) iCLIP binding at all RDH transcripts from the TSS to the SL. iCLIP data k-means clustered, based on RNA-seq expression (high, medium and low). n=4 biologically independent experiments.

c, Biodalliance genome browser view of F-CDK11, F-CDK11 (226-783) and uncrosslinked control (no UV) iCLIP binding at *HIST1H3B* transcript. Stem loop (SL) is indicated by a black line.

d, Western blot analysis of association of the indicated factors in soluble and insoluble fractions of chromatin either treated or not treated with RNase A/T1. Arrows mark phosphorylated (upper) and non-phosphorylated (lower) forms of RNAPII. For CDK11, long and short exposures of the film are shown.

e, CDK11 ChIP-qPCR on RDH genes in HCT116 cells expressing stably integrated F-CDK11 and treated either with Amanitin (4 μg/ml) or Triptolide (10 μM) or untreated

(CTRL). n=4 biologically independent experiments, error bars=SEM, *P<0.05, Student´s t-test, Ir=intergenic region. Source data for panel e are available with the paper on line.

Figure 5 CDK11 promotes transcriptional elongation of RDH genes.

a, GST-CTD or BSA was incubated with the indicated cyclins/CDKs in the presence of [γ-^{32}P] ATP, the resulting kinase reactions (*IVKA*) were resolved on SDS-PAGE gel and visualized by autoradiography. Phosphorylated GST-CTD (P-GST-CTD) and autophosphorylated CDK11 is shown (upper panel). Equal input of flag-tagged cyclins/CDKs and GST-CTD to the *IVKA* were confirmed by western blotting with anti-flag antibody (middle panel) or by Coomassie staining (lower panel), respectively.

b, Displayed cyclins/CDKs purified from HCT116 cells were incubated with GST-CTD in *IVKA*. Phosphorylation was monitored by the indicated antibodies by Western blotting (upper panel). Input of equal amounts of flag-tagged CDKs into *IVKA* was validated by flag antibody (lower panel). F=flag tag, X=xpress tag, KD=kinase dead mutant, end=endogenous, EV=empty vector.

c, ChIP-seq analyses of RNAPII and P-Ser2 occupancies on expressed RDH genes in HCT116 cells treated with either control (CTRL) or CDK11 siRNA. Transcription elongation "transition" point is indicated by dashed line. n=3 biologically independent experiments.

d, P-Ser2/RNAPII normalized ChIP-seq log2 fold change on RDH genes after CDK11 knockdown within differential P-Ser2 MACS2 peaks (depicted as P-Ser2 start (vertical dashed line) and P-Ser2 end).

e, *HIST1H4E* gene tracks with raw RNAPII and P-Ser2 ChIP-seq data and RNAPII, P-Ser2 and P-Ser2/RNAPII log2 fold change after CDK11 depletion. Black line indicates differential peaks identified by MACS2 program (p<0.05).

f, CDK11 ChIP-seq occupancy is most abundant just upstream of the differential P-Ser2 MACS2 peaks in RDH genes. The start of the P-Ser2 peaks is indicated by vertical dashed line (see also Fig. 5d for metaplot and heatmap).

g, Violin-plots measure RNAPII occupancy on the TSS (top panel, flank 500 nt) and SL or TES (bottom panel, 250 nt upstream and 750 nt downstream) of expressed RDH and 200 highly expressed and randomized genes.

Figure 6 Recruitment of 3´ end processing factor CPSF100 to the RDH genes depends on CDK11-mediated phosphorylation of Ser2.

a, b, c, Graphs present ChIP-qPCR data for CPSF100 (a), RNAPII (b) and P-Ser2 (c) in HCT116 cells transfected with control (siCTRL) or CDK11 (siCDK11) siRNA. qPCR primers were designed in coding regions of RDH genes. n=4, n=3 and n=3 biologically independent experiments for (a), (b) and (c), respectively; error bars=SEM, Ir = intergenic region.

d, e. Graphs present ratios of CPSF100/RNAPII (d) and CPSF100/P-Ser2 (e) ChIP-qPCR signals. n=4 and 3 biologically independent experiments for (d) and (e), respectively; error bars=SEM, *P<0.05, Student´s two-sided t-test.

f, Subtracted RNA-seq (siCDK11 - siCTRL) RPKM normalized downstream of the SL until the next conserved polyadenylation site (33 RDH genes; distance from 27 nt to 15 kb) (upper panel). The read-through is depicted for indicated individual RDH genes carrying cryptic polyadenylation site downstream of SL (lower panel).

Source data for panels a-e are available with the paper on line.

Figure 7 Summary of iCLIP and ChIP-seq data and working model.

a, Each column in the table depicts distribution of iCLIP and ChIP-seq peaks over selected genes either affected or not in CDK11 RNA-seq (Fig. 1a). See Online Methods for further description. iCLIP peaks density of significant cross links cDNA normalized by gene length

(sig. > 0.01). CDK11 ChIP-seq bound RPGS inside MACS2 significant peak ($p<0.05$) to selected genes (sig. > 0.01). RNA-seq DE-Seq2 differentially expressed genes ($-1>\log_2\text{FoldChange}>1$, p-adj < 0.05). P-Ser2 ChIP-seq RPGC siCDK11 log2 fold change inside the MACS2 differential expressed peaks ($-1>\log_2\text{FoldChange}>1$, p-adj < 0.05, supplementary table 5). RNAPII ChIP-seq RPGC siCDK11 log2 fold change inside the differentially expressed peaks ($-1>\log_2\text{FoldChange}>1$, p-adj < 0.05, supplementary table 6). P-Ser2/RNAPII normalised ChIP RPGC siCDK11 log2 fold change inside the differential expressed peaks ($-1>\log_2\text{FoldChange}>1$, p-adj < 0.05, supplementary table 6). The groups of genes: 44 regulated RDH (base mean expression>10); 39 low- and non-expressed RDH (base mean expression<10); 10 most down- and up-regulated genes in CDK11 RNA-seq (in fuchsia and blue, respectively), 10 selected cell cycle-related genes (in green). All genes were sorted by base mean expression within each group. Gene symbols are shown on the right.

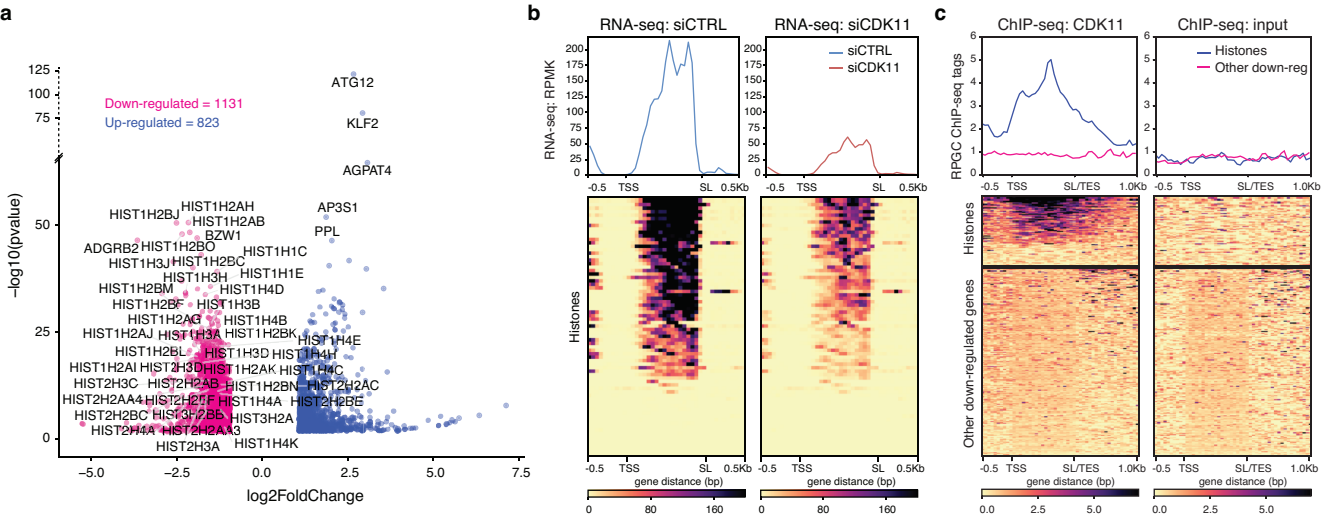
b, Schematic working model. CDK11 regulates transcription elongation of RDH genes and contributes to their 3´end processing. FLASH (grey flash) recruits CDK11 (red oval) collaboratively with nascent RDH mRNAs (black line) to chromatin of RDH genes (grey double helix) and phosphorylates (arrow) Ser2 (red ball) in the CTD (red and grey balls) of RNAPII (violet oval). The Ser2 phosphorylation promotes the RNAPII elongation on RDH genes. CDK11 also phosphorylates FLASH in S-phase which may be needed for its stability and/or yet unknown function in transcription/3´end processing of RDH genes. CDK11 is bound abundantly at the 3´end of RDH mRNAs and this binding likely occurs on or in the close vicinity of RDH chromatin. CDK11-dependent phosphorylation of Ser2 contributes to the recruitment of 3´end processing HCC complex (SYMPLEKIN (blue oval), CPSF100 (green circle), CstF64 (brown circle) and CPSF73 (yellow circle) allowing CPSF73 to cleave nascent RDH mRNA (black line). FLASH interaction with U7 snRNP (white/blue circular complex) also contributes to the recruitment of the HCC to pre-mRNA [11].

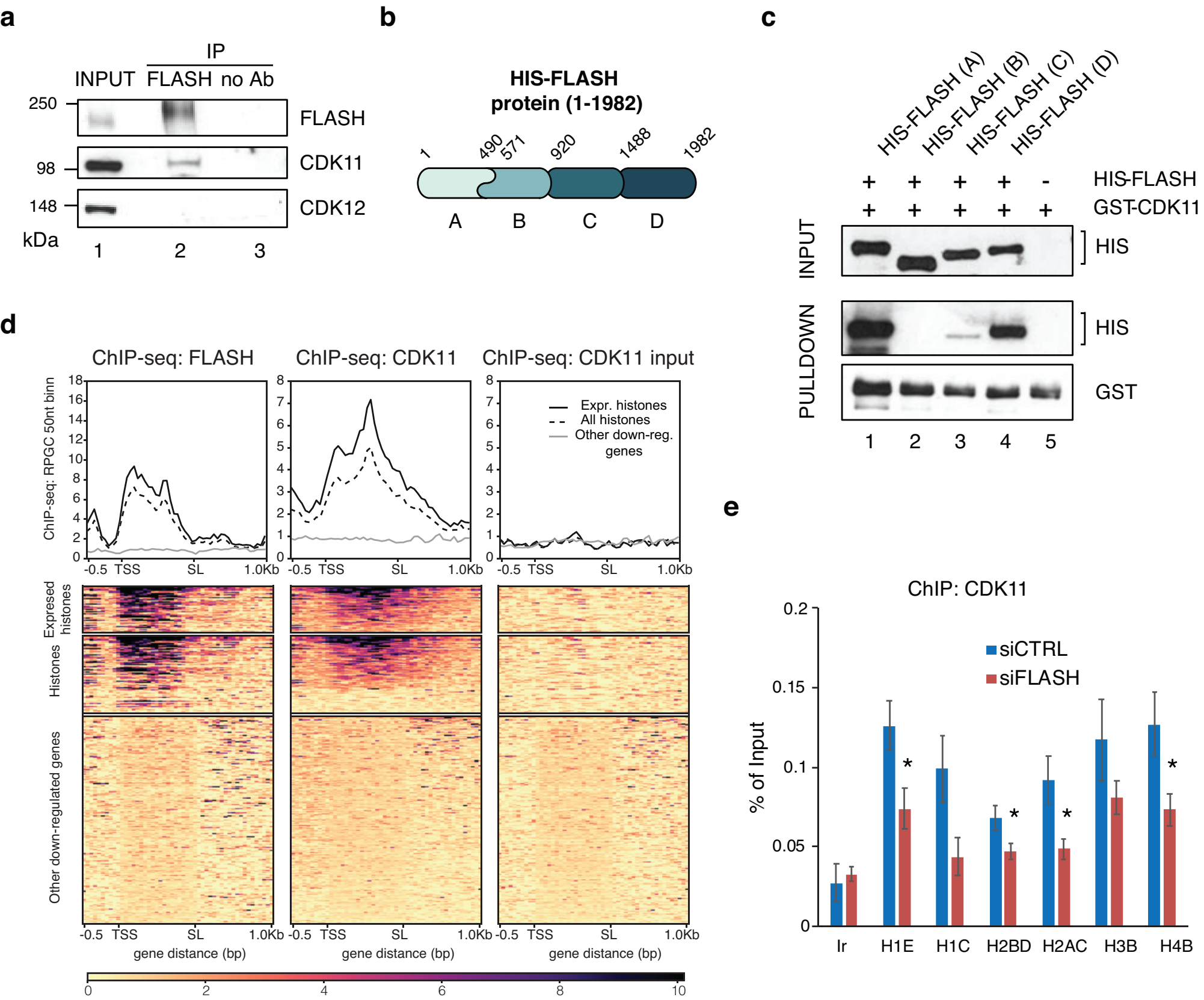
References

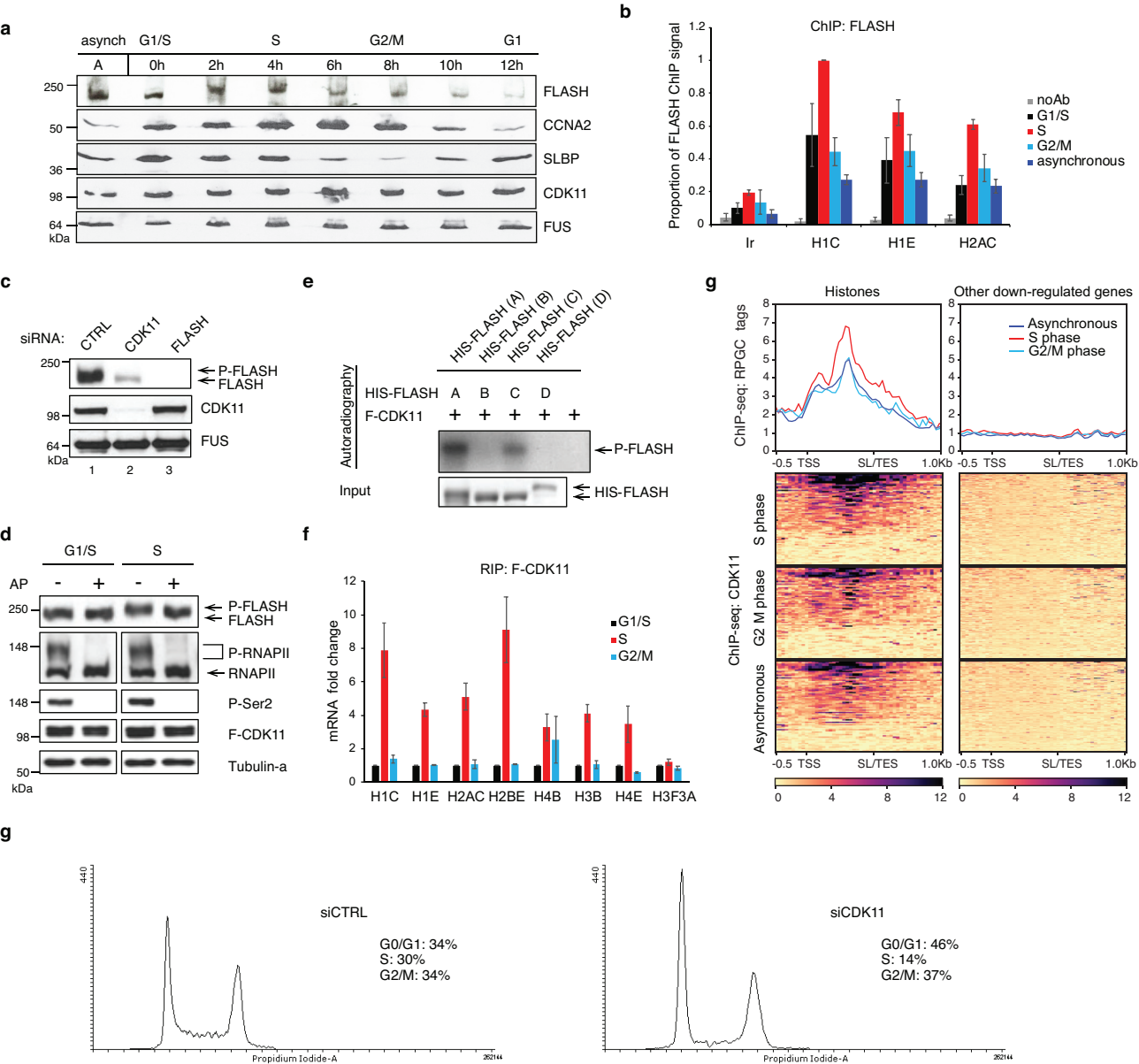
1. Adelman, K. & Lis, J.T. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet* **13**, 720-31 (2012).
2. Fuda, N.J., Ardehali, M.B. & Lis, J.T. Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature* **461**, 186-92 (2009).
3. Proudfoot, N.J. Transcriptional termination in mammals: Stopping the RNA polymerase II juggernaut. *Science* **352**, aad9926 (2016).
4. Eick, D. & Geyer, M. The RNA polymerase II carboxy-terminal domain (CTD) code. *Chem Rev* **113**, 8456-90 (2013).
5. Harlen, K.M. & Churchman, L.S. The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain. *Nat Rev Mol Cell Biol* **18**, 263-273 (2017).
6. Zaborowska, J., Egloff, S. & Murphy, S. The pol II CTD: new twists in the tail. *Nat Struct Mol Biol* **23**, 771-7 (2016).
7. Jeronimo, C., Collin, P. & Robert, F. The RNA Polymerase II CTD: The Increasing Complexity of a Low-Complexity Protein Domain. *J Mol Biol* **428**, 2607-2622 (2016).
8. Bentley, D.L. Coupling mRNA processing with transcription in time and space. *Nat Rev Genet* **15**, 163-75 (2014).
9. Hsin, J.P. & Manley, J.L. The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes Dev* **26**, 2119-37 (2012).
10. Herzel, L., Ottoz, D.S.M., Alpert, T. & Neugebauer, K.M. Splicing and transcription touch base: co-transcriptional spliceosome assembly and function. *Nat Rev Mol Cell Biol* **18**, 637-650 (2017).
11. Marzluff, W.F. & Koreski, K.P. Birth and Death of Histone mRNAs. *Trends Genet* **33**, 745-759 (2017).
12. Duronio, R.J. & Marzluff, W.F. Coordinating cell cycle-regulated histone gene expression through assembly and function of the Histone Locus Body. *RNA Biol* **14**, 726-738 (2017).
13. Sullivan, K.D., Steiniger, M. & Marzluff, W.F. A core complex of CPSF73, CPSF100, and Symplekin may form two different cleavage factors for processing of poly(A) and histone mRNAs. *Mol Cell* **34**, 322-32 (2009).
14. Kohn, M., Ihling, C., Sinz, A., Krohn, K. & Huttelmaier, S. The Y3** ncRNA promotes the 3' end processing of histone mRNAs. *Genes Dev* **29**, 1998-2003 (2015).
15. Pirngruber, J. et al. CDK9 directs H2B monoubiquitination and controls replication-dependent histone mRNA 3'-end processing. *EMBO Rep* **10**, 894-900 (2009).
16. Narita, T. et al. NELF interacts with CBC and participates in 3' end processing of replication-dependent histone mRNAs. *Mol Cell* **26**, 349-65 (2007).
17. Saldi, T., Fong, N. & Bentley, D.L. Transcription elongation rate affects nascent histone pre-mRNA folding and 3' end processing. *Genes Dev* **32**, 297-308 (2018).
18. Hsin, J.P., Sheth, A. & Manley, J.L. RNAP II CTD phosphorylated on threonine-4 is required for histone mRNA 3' end processing. *Science* **334**, 683-6 (2011).
19. Medlin, J. et al. P-TEFb is not an essential elongation factor for the intronless human U2 snRNA and histone H2b genes. *EMBO J* **24**, 4154-65 (2005).
20. Hintermair, C. et al. Threonine-4 of mammalian RNA polymerase II CTD is targeted by Polo-like kinase 3 and required for transcriptional elongation. *EMBO J* **31**, 2784-97 (2012).

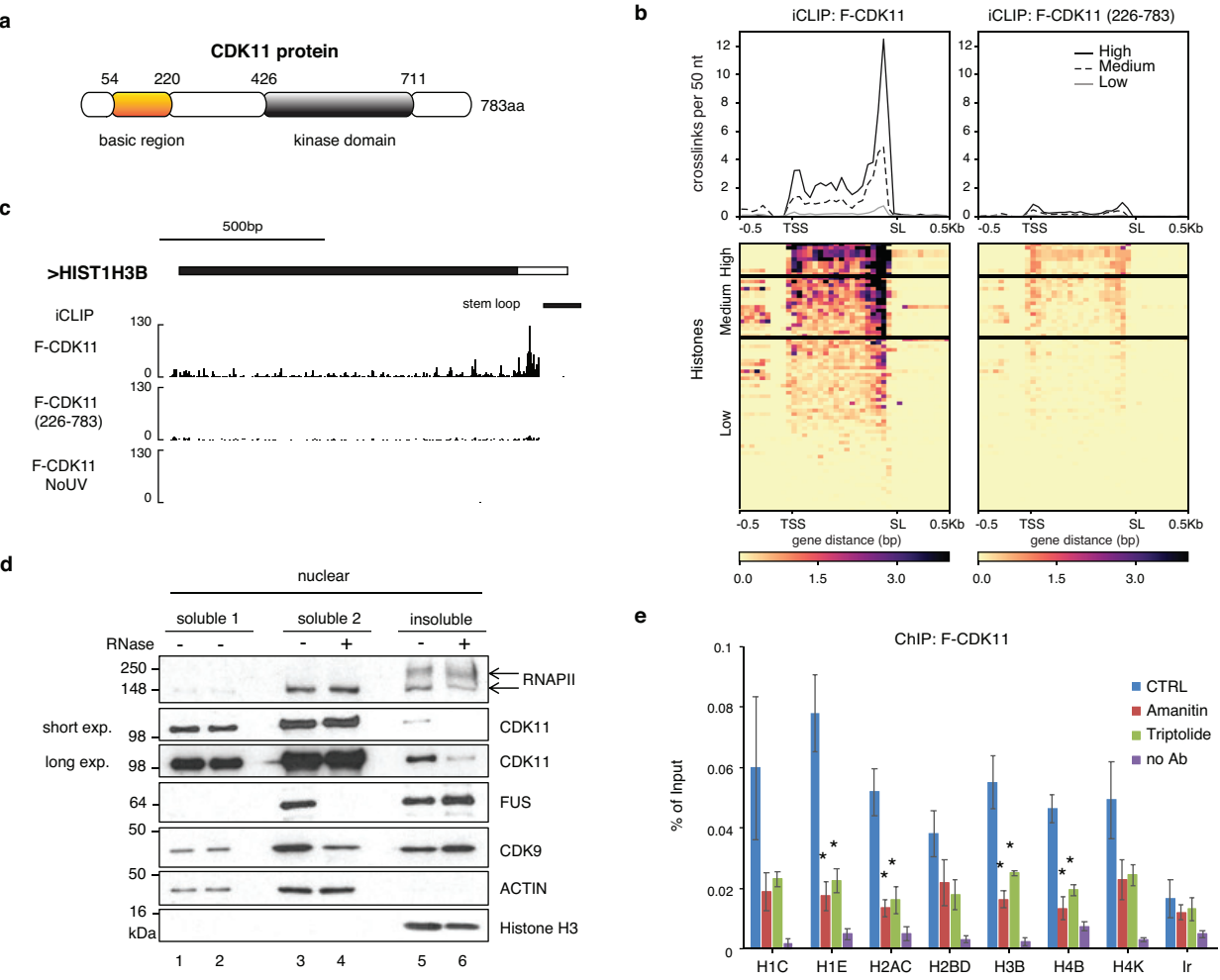
21. Loyer, P. et al. Characterization of cyclin L1 and L2 interactions with CDK11 and splicing factors: influence of cyclin L isoforms on splice site selection. *J Biol Chem* **283**, 7721-32 (2008).
22. Cornelis, S. et al. Identification and characterization of a novel cell cycle-regulated internal ribosome entry site. *Mol Cell* **5**, 597-605 (2000).
23. Hu, D., Valentine, M., Kidd, V.J. & Lahti, J.M. CDK11(p58) is required for the maintenance of sister chromatid cohesion. *J Cell Sci* **120**, 2424-34 (2007).
24. Petretti, C. et al. The PITSLRE/CDK11p58 protein kinase promotes centrosome maturation and bipolar spindle formation. *EMBO Rep* **7**, 418-24 (2006).
25. Zhou, Y., Shen, J.K., Hornicek, F.J., Kan, Q. & Duan, Z. The emerging roles and therapeutic potential of cyclin-dependent kinase 11 (CDK11) in human cancer. *Oncotarget* **7**, 40846-40859 (2016).
26. Li, T., Inoue, A., Lahti, J.M. & Kidd, V.J. Failure to proliferate and mitotic arrest of CDK11(p110/p58)-null mutant mice at the blastocyst stage of embryonic cell development. *Mol Cell Biol* **24**, 3188-97 (2004).
27. Hu, D., Mayeda, A., Trembley, J.H., Lahti, J.M. & Kidd, V.J. CDK11 complexes promote pre-mRNA splicing. *J Biol Chem* **278**, 8623-9 (2003).
28. Trembley, J.H. et al. PITSLRE p110 protein kinases associate with transcription complexes and affect their activity. *J Biol Chem* **277**, 2589-96 (2002).
29. Valente, S.T., Gilmartin, G.M., Venkatarama, K., Arriagada, G. & Goff, S.P. HIV-1 mRNA 3' end processing is distinctively regulated by eIF3f, CDK11, and splice factor 9G8. *Mol Cell* **36**, 279-89 (2009).
30. Tiedemann, R.E. et al. Identification of molecular vulnerabilities in human multiple myeloma cells by RNA interference lethality screening of the druggable genome. *Cancer Res* **72**, 757-68 (2012).
31. Chi, Y. et al. Abnormal expression of CDK11p58 in prostate cancer. *Cancer Cell Int* **14**, 2 (2014).
32. Duan, Z. et al. Systematic kinome shRNA screening identifies CDK11 (PITSLRE) kinase expression is critical for osteosarcoma cell growth and proliferation. *Clin Cancer Res* **18**, 4580-8 (2012).
33. Kren, B.T. et al. Preclinical evaluation of cyclin dependent kinase 11 and casein kinase 2 survival kinases as RNA interference targets for triple negative breast cancer therapy. *Breast Cancer Res* **17**, 524 (2015).
34. Liu, X. et al. Cyclin-Dependent Kinase 11 (CDK11) Is Required for Ovarian Cancer Cell Growth In Vitro and In Vivo, and Its Inhibition Causes Apoptosis and Sensitizes Cells to Paclitaxel. *Mol Cancer Ther* **15**, 1691-701 (2016).
35. Du, Y. et al. CDK11(p110) plays a critical role in the tumorigenicity of esophageal squamous cell carcinoma cells and is a potential drug target. *Cell Cycle* **18**, 452-466 (2019).
36. Sokolova, M. et al. Genome-wide screen of cell-cycle regulators in normal and tumor cells identifies a differential response to nucleosome depletion. *Cell Cycle* **16**, 189-199 (2017).
37. Yang, X.C., Burch, B.D., Yan, Y., Marzluff, W.F. & Dominski, Z. FLASH, a proapoptotic protein involved in activation of caspase-8, is essential for 3' end processing of histone pre-mRNAs. *Mol Cell* **36**, 267-78 (2009).
38. Kohoutek, J. & Blazek, D. Cyclin K goes with Cdk12 and Cdk13. *Cell Div* **7**, 12 (2012).
39. Marzluff, W.F., Wagner, E.J. & Duronio, R.J. Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail. *Nat Rev Genet* **9**, 843-54 (2008).

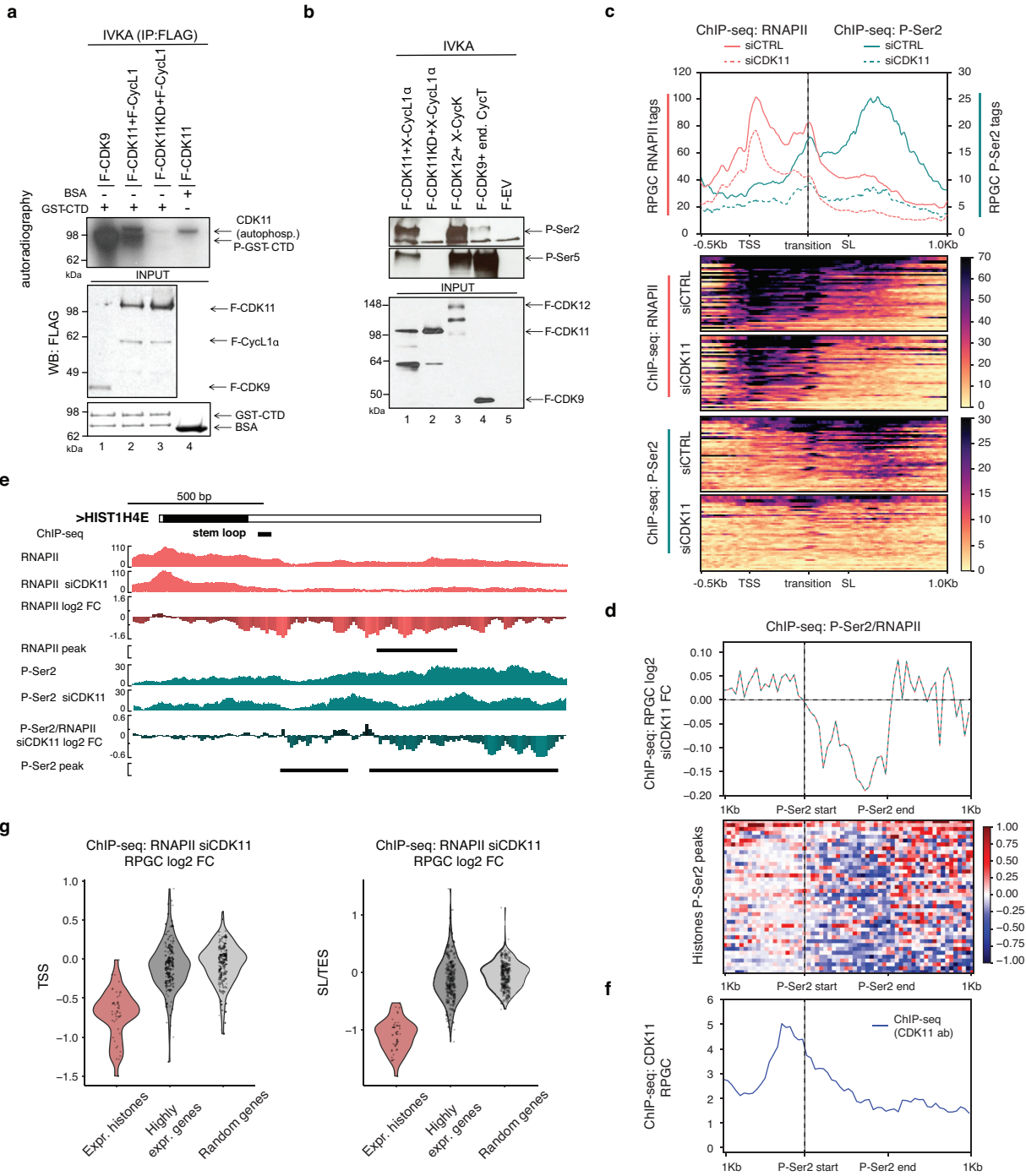
40. Zhao, J. et al. NPAT links cyclin E-Cdk2 to the regulation of replication-dependent histone gene transcription. *Genes Dev* **14**, 2283-97 (2000).
41. Castello, A. et al. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* **149**, 1393-406 (2012).
42. Baltz, A.G. et al. The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. *Mol Cell* **46**, 674-90 (2012).
43. Konig, J. et al. iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. *Nat Struct Mol Biol* **17**, 909-15 (2010).
44. Van Nostrand, E.L. et al. Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). *Nat Methods* **13**, 508-14 (2016).
45. Beltran, M. et al. The interaction of PRC2 with RNA or chromatin is mutually antagonistic. *Genome Res* **26**, 896-907 (2016).
46. Trembley, J.H., Hu, D., Slaughter, C.A., Lahti, J.M. & Kidd, V.J. Casein kinase 2 interacts with cyclin-dependent kinase 11 (CDK11) in vivo and phosphorylates both the RNA polymerase II carboxyl-terminal domain and CDK11 in vitro. *J Biol Chem* **278**, 2265-70 (2003).
47. Pak, V. et al. CDK11 in TREX/THOC Regulates HIV mRNA 3' End Processing. *Cell Host Microbe* **18**, 560-70 (2015).
48. Peterlin, B.M. & Price, D.H. Controlling the elongation phase of transcription with P-TEFb. *Mol Cell* **23**, 297-305 (2006).
49. Bosken, C.A. et al. The structure and substrate specificity of human Cdk12/Cyclin K. *Nat Commun* **5**, 3505 (2014).
50. Lyons, S.M. et al. A subset of replication-dependent histone mRNAs are expressed as polyadenylated RNAs in terminally differentiated tissues. *Nucleic Acids Res* **44**, 9190-9205 (2016).
51. Larochelle, S. et al. Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. *Nat Struct Mol Biol* **19**, 1108-15 (2012).
52. Gruber, J.J. et al. Ars2 promotes proper replication-dependent histone mRNA 3' end formation. *Mol Cell* **45**, 87-98 (2012).
53. Sullivan, K.D., Mullen, T.E., Marzluff, W.F. & Wagner, E.J. Knockdown of SLBP results in nuclear retention of histone mRNA. *RNA* **15**, 459-72 (2009).
54. Drogat, J. et al. Cdk11-cyclinL controls the assembly of the RNA polymerase II mediator complex. *Cell Rep* **2**, 1068-76 (2012).
55. Kim, D.U. et al. Analysis of a genome-wide set of gene deletions in the fission yeast Schizosaccharomyces pombe. *Nat Biotechnol* **28**, 617-623 (2010).
56. Kurat, C.F. et al. Regulation of histone gene transcription in yeast. *Cell Mol Life Sci* **71**, 599-613 (2014).
57. Barcaroli, D. et al. FLASH is required for histone transcription and S-phase progression. *Proc Natl Acad Sci U S A* **103**, 14808-12 (2006).
58. Chapman, R.D. et al. Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7. *Science* **318**, 1780-2 (2007).
59. Schuller, R. et al. Heptad-Specific Phosphorylation of RNA Polymerase II CTD. *Mol Cell* **61**, 305-14 (2016).
60. Lin, A. et al. Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. *Sci Transl Med* **11**(2019).

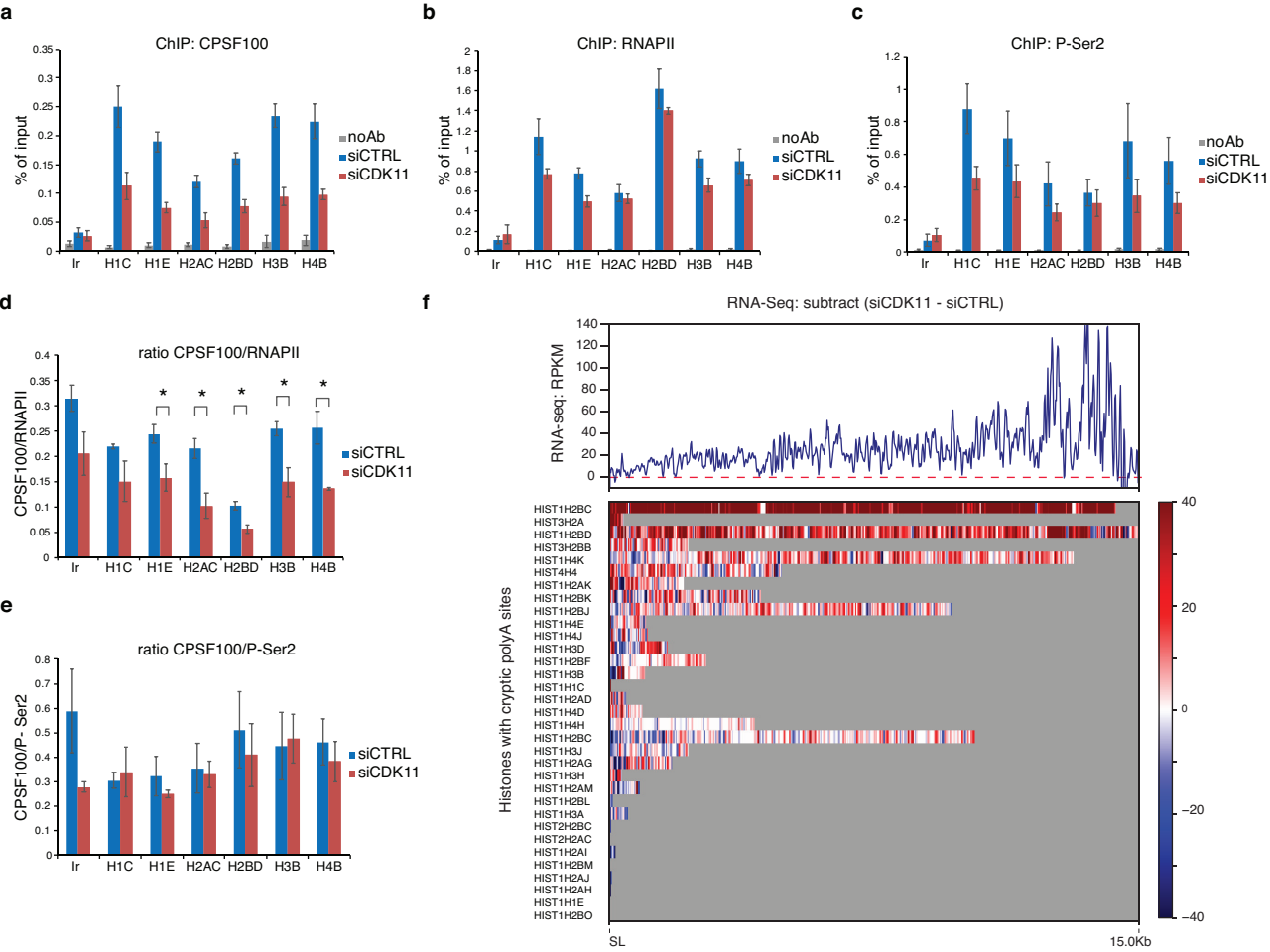

a
Down-regulated = 1131
Up-regulated = 823
ATG12
KLF2
AGPAT4
AP3S1
PPL
HIST1H2AH
HIST1H2BJ
HIST1H2AB
BZW1
ADGRB2
HIST1H2BO
HIST1H1C
HIST1H3J
HIST1H2BC
HIST1H3H
HIST1H1E
HIST1H2BM
HIST1H4D
HIST1H2BF
HIST1H3B
HIST1H2AG
HIST1H4B
HIST1H2AJ
HIST1H3A
HIST1H2BK
HIST1H4E
HIST1H2BL
HIST1H3D
HIST1H4I
HIST1H2AI
HIST2H3D
HIST1H2AK
HIST1H4C
HIST2H3C
HIST2H2AB
HIST1H2BN
HIST2H2AC
HIST2H2AA4
HIST2H2EF
HIST1H4A
HIST2H2BE
HIST2H2BC
HIST3H2BB
HIST3H2A
HIST2H4A
HIST2H2AA3
HIST2H3A
HIST1H4K
−log10(pvalue)
log2FoldChange
b
RNA-seq: siCTRL
RNA-seq: siCDK11
siCTRL
siCDK11
RNA-seq: RPMK
Histones
TSS
SL
gene distance (bp)
c
ChIP-seq: CDK11
ChIP-seq: input
Histones
Other down-reg
RPGC ChIP-seq tags
SL/TES
Other down-regulated genes

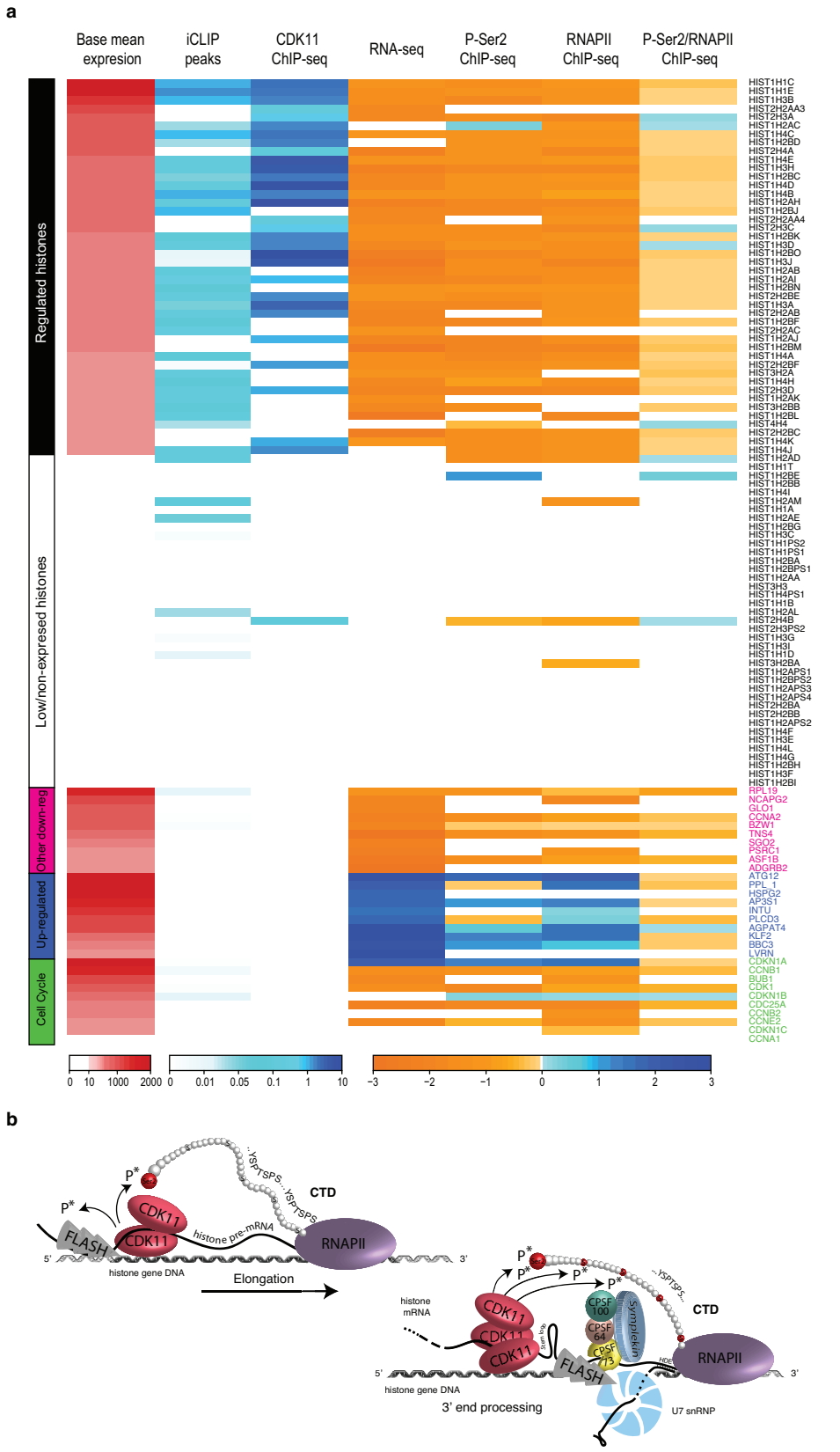

a
IP
INPUT
FLASH
no Ab
250
98
148
kDa
FLASH
CDK11
CDK12
1
2
3
b
HIS-FLASH
protein (1-1982)
1
490
571
920
1488
1982
A
B
C
D
c
HIS-FLASH (A)
HIS-FLASH (B)
HIS-FLASH (C)
HIS-FLASH (D)
HIS-FLASH
GST-CDK11
INPUT
PULLDOWN
HIS
HIS
GST
1
2
3
4
5
d
ChIP-seq: FLASH
ChIP-seq: CDK11
ChIP-seq: CDK11 input
ChIP-seq: RPGC 50nt binn
Expr. histones
All histones
Other down-reg. genes
-0.5
TSS
SL
1.0Kb
Expresed histones
Histones
Other down-regulated genes
gene distance (bp)
0
2
4
6
8
10
e
ChIP: CDK11
siCTRL
siFLASH
% of Input
0.2
0.15
0.1
0.05
0
Ir
H1E
H1C
H2BD
H2AC
H3B
H4B

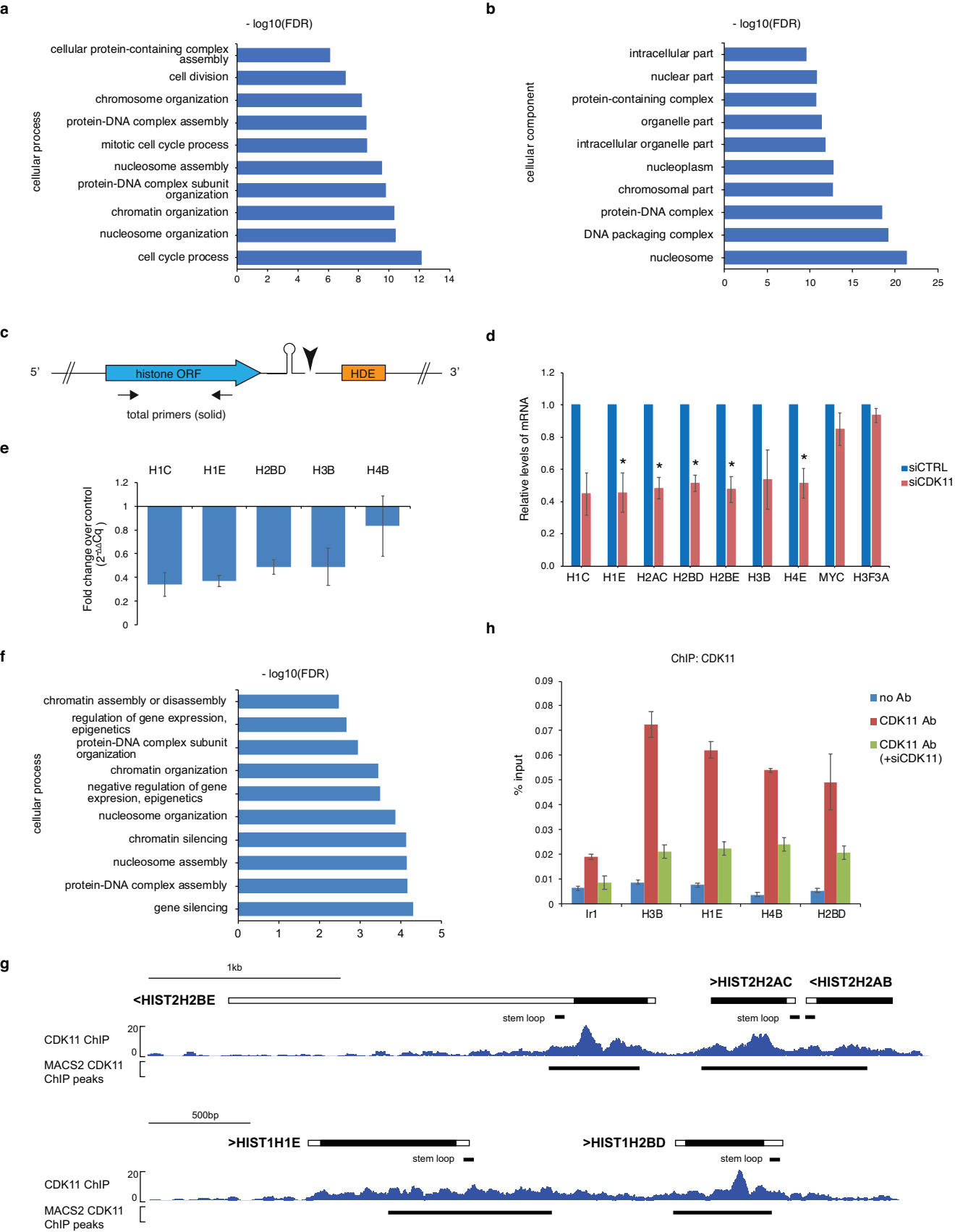

a
asynch
G1/S
S
G2/M
G1
A
0h
2h
4h
6h
8h
10h
12h
250
50
36
98
64
kDa
FLASH
CCNA2
SLBP
CDK11
FUS
b
ChIP: FLASH
Proportion of FLASH ChIP signal
1.2
1
0.8
0.6
0.4
0.2
0
Ir
H1C
H1E
H2AC
noAb
G1/S
S
G2/M
asynchronous
c
siRNA:
CTRL
CDK11
FLASH
250
98
64
kDa
P-FLASH
FLASH
CDK11
FUS
1
2
3
d
G1/S
S
AP
-
+
-
+
250
148
148
98
50
kDa
P-FLASH
FLASH
P-RNAPII
RNAPII
P-Ser2
F-CDK11
Tubulin-a
e
HIS-FLASH (A)
HIS-FLASH (B)
HIS-FLASH (C)
HIS-FLASH (D)
Autoradiography
HIS-FLASH
A
B
C
D
F-CDK11
+
+
+
+
+
P-FLASH
Input
HIS-FLASH
f
RIP: F-CDK11
mRNA fold change
12
10
8
6
4
2
0
G1/S
S
G2/M
H1C
H1E
H2AC
H2BE
H4B
H3B
H4E
H3F3A
g
Histones
Other down-regulated genes
ChIP-seq: RPGC tags
8
7
6
5
4
3
2
1
0
Asynchronous
S phase
G2/M phase
-0.5 TSS
SL/TES
1.0Kb
ChIP-seq: CDK11
S phase
G2 M phase
Asynchronous
0
4
8
12
g
siCTRL
G0/G1: 34%
S: 30%
G2/M: 34%
siCDK11
G0/G1: 46%
S: 14%
G2/M: 37%
440
Propidium Iodide-A
262144

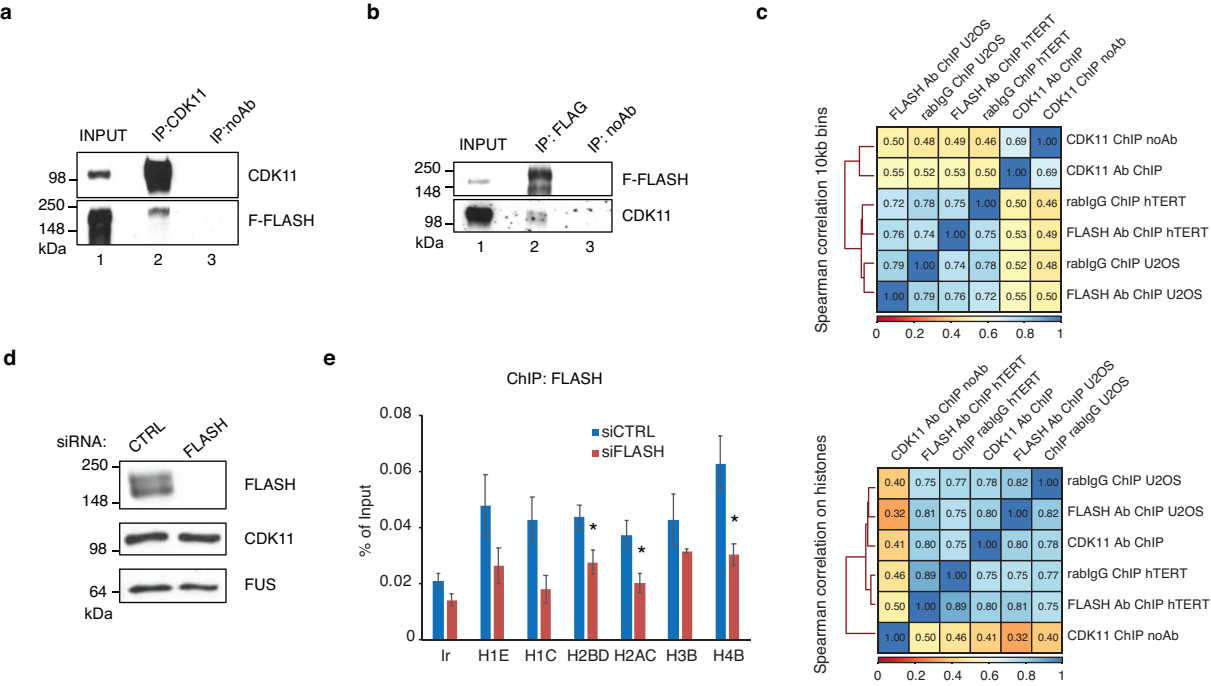

a
CDK11 protein
54
220
426
711
783aa
basic region
kinase domain
b
iCLIP: F-CDK11
iCLIP: F-CDK11 (226-783)
crosslinks per 50 nt
High
Medium
Low
-0.5
TSS
SL
0.5Kb
Histones
gene distance (bp)
0.0
1.5
3.0
c
500bp
>HIST1H3B
stem loop
iCLIP
F-CDK11
F-CDK11 (226-783)
F-CDK11 NoUV
130
0
d
nuclear
soluble 1
soluble 2
insoluble
RNase
RNAPII
short exp.
long exp.
CDK11
FUS
CDK9
ACTIN
Histone H3
kDa
e
ChIP: F-CDK11
% of Input
CTRL
Amanitin
Triptolide
no Ab
H1C
H1E
H2AC
H2BD
H3B
H4B
H4K
Ir

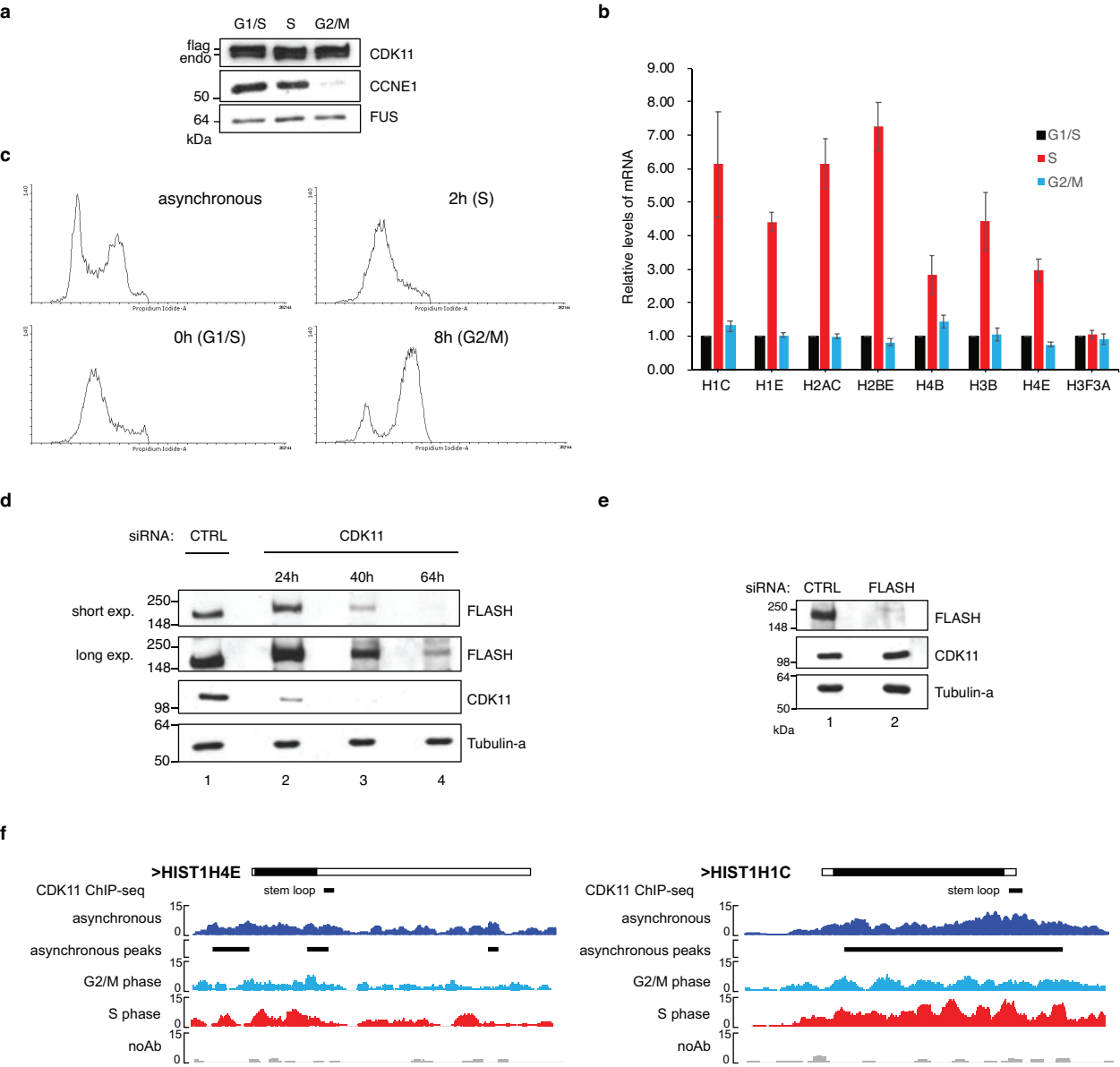

a
IVKA (IP:FLAG)
F-CDK9
F-CDK11+F-CycL1
F-CDK11KD+F-CycL1
F-CDK11
BSA
GST-CTD
autoradiography
CDK11 (autophosp.)
P-GST-CTD
INPUT
WB: FLAG
F-CDK11
F-CycL1α
F-CDK9
GST-CTD
BSA
kDa
b
IVKA
F-CDK11+X-CycL1α
F-CDK11KD+X-CycL1α
F-CDK12+ X-CycK
F-CDK9+ end. CycT
F-EV
P-Ser2
P-Ser5
INPUT
F-CDK12
F-CDK11
F-CDK9
c
ChIP-seq: RNAPII
ChIP-seq: P-Ser2
siCTRL
siCDK11
RPGC RNAPII tags
RPGC P-Ser2 tags
-0.5Kb
TSS
transition
SL
1.0Kb
ChIP-seq: RNAPII
ChIP-seq: P-Ser2
d
ChIP-seq: P-Ser2/RNAPII
ChIP-seq: RPGC log2 siCDK11 FC
1Kb
P-Ser2 start
P-Ser2 end
Histones P-Ser2 peaks
e
500 bp
>HIST1H4E
stem loop
ChIP-seq
RNAPII
RNAPII siCDK11
RNAPII log2 FC
RNAPII peak
P-Ser2
P-Ser2 siCDK11
P-Ser2/RNAPII siCDK11 log2 FC
P-Ser2 peak
f
ChIP-seq: CDK11 RPGC
ChIP-seq (CDK11 ab)
g
ChIP-seq: RNAPII siCDK11 RPGC log2 FC
TSS
SL/TES
Expr. histones
Highly expr. genes
Random genes

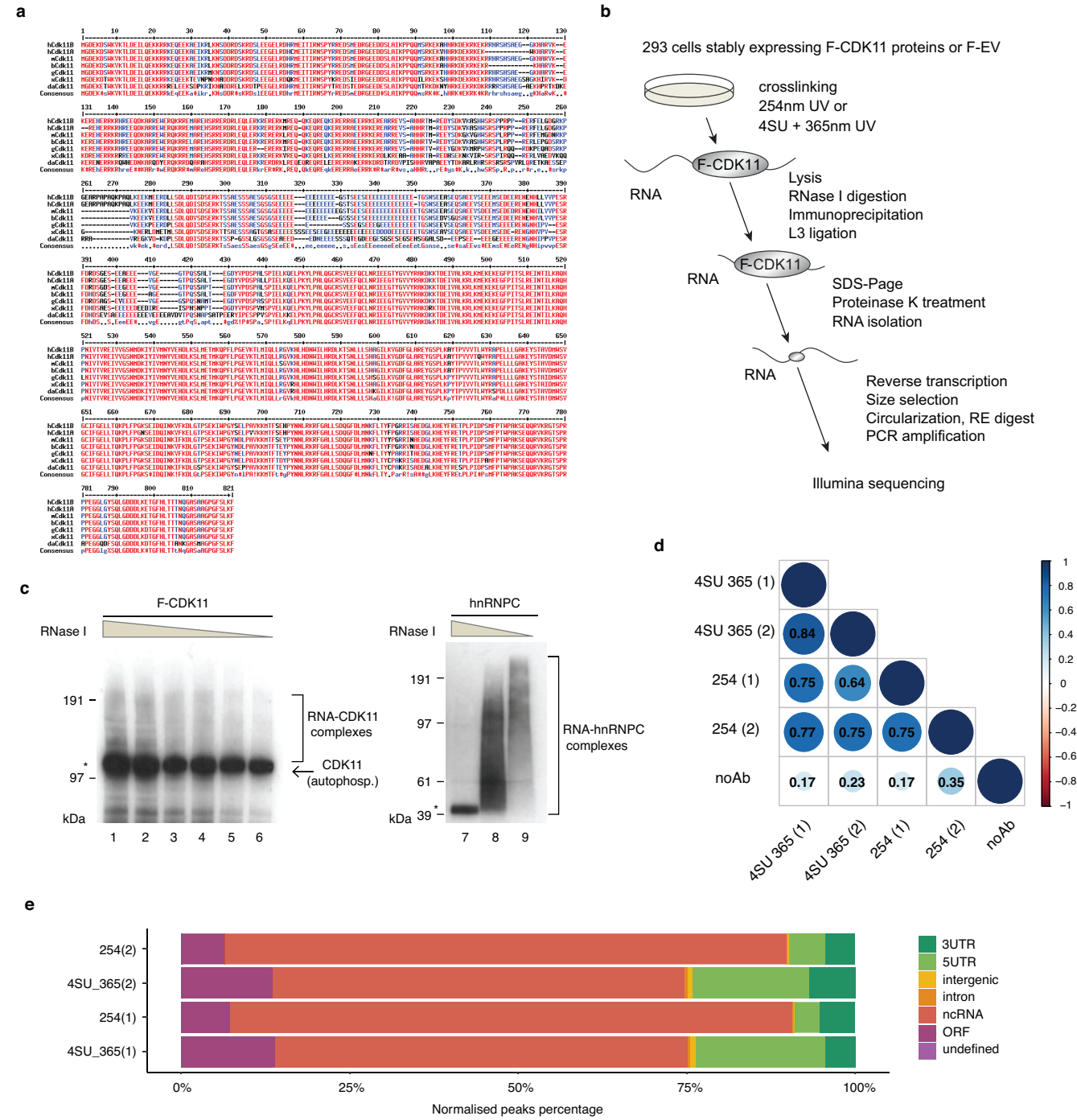

a
ChIP: CPSF100
% of input
noAb
siCTRL
siCDK11
Ir
H1C
H1E
H2AC
H2BD
H3B
H4B
b
ChIP: RNAPII
% of input
noAb
siCTRL
siCDK11
Ir
H1C
H1E
H2AC
H2BD
H3B
H4B
c
ChIP: P-Ser2
% of input
noAb
siCTRL
siCDK11
Ir
H1C
H1E
H2AC
H2BD
H3B
H4B
d
ratio CPSF100/RNAPII
CPSF100/RNAPII
siCTRL
siCDK11
Ir
H1C
H1E
H2AC
H2BD
H3B
H4B
e
ratio CPSF100/P-Ser2
CPSF100/P- Ser2
siCTRL
siCDK11
Ir
H1C
H1E
H2AC
H2BD
H3B
H4B
f
RNA-Seq: subtract (siCDK11 - siCTRL)
RNA-seq: RPKM
Histones with cryptic polyA sites
HIST1H2BC
HIST3H2A
HIST1H2BD
HIST3H2BB
HIST1H4K
HIST4H4
HIST1H2AK
HIST1H2BK
HIST1H2BJ
HIST1H4E
HIST1H4J
HIST1H3D
HIST1H2BF
HIST1H3B
HIST1H1C
HIST1H2AD
HIST1H4D
HIST1H4H
HIST1H2BC
HIST1H3J
HIST1H2AG
HIST1H3H
HIST1H2AM
HIST1H2BL
HIST1H3A
HIST2H2BC
HIST2H2AC
HIST1H2AI
HIST1H2BM
HIST1H2AJ
HIST1H2AH
HIST1H1E
HIST1H2BO
SL
15.0Kb

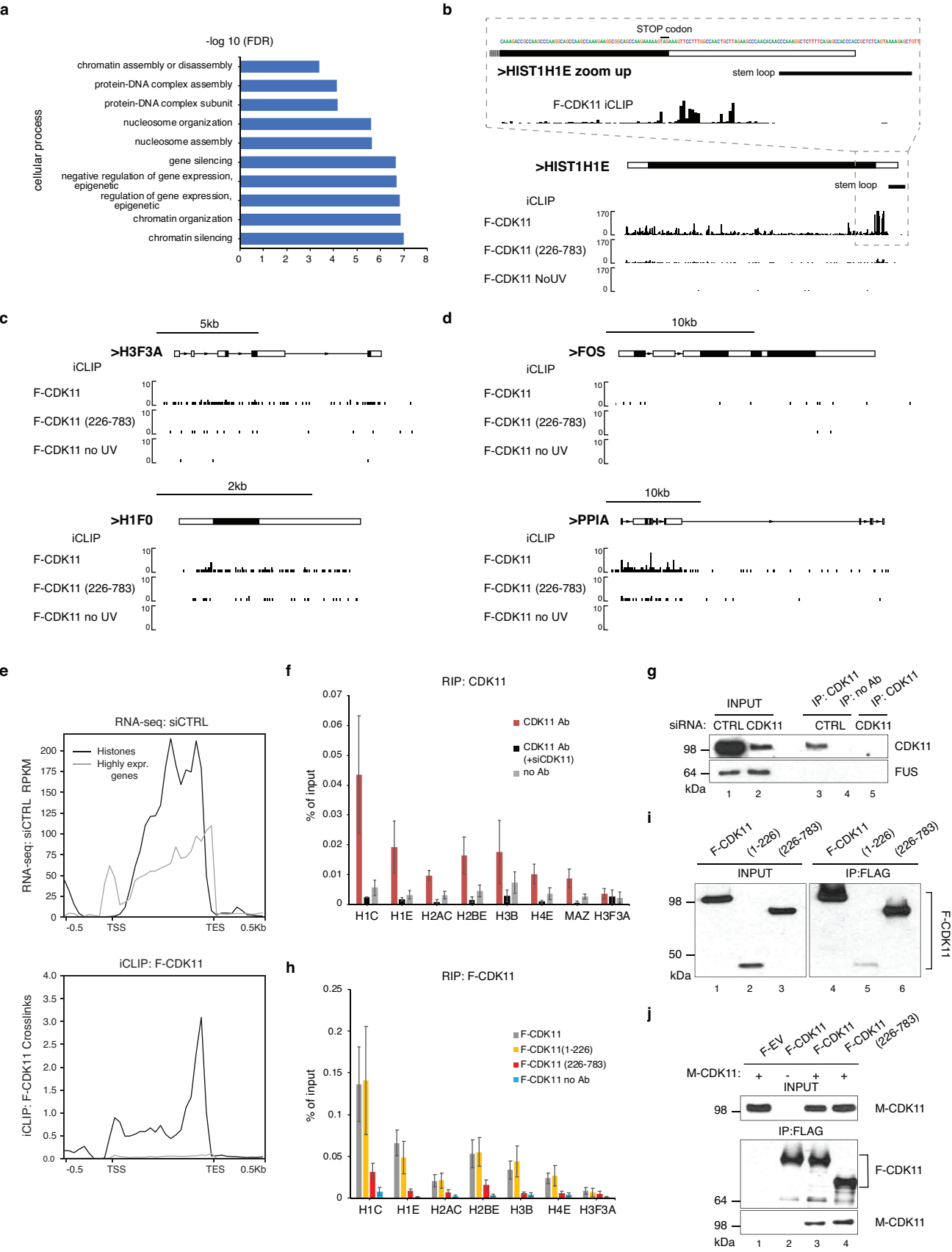

a
Base mean expresion
iCLIP peaks
CDK11 ChIP-seq
RNA-seq
P-Ser2 ChIP-seq
RNAPII ChIP-seq
P-Ser2/RNAPII ChIP-seq
Regulated histones
Low/non-expresed histones
Other down-reg
Up-regulated
Cell Cycle
HIST1H1C
HIST1H1E
HIST1H3B
HIST2H2AA3
HIST2H3A
HIST1H2AC
HIST1H4C
HIST1H2BD
HIST2H4A
HIST1H4E
HIST1H3H
HIST1H2BC
HIST1H4D
HIST1H4B
HIST1H2AH
HIST1H2BJ
HIST2H2AA4
HIST2H3C
HIST1H2BK
HIST1H3D
HIST1H2BO
HIST1H3J
HIST2H2AB
HIST1H2AI
HIST1H2BN
HIST2H2BE
HIST1H3A
HIST2H2AB
HIST1H2BF
HIST2H2AC
HIST1H2AJ
HIST1H2BM
HIST1H4A
HIST2H2BF
HIST3H2A
HIST1H4H
HIST2H3D
HIST1H2AK
HIST3H2BB
HIST1H2BL
HIST4H4
HIST2H2BC
HIST1H4K
HIST1H4J
HIST1H2AD
HIST1H1T
HIST1H2BE
HIST1H2BB
HIST1H4I
HIST1H2AM
HIST1H1A
HIST1H2AE
HIST1H2BG
HIST1H3C
HIST1H1PS2
HIST1H1PS1
HIST1H2BA
HIST1H2BPS1
HIST1H2AA
HIST3H3
HIST1H4PS1
HIST1H1B
HIST1H2AL
HIST2H4B
HIST2H3PS2
HIST1H3G
HIST1H3I
HIST1H1D
HIST3H2BA
HIST1H2APS1
HIST1H2BPS2
HIST1H2APS3
HIST1H2APS4
HIST2H2BA
HIST2H2BB
HIST1H2APS2
HIST1H4F
HIST1H3E
HIST1H4L
HIST1H4G
HIST1H2BH
HIST1H3F
HIST1H2BI
RPL19
NCAPG2
GLO1
CCNA2
B2W1
TNS4
SGO2
PSRC1
ASF1B
ADGRB2
ATG12
PPL_1
HSPG2
AP3S1
INTU
PLCD3
AGPAT4
KLF2
BBC3
LVRN
CDKN1A
CCNB1
BUB1
CDK1
CDKN1B
CDC25A
CCNB2
CCNE2
CDKN1C
CCNA1
0 10 1000 2000
0 0.01 0.05 0.1 1 10
−3 −2 −1 0 1 2 3
b
P*
CDK11
CDK11
FLASH
YSPTSPS
CTD
RNAPII
histone pre-mRNA
5'
3'
histone gene DNA
Elongation
histone mRNA
Stem loop
CPSF 100
CPSF 64
CPSF 73
Symplekin
WDR82
U7 snRNP
3' end processing

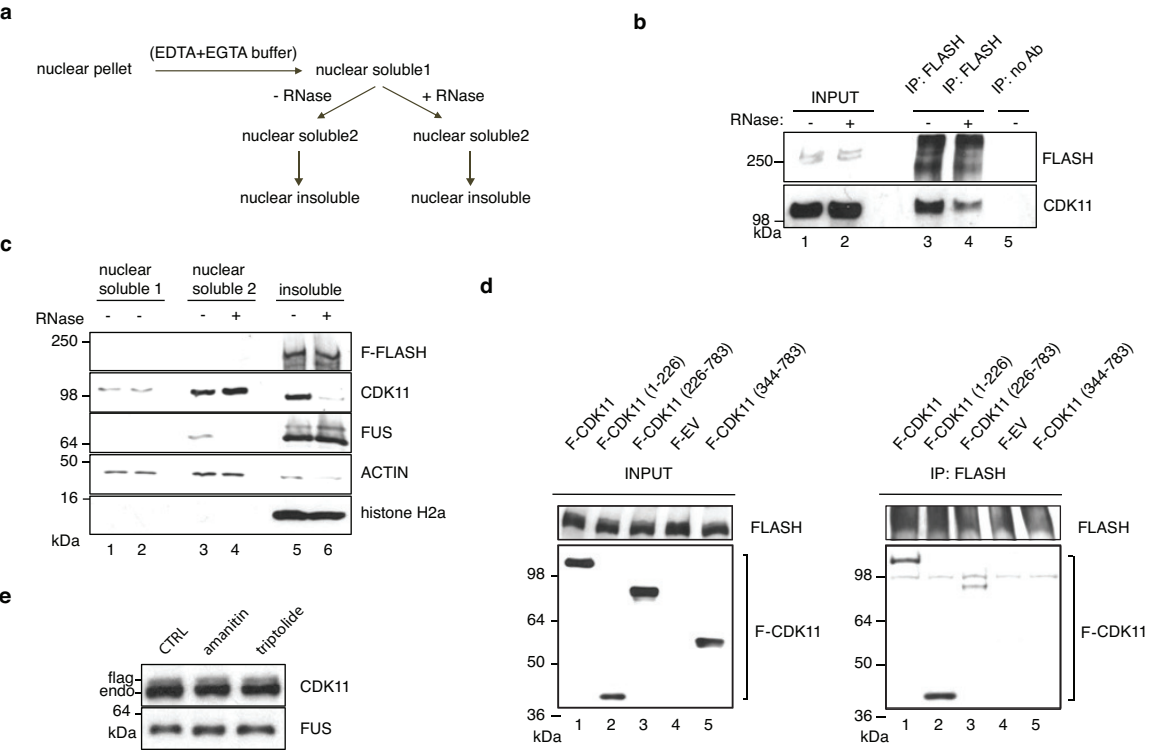

a
- log10(FDR)
cellular process
cellular protein-containing complex assembly
cell division
chromosome organization
protein-DNA complex assembly
mitotic cell cycle process
nucleosome assembly
protein-DNA complex subunit organization
chromatin organization
nucleosome organization
cell cycle process
0 2 4 6 8 10 12 14
b
- log10(FDR)
cellular component
intracellular part
nuclear part
protein-containing complex
organelle part
intracellular organelle part
nucleoplasm
chromosomal part
protein-DNA complex
DNA packaging complex
nucleosome
0 5 10 15 20 25
c
5'
histone ORF
HDE
3'
total primers (solid)
d
Relative levels of mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
H1C H1E H2AC H2BD H2BE H3B H4E MYC H3F3A
siCTRL
siCDK11
e
H1C H1E H2BD H3B H4B
Fold change over control (2^-ΔΔCq)
1.2
1
0.8
0.6
0.4
0.2
0
f
- log10(FDR)
cellular process
chromatin assembly or disassembly
regulation of gene expression, epigenetics
protein-DNA complex subunit organization
chromatin organization
negative regulation of gene expresion, epigenetics
nucleosome organization
chromatin silencing
nucleosome assembly
protein-DNA complex assembly
gene silencing
0 1 2 3 4 5
h
ChIP: CDK11
% input
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
no Ab
CDK11 Ab
CDK11 Ab (+siCDK11)
Ir1 H3B H1E H4B H2BD
g
1kb
<HIST2H2BE
>HIST2H2AC
<HIST2H2AB
stem loop
stem loop
stem loop
CDK11 ChIP
20
0
MACS2 CDK11 ChIP peaks
500bp
>HIST1H1E
>HIST1H2BD
stem loop
stem loop
CDK11 ChIP
20
0
MACS2 CDK11 ChIP peaks

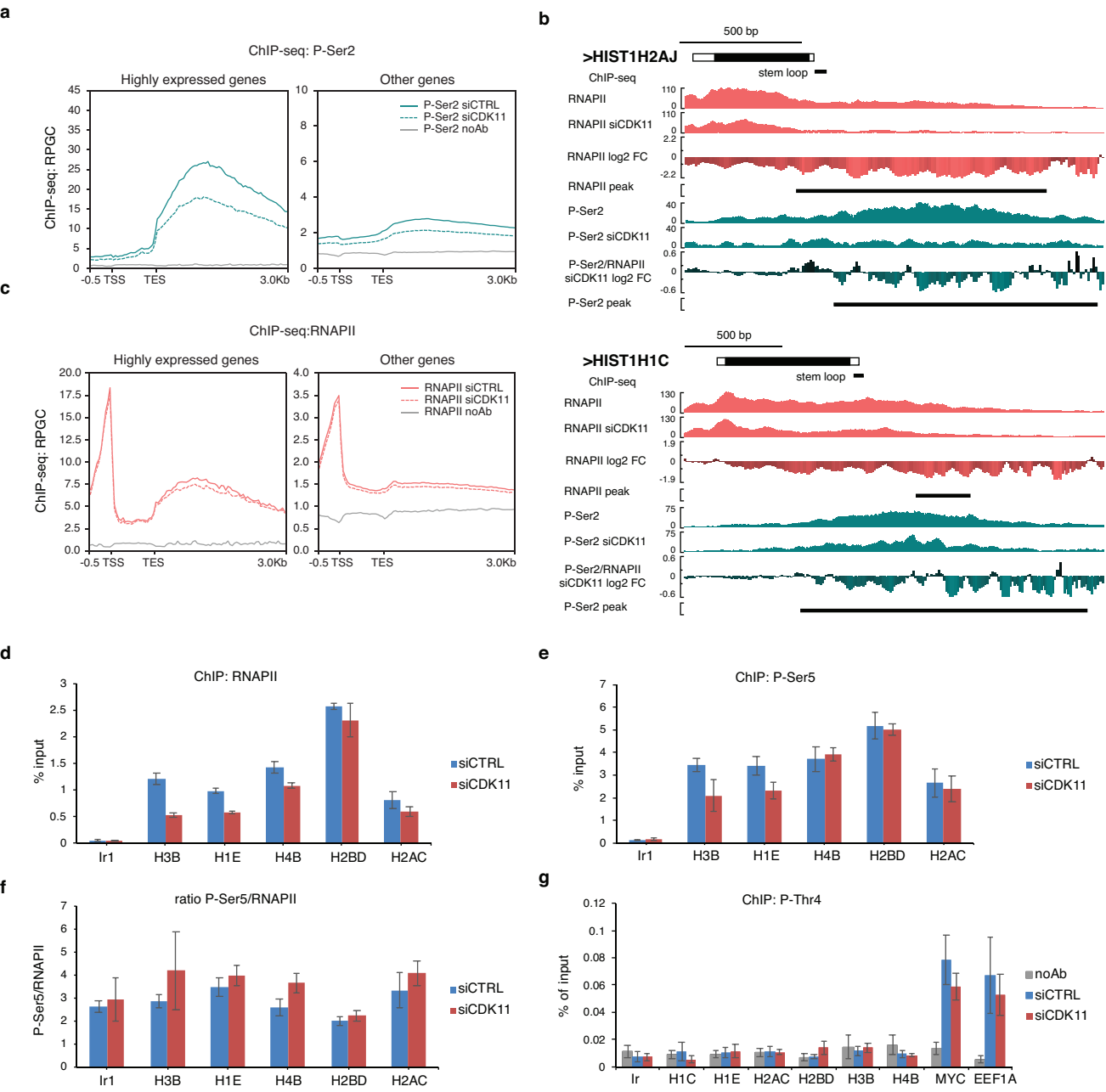

a
INPUT
IP:CDK11
IP:noAb
98
250
148
kDa
CDK11
F-FLASH
1
2
3
b
INPUT
IP: FLAG
IP: noAb
250
148
98
kDa
F-FLASH
CDK11
1
2
3
c
FLASH Ab ChIP U2OS
rabIgG ChIP U2OS
FLASH Ab ChIP hTERT
rabIgG ChIP hTERT
CDK11 Ab ChIP
CDK11 ChIP noAb
Spearman correlation 10kb bins
0.50 0.48 0.49 0.46 0.69 1.00 CDK11 ChIP noAb
0.55 0.52 0.53 0.50 1.00 0.69 CDK11 Ab ChIP
0.72 0.78 0.75 1.00 0.50 0.46 rabIgG ChIP hTERT
0.76 0.74 1.00 0.75 0.53 0.49 FLASH Ab ChIP hTERT
0.79 1.00 0.74 0.78 0.52 0.48 rabIgG ChIP U2OS
1.00 0.79 0.76 0.72 0.55 0.50 FLASH Ab ChIP U2OS
0 0.2 0.4 0.6 0.8 1
d
siRNA:
CTRL
FLASH
250
148
98
64
kDa
FLASH
CDK11
FUS
e
ChIP: FLASH
siCTRL
siFLASH
% of Input
0.08
0.06
0.04
0.02
0
Ir
H1E
H1C
H2BD
H2AC
H3B
H4B
*
CDK11 Ab ChIP noAb
FLASH Ab ChIP hTERT
ChIP rabIgG hTERT
CDK11 Ab ChIP
FLASH Ab ChIP U2OS
ChIP rabIgG U2OS
Spearman correlation on histones
0.40 0.75 0.77 0.78 0.82 1.00 rabIgG ChIP U2OS
0.32 0.81 0.75 0.80 1.00 0.82 FLASH Ab ChIP U2OS
0.41 0.80 0.75 1.00 0.80 0.78 CDK11 Ab ChIP
0.46 0.89 1.00 0.75 0.75 0.77 rabIgG ChIP hTERT
0.50 1.00 0.89 0.80 0.81 0.75 FLASH Ab ChIP hTERT
1.00 0.50 0.46 0.41 0.32 0.40 CDK11 ChIP noAb
0 0.2 0.4 0.6 0.8 1

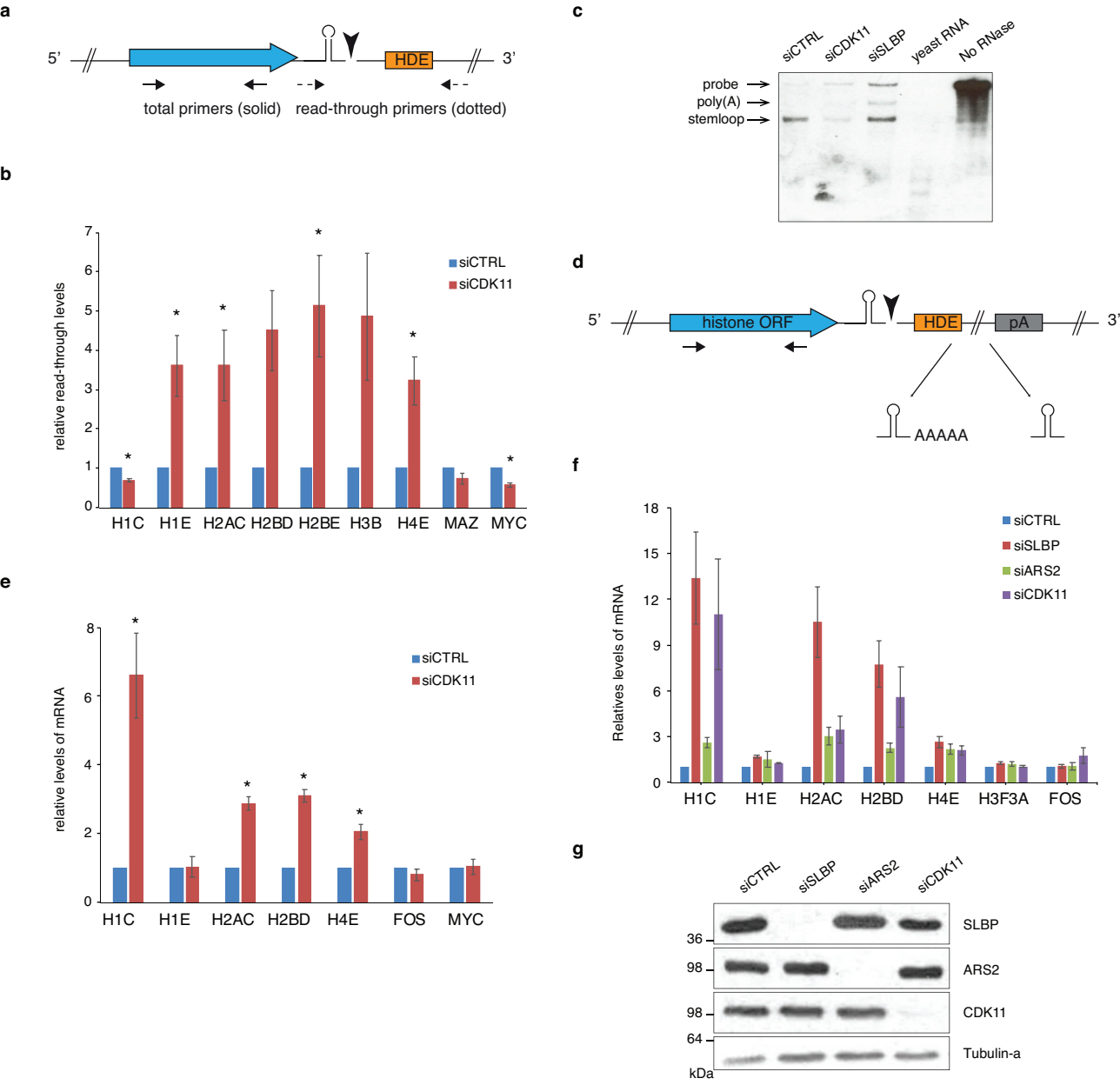

a
G1/S
S
G2/M
flag
endo
CDK11
CCNE1
50
64
FUS
kDa
b
Relative levels of mRNA
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
G1/S
S
G2/M
H1C
H1E
H2AC
H2BE
H4B
H3B
H4E
H3F3A
c
asynchronous
2h (S)
0h (G1/S)
8h (G2/M)
Propidium Iodide-A
d
siRNA:
CTRL
CDK11
24h
40h
64h
short exp.
long exp.
250
148
98
64
50
FLASH
CDK11
Tubulin-a
1
2
3
4
e
siRNA:
CTRL
FLASH
250
148
98
64
50
FLASH
CDK11
Tubulin-a
kDa
1
2
f
>HIST1H4E
>HIST1H1C
CDK11 ChIP-seq
stem loop
asynchronous
asynchronous peaks
G2/M phase
S phase
noAb
15
0

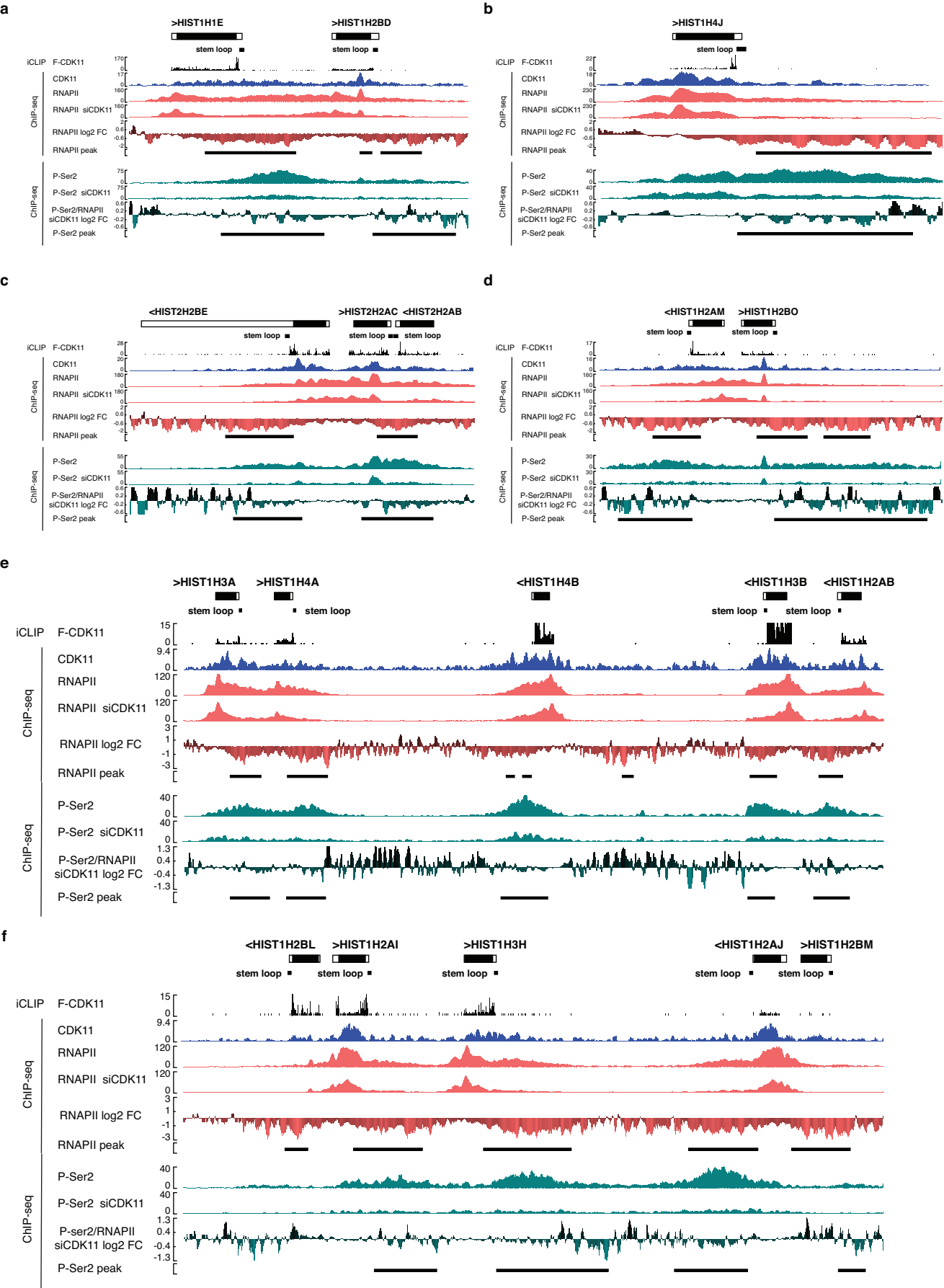

a
b
293 cells stably expressing F-CDK11 proteins or F-EV
crosslinking
254nm UV or
4SU + 365nm UV
F-CDK11
RNA
Lysis
RNase I digestion
Immunoprecipitation
L3 ligation
F-CDK11
RNA
SDS-Page
Proteinase K treatment
RNA isolation
RNA
Reverse transcription
Size selection
Circularization, RE digest
PCR amplification
Illumina sequencing
c
F-CDK11
hnRNPC
RNase I
RNase I
191
97
kDa
RNA-CDK11
complexes
CDK11
(autophosp.)
191
97
61
39
kDa
RNA-hnRNPC
complexes
1 2 3 4 5 6
7 8 9
d
4SU 365 (1)
4SU 365 (2)
254 (1)
254 (2)
noAb
0.84
0.75
0.64
0.77
0.75
0.75
0.17
0.23
0.17
0.35
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1
e
254(2)
4SU_365(2)
254(1)
4SU_365(1)
0%
25%
50%
75%
100%
Normalised peaks percentage
3UTR
5UTR
intergenic
intron
ncRNA
ORF
undefined


a
-log 10 (FDR)
cellular process
chromatin assembly or disassembly
protein-DNA complex assembly
protein-DNA complex subunit
nucleosome organization
nucleosome assembly
gene silencing
negative regulation of gene expression, epigenetic
regulation of gene expression, epigenetic
chromatin organization
chromatin silencing
0 1 2 3 4 5 6 7 8
b
STOP codon
>HIST1H1E zoom up
stem loop
F-CDK11 iCLIP
>HIST1H1E
stem loop
iCLIP
F-CDK11
F-CDK11 (226-783)
F-CDK11 NoUV
c
5kb
>H3F3A
iCLIP
F-CDK11
F-CDK11 (226-783)
F-CDK11 no UV
2kb
>H1F0
iCLIP
F-CDK11
F-CDK11 (226-783)
F-CDK11 no UV
d
10kb
>FOS
iCLIP
F-CDK11
F-CDK11 (226-783)
F-CDK11 no UV
10kb
>PPIA
iCLIP
F-CDK11
F-CDK11 (226-783)
F-CDK11 no UV
e
RNA-seq: siCTRL
Histones
Highly expr. genes
RNA-seq: siCTRL RPKM
-0.5 TSS TES 0.5Kb
iCLIP: F-CDK11
iCLIP: F-CDK11 Crosslinks
-0.5 TSS TES 0.5Kb
f
RIP: CDK11
CDK11 Ab
CDK11 Ab (+siCDK11)
no Ab
% of input
H1C H1E H2AC H2BE H3B H4E MAZ H3F3A
g
INPUT
IP: CDK11
IP: no Ab
IP: CDK11
siRNA: CTRL CDK11 CTRL CDK11
98
64
kDa
CDK11
FUS
1 2 3 4 5
h
RIP: F-CDK11
F-CDK11
F-CDK11(1-226)
F-CDK11 (226-783)
F-CDK11 no Ab
% of input
H1C H1E H2AC H2BE H3B H4E H3F3A
i
F-CDK11
(1-226)
(226-783)
INPUT
IP:FLAG
98
50
kDa
F-CDK11
1 2 3 4 5 6
j
F-EV
F-CDK11
F-CDK11
F-CDK11 (226-783)
M-CDK11:
INPUT
IP:FLAG
98
64
kDa
M-CDK11
F-CDK11
M-CDK11
1 2 3 4


a
nuclear pellet
(EDTA+EGTA buffer)
nuclear soluble1
- RNase
+ RNase
nuclear soluble2
nuclear soluble2
nuclear insoluble
nuclear insoluble
b
INPUT
IP: FLASH
IP: FLASH
IP: no Ab
RNase:
- + - + -
250
98
kDa
FLASH
CDK11
1 2 3 4 5
c
nuclear soluble 1
nuclear soluble 2
insoluble
RNase - - - + - +
250
98
64
50
16
kDa
F-FLASH
CDK11
FUS
ACTIN
histone H2a
1 2 3 4 5 6
d
F-CDK11
F-CDK11 (1-226)
F-CDK11 (226-783)
F-EV
F-CDK11 (344-783)
INPUT
IP: FLASH
FLASH
98
64
50
36
kDa
F-CDK11
1 2 3 4 5
e
CTRL
amanitin
triptolide
flag
endo
64
kDa
CDK11
FUS


a
ChIP-seq: P-Ser2
Highly expressed genes
Other genes
ChIP-seq: RPGC
P-Ser2 siCTRL
P-Ser2 siCDK11
P-Ser2 noAb
-0.5 TSS
TES
3.0Kb
b
500 bp
>HIST1H2AJ
stem loop
ChIP-seq
RNAPII
RNAPII siCDK11
RNAPII log2 FC
RNAPII peak
P-Ser2
P-Ser2 siCDK11
P-Ser2/RNAPII siCDK11 log2 FC
P-Ser2 peak
>HIST1H1C
c
ChIP-seq:RNAPII
RNAPII siCTRL
RNAPII siCDK11
RNAPII noAb
d
ChIP: RNAPII
% input
siCTRL
siCDK11
Ir1
H3B
H1E
H4B
H2BD
H2AC
e
ChIP: P-Ser5
f
ratio P-Ser5/RNAPII
P-Ser5/RNAPII
g
ChIP: P-Thr4
% of input
noAb
Ir
H1C
MYC
EEF1A


a
5'
HDE
3'
total primers (solid)
read-through primers (dotted)
b
relative read-through levels
siCTRL
siCDK11
H1C
H1E
H2AC
H2BD
H2BE
H3B
H4E
MAZ
MYC
c
siCTRL
siCDK11
siSLBP
yeast RNA
No RNase
probe
poly(A)
stemloop
d
5'
histone ORF
HDE
pA
3'
AAAAA
e
relative levels of mRNA
siCTRL
siCDK11
H1C
H1E
H2AC
H2BD
H4E
FOS
MYC
f
Relatives levels of mRNA
siCTRL
siSLBP
siARS2
siCDK11
H1C
H1E
H2AC
H2BD
H4E
H3F3A
FOS
g
siCTRL
siSLBP
siARS2
siCDK11
SLBP
ARS2
CDK11
Tubulin-a
36
98
98
64
kDa


a
>HIST1H1E
>HIST1H2BD
stem loop
stem loop
iCLIP F-CDK11
ChIP-seq CDK11
RNAPII
RNAPII siCDK11
RNAPII log2 FC
RNAPII peak
ChIP-seq P-Ser2
P-Ser2 siCDK11
P-Ser2/RNAPII siCDK11 log2 FC
P-Ser2 peak
b
>HIST1H4J
stem loop
c
<HIST2H2BE
>HIST2H2AC
<HIST2H2AB
stem loop
stem loop
stem loop
d
<HIST1H2AM
>HIST1H2BO
stem loop
stem loop
e
>HIST1H3A
>HIST1H4A
<HIST1H4B
<HIST1H3B
<HIST1H2AB
stem loop
stem loop
stem loop
stem loop
f
<HIST1H2BL
>HIST1H2AI
>HIST1H3H
<HIST1H2AJ
>HIST1H2BM
stem loop
stem loop
stem loop
stem loop
stem loop