Tyrosine phosphorylation of histone H2A by CK2 regulates transcriptional elongation

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Post-translational histone modifications have a critical role in regulating transcription, the cell cycle, DNA replication and DNA damage repair. The identification of new histone modifications critical for transcriptional regulation at initiation, elongation or termination is of particular interest. Here we report a new layer of regulation in transcriptional elongation that is conserved from yeast to mammals. This regulation is based on the phosphorylation of a highly conserved tyrosine residue, Tyr 57, in histone H2A and is mediated by the unsuspected tyrosine kinase activity of casein kinase 2 (CK2). Mutation of Tyr 57 in H2A in yeast or inhibition of CK2 activity impairs transcriptional elongation in yeast as well as in mammalian cells. Genome-wide binding analysis reveals that CK2α, the catalytic subunit of CK2, binds across RNA-polymerase-II-transcribed coding genes and active enhancers. Mutation of Tyr 57 causes a loss of H2B mono-ubiquitination as well as H3K4me3 and H3K79me3, histone marks associated with active transcription. Mechanistically, both CK2 inhibition and the H2A(Y57F) mutation enhance H2B deubiquitination activity of the Spt-Ada-Gcn5 acetyltransferase (SAGA) complex, suggesting a critical role of this phosphorylation in coordinating the activity of the SAGA complex during transcription. Together, these results identify a new component of regulation in transcriptional elongation based on CK2-dependent tyrosine phosphorylation of the globular domain of H2A.

To assess potential tyrosine phosphorylation events in H2A, we individually mutated every tyrosine residue in H2A to phenylalanine and expressed the mutants in 293T cells. Mutation of Tyr 39 and Tyr 57 resulted in a decrease in tyrosine phosphorylation compared to the wild-type protein, indicating that these residues might be phosphorylated (Fig. 1a). Mass spectrometry confirmed phosphorylation of these residues in histone extracts from 293T cells (Supplementary Table 1). The Tyr 57 residue, along with neighbouring residues, is conserved from yeast to mammals (Fig. 1b), and is present in all variants of H2A (Extended Data Fig. 1a). In budding yeast, where genetic manipulation of histones is possible, mutation of the corresponding residue to alanine is lethal, suggesting a critical structural and/or functional contribution of this tyrosine residue2,3. Analysis of histones in wild-type mononucleosomes and those containing the H2A(Y57F) mutant showed similar stoichiometry, suggesting that the Y57F mutation is unlikely to affect the structural integrity of nucleosomes (Extended Data Fig. 1b). Hence, we tested whether the structurally conservative substitution of tyrosine with phenylalanine would

Figure 1 | The conserved Tyr 57 residue in H2A is phosphorylated. a, Tyr 57 in H2A is phosphorylated in 293T cells. Flag-tagged H2A mutants were expressed in 293T cells, immunoprecipitated (IP) under denaturing conditions, and immunoblotted (IB) as indicated. Vec., vector; WT, wild type. b, Tyr 57 in H2A is highly conserved. Comparison of H2A sequence surrounding the Tyr 57 residue (arrow) in different organisms. H., Homo; G., Gallus; X., Xenopus; S., Saccharomyces (for cerevisiae); S., Schizosaccharomyces (for pombe). c, Tyr 58 in H2A is functionally important in yeast. Fivefold serial dilutions of the yeast strains lacking H2A (hta) and H2B (htb) but containing pHJ33 (HTA1-HTB1 HHF2-HHT2 URA3 CEN) were transformed as indicated, and the transformants were plated on SC (synthetic complete supplement)—His–Ura for growth control and 5-fluoroorotic acid (5-FOA) for the removal of pHJ33. d, e, Tyr 58 in H2A is phosphorylated in S. cerevisiae. d, Flag-tagged wild-type H2A and H2A(Y58F) were immunoprecipitated under denaturing conditions and immunoblotted as indicated. e, Whole-cell extracts from yeast strains were prepared under denaturing conditions and immunoblotted as indicated. Data represent three independent experiments.
produce a different phenotype in yeast. Tyr 58 in yeast H2A corresponds to Tyr 57 in mammalian H2A. The H2A(Y58F) mutant was viable and exhibited a slow growth phenotype (Fig. 1c). Notably, the same mutation proved to be lethal in the HTZ1 (the gene encoding H2AZ also known as H2AFZ) null background, and double mutation of the tyrosine residue in both H2A and H2AZ resulted in an extremely slow growth phenotype (Fig. 1c and Extended Data Fig. 1c). Next, we tested if this site is phosphorylated in yeast. Immunoprecipitated Flag-tagged H2A(Y58F) showed reduced tyrosine phosphorylation compared to the wild-type protein (Fig. 1d), suggesting that this residue is phosphorylated.

To confirm Tyr 57 phosphorylation and investigate its function, an antibody specific for phosphorylated Tyr (pTyr) 57 H2A was developed. This antibody detected proteins corresponding to the size of H2A and ubiquitinated H2A in 293T cells (Extended Data Fig. 1d). Peptide blocking assays and dot blot assays verified the specificity of the antibody and treatment with calf intestinal phosphatase further validated its phosphospecificity (Extended Data Fig. 1d–i). Use of this antibody confirmed Tyr 58 phosphorylation in yeast H2A (Fig. 1e). Collectively, these results demonstrate that Tyr 57 in H2A is phosphorylated and that this phosphorylation is conserved from yeast to mammals.

To identify the kinase(s) that mediate(s) phosphorylation of Tyr 57 in H2A in 293T cells, we performed mass spectrometry analysis of proteins interacting with H2A in 293T cells. To be consistent with yeast, we used H2AX (also known as H2AFX), a common H2A variant that has closer sequence homology to yeast H2A, for co-immunoprecipitation and interacting with H2A in 293T cells. To be consistent with yeast, we used kinase assays were performed using recombinant glutathione S-transferase (GST)-CK2α, and full-length or nucleosomal Flag-tagged H2AX purified from 293T cells, and were immunoblotted to examine tyrosine phosphorylation. c, CK2 phosphorylates Tyr 57 in H2A in vivo. c, CK2α was knocked down in 293T cells, and nuclear extracts were immunoblotted. Ctrl indicates scrambled short interfering RNA (siRNA). d, Nuclear extract from 293T cells treated with vehicle (Veh.) or TBBz for 3 h were immunoblotted. Data represent three independent experiments.

Next we investigated whether CK2 is necessary for H2A Tyr 57 phosphorylation in vivo. CK2α knockdown in 293T cells reduced the level of Tyr 57 phosphorylation in H2A (Fig. 2c), supporting an in vivo role of CK2α in regulating this phosphorylation. Moreover, a dose-dependent decrease in H2A Tyr 57 phosphorylation was observed upon treatment with TBBz (Fig. 2d), further supporting the role of CK2α in 57 phosphorylation in H2A. Together, these results provide strong evidence for a function of CK2α in H2A Tyr 57 phosphorylation.

To investigate the physiological significance of Tyr 57 phosphorylation in H2A, we examined in yeast the impact of the H2A(Y58F) mutation on other important histone marks. We found that the H2A(Y58F) mutation resulted in a loss of H2B mono-ubiquitination, as well as tri-methylation of H3K4 and H3K79 (Fig. 3a). H3K27 acetylation showed a modest increase, and all other histone modifications tested were unaffected (Extended Data Fig. 3a). Notably, the Y57F mutation also lowered the level of H2A mono-ubiquitination in 293T cells (Extended Data Fig. 3b, c). The role of H2A Tyr 58 phosphorylation as a potential regulator of H2B mono-ubiquitination is of particular significance because this modification has an established role in transcriptional elongation, thus potentially linking H2A Tyr 58 phosphorylation to transcriptional elongation. Further, yeast with the H2A(Y58F) mutation exhibited increased sensitivity to 6-azauracil (Extended Data Fig. 3d), indicating a defect in transcriptional elongation.

To assess the role of Tyr 58 phosphorylation in transcriptional elongation, binding of RNA polymerase II (Pol II) in actively transcribed genes was evaluated by chromatin immunoprecipitation (ChIP) followed by quantitative real-time polymerase chain reaction (qPCR). Pol II binding was reduced in the gene body of a housekeeping gene, PKY1, as well as a number of heat-shock-induced genes upon heat shock in the H2A(Y58F) mutant (Fig. 3b). Pol II binding was further reduced in H2A(Y58F) and H2AZ(Y65F) mutants (Fig. 3b). The decrease in Pol II binding in the H2A(Y58F) mutant was not due to reduced Pol II expression (Extended Data Fig. 3e). Consistent with the defect in transcriptional elongation, a decreased level of messenger RNA of the corresponding genes was observed in H2A(Y58F) mutants (Extended Data Fig. 3f), whereas H2AZ(Y65F) mutation alone caused only a mild defect in transcription of heat-shock-induced genes (Extended Data Fig. 3g). Furthermore, in 293T cells, H2A Tyr 57 phosphorylation, like H2B mono-ubiquitination, was correlated with transcriptional elongation events, as demonstrated when transcriptional elongation was blocked with flavopiridol treatment, and induced by washing out the drug (Extended Data Fig. 3h). H3K4me2, a control histone mark, did not change in this assay (Extended Data Fig. 3h). Collectively, these results indicate a conserved role of Tyr 57/58 phosphorylation in H2A in regulating transcriptional elongation.
To address the mechanism through which H2A Tyr 57/58 phosphorylation regulates H2B mono-ubiquitination, we examined the recruitment of proteins known to be involved in establishing H2B mono-ubiquitination, such as Paf1, Rtf1 and Rad6 (refs 13–15), by ChIP-qPCR. The binding of Paf1 and Rtf1 was comparable in wild-type and H2A(Y58F) yeast strains, whereas Rad6 binding was slightly reduced in the genes tested in the H2A(Y58F) mutant strain (Extended Data Fig. 4a–c). The effect of the H2A(Y58F) mutation was further evaluated in yeast lacking UBP8, which encodes a major H2B deubiquitinase that is a component of the SAGA complex. Deletion of UBP8 restored H2B mono-ubiquitination in the H2A(Y58F) mutant to the wild-type level (Fig. 3c), suggesting that the defect in H2B mono-ubiquitination in the H2A(Y58F) mutant occurs through Ubp8 deubiquitinase activity, and not through defective ubiquitination machinery. Likewise, CK2 inhibition reduced H2B mono-ubiquitination in wild-type yeast while having no effect in UBP8 mutants (Fig. 3d), further supporting the role of CK2-mediated H2A Tyr 58 phosphorylation in preventing H2B deubiquitination. Notably, despite the complete rescue of H2B mono-ubiquitination, UBP8 deletion only partially rescued H3K79me3, and the level of H3K4me3 remained low in the H2A(Y58F) mutant (Fig. 3c). Furthermore, deletion of UBP8 did not rescue defects in Pol II binding, the transcript levels, or the slow growth phenotype of the H2A(Y58F) mutant (Extended Data Fig. 4d–f). These results suggest that the physiological effects of Tyr 58 mutation in H2A are linked to, but extend beyond, the loss of H2B mono-ubiquitination.

Next, we investigated whether knockdown or inhibition of CK2 phenocopies the effects of H2A(Y58F) mutation in the regulation of transcriptional elongation. Consistent with the result in yeast, H2B mono-ubiquitination was reduced upon CK2α knockdown or inhibition of CK2 kinase activity in 293T cells (Fig. 2c, d). Likewise, Pol II binding in active genes in LNCaP human prostate carcinoma cells was impaired in gene bodies but not in the promoter regions upon CK2 inhibition, as determined by ChIP followed by sequencing (ChIP-seq) (Fig. 4a). A travelling ratio plot of Pol II16,17 showed a significant shift in CK2-inhibited cells (Fig. 4a), suggesting that CK2 kinase activity is required for transcriptional elongation in gene bodies. In agreement with this, dihydrotestosterone (DHT)-induced transcriptional activation of androgen-receptor-regulated genes was impaired in LNCaP cells treated with TBBz (Extended Data Fig. 5a).

To understand the molecular aspects of the role of CK2 in transcriptional elongation, genome-wide localization of CK2 in LNCaP cells was determined by ChIP-seq. Like Pol II, CK2α showed binding to actively transcribed genes across gene bodies, although its binding profile was distinct from that of Pol II (Fig. 4b). Meta-analysis of the top 10% of active genes, based on global run-on sequencing results in LNCaP cells18, revealed that CK2α globally co-localizes with Pol II (Fig. 4c). Consistent with the genome-wide binding pattern, CK2α immunoprecipitated with the phosphorylated carboxy-terminal domain (CTD) of Pol II, which is localized in the promoters (pSer 5) and gene bodies (pSer 2) of active genes (Extended Data Fig. 5b). We also found CK2α binding in intergenic regions that co-localized with H3K4me1 and H3K27ac marks, histone modifications that co-localize with active enhancers19, and LNCaP cell-type-specific androgen receptor enhancers (Fig. 4d and Extended Data Fig. 5c), suggesting that the intergenic CK2α peaks are in enhancer regions. Inhibition of CK2α also caused stalling of Pol II in the androgen-receptor-bound enhancers (Fig. 4e, Extended Data Fig. 5d), underpinning the function of CK2 in transcriptional elongation in both gene bodies and enhancers.

We asked if CK2α also regulates transcriptional elongation in yeast, and if so, whether H2A Tyr 58 phosphorylation is a key player, among the other many other substrates of CK220. Inhibition of CK2 kinase activity resulted in a decrease in the recruitment of Pol II in both the promoter region as well as gene bodies of the tested genes in wild-type yeast, but did not have additive effects in the H2A(Y58F) yeast (Fig. 4f). It is noteworthy that both CK2 inhibition and H2A(Y58F) mutation did not result in promoter-proximal pausing in the genes tested, consistent with the observation that regulation by promoter-proximal pausing is rare in yeast21. Collectively, these results demonstrate that CK2 has a deeply conserved role in transcriptional elongation both in gene bodies and enhancers, and that H2A Tyr 58 phosphorylation is critical for this regulation.

This study identifies a new H2A modification, phosphorylation of Tyr 57/58, which provides new insight into how two important protein complexes, SAGA and Paf1, with opposite enzymatic effects on H2B mono-ubiquitination, might be coordinated during transcriptional elongation. The data further emphasize the key significance of this delicate coordination as demonstrated by defects in transcriptional elongation upon mutation of the conserved phosphorylation site. A moderate increase
in Gcn5–SAGA-mediated H3K27 acetylation in the H2A(Y58F) mutant yeast suggests that phosphorylation may antagonize multiple activities of SAGA. Such antagonism could explain the partial rescue of the defects in the H2A(Y58F) mutant upon deletion of UBP8, as the other modules of SAGA remain functional in the absence of Ubp8. Although unlikely, the potential role in transcriptional elongation of the hydroxyl group of Tyr 58, rather than phosphorylation itself, cannot yet be dismissed. Assays using synthetic nucleosomes with constitutively phosphorylated H2A Tyr 57/58 may ultimately be used to further define the role of this site in transcriptional elongation. This study also emphasizes the functional significance of the tyrosine kinase activity of CK2, and encourages the search for other tyrosine substrates of CK2. Importantly, the identification of a highly conserved role of CK2 in regulating transcriptional elongation in both gene bodies and enhancer regions adds yet another layer to understanding transcription.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information ChIP-seq data has been deposited in the Gene Expression Omnibus database under accession number GSE58607. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.G.R. (mrosenfeld@ucsd.edu) or L.P. (lpillus@ucsd.edu).
METHODS

Cell culture, short interfering RNA, primers, plasmids, transfection, antibodies and kinase inhibitors. LNCaP and 293T cells were cultured in F12 medium supplemented with 10% fetal bovine serum (FBS) and glutamine. For the DHT-treatment experiments, LNCaP cells were cultured in deficient DME high glucose medium with 5% FBS (charcoal dextran filtered) for 3–4 days. Short interfering RNA (siRNA) against CK2β was from Santa Cruz (sc-29918). Cells were transfected using lipofectamine 2000 (Invitrogen) using the manufacturer’s protocol. Mutagenesis was done using Quikchange Lightening Mutagenesis Kit following the manufacturer’s recommended protocol. The following antibodies were used in this study: pTyr 57 H2A antibody was generated by Biomatik Company using pTyr 57 H2A peptide as an antigen (LE(P)YLTAEILEAGNC), purified, and positively selected using a pTyr 57 H2A peptide column, and negatively selected using a Tyr 57 H2A peptide column; anti-H2A (Abcam ab330), anti-CK2α (Abcam ab 70774), anti-H2BK120ub1 (Cell Signaling no. 35665), anti-Flag (Sigma M2), anti-RNA polymerase II (Santa Cruz N-20 (for mammalian cells), Abcam no. ab817 (for yeast cells)), anti-pSer 2 Pol II (Abcam ab3095), anti-pSer 5 Pol II (Abcam ab5131), anti-pTyr (Millipore no. 05-321 (4G10)), anti-H2A (yeast) (Active Motif no. 39236), anti-H4K16me3 (Active Motif no. 39159), anti-H3K4me2 (Active Motif no. 07-030), anti-H3K4me1 (Millipore no. 07-436), anti-pSer 10-H3 (Millipore no. 06-570), anti-H3K36me2 (Active Motif no. 39255) and anti-H2AK119ub (Cell Signaling no. 82405). TBBz and flavopiridol were from Sigma. Primers used in this study are listed in Supplementary Table 3.

Chromatin immunoprecipitation. Cells were grown to 90–95% confluence, fixed with 1% formaldehyde for 15 min for Pol II ChIP, and with di-succinimidyl glutarate (DSG) for 45 min followed by 10 min fixation with 1% formaldehyde for CK2 inhibitor treatment. Cells were fixed for 15 min, and then incubated with glycine (1.25 mM) for 10 min. Nuclei were prepared using a pTyr 57 H2A peptide column, and negatively selected using a Tyr 57 H2A peptide column; anti-H2A (Abcam ab330), anti-CK2α (Abcam ab 70774), anti-H2BK120ub1 (Cell Signaling no. 35665), anti-Flag (Sigma M2), anti-RNA polymerase II (Santa Cruz N-20 (for mammalian cells), Abcam no. ab817 (for yeast cells)), anti-pSer 2 Pol II (Abcam ab3095), anti-pSer 5 Pol II (Abcam ab5131), anti-pTyr (Millipore no. 05-321 (4G10)), anti-H2A (yeast) (Active Motif no. 39236), anti-H4K16me3 (Active Motif no. 39159), anti-H3K4me2 (Active Motif no. 07-030), anti-H3K4me1 (Millipore no. 07-436), anti-pSer 10-H3 (Millipore no. 06-570), anti-H3K36me2 (Active Motif no. 39255) and anti-H2AK119ub (Cell Signaling no. 82405). TBBz and flavopiridol were from Sigma. Primers used in this study are listed in Supplementary Table 3.

In vitro kinase assay and phosphoamino acid analysis (PAA). In vitro kinase reactions were performed with 100 ng of recombinant GST-tagged CK2β (expressed in E. coli with 0.2 mM isopropyl-β-D-thiogalactoside induction for 3 h at 30°C) in 1× kinase buffer (20 mM Tris-HCl, 50 mM KCl, 10 mM MgCl₂, pH 7.5) with the addition of 0.2 mM ATP for cold reactions (and 10 mM ATP mixed with 10 μCi of γ-[32P]ATP for the radioactive reactions). The substrates (Flag-tagged wild-type H2AX and H2AX(T53F)) were purified from 293T cells expressing Flag-tagged H2AX constructs. For the full-length proteins, histone extracts were immunoprecipitated using Flag antibody, then washed several times with wash buffer (1% Triton X-100, 900 mM NaCl, 20 mM Tris 8.0), treated with calf intestinal phosphatase for 30 min at 37°C, washed a few more times with wash buffer, and eluted with 3× Flag peptides. Mononucleosomes were prepared as described with minor changes in micrococcal nuclease digestion. In brief, nuclei were isolated from 15-cm diameter dishes, and DNA was digested in 1.2 ml total volume with 25 μl micrococcal nuclease (NEB no. M0247S) for 10 min at 37°C, and the reaction was stopped by adding 5 mM EGTA, and mononucleosomes were collected by centrifugation. Mononucleosomes were immunoprecipitated with Flag antibody, washed four times with buffer A (340 mM sucrose, 10 mM HEPS pH 7.5, 10% glycerol, 1.5 mM MgCl₂, 10 mM KCl) followed by three washes with kinase reaction buffer, then treatment with 500 μM FSa (Sigma no. F9128-25MG) for 25 min at 37°C to irreversibly inhibit any potential kinases interacting with the nucleosome. Samples were washed three times with kinase buffer, treated with calf intestinal phosphatase for 30 min at 37°C, and washed a further three times withbuffer A. The bound nucleosomes were eluted with 3× Flag peptides in buffer A. The kinase reactions were carried out for 1 h at 37°C. For PAA, the samples were separated by SDS–PAGE, transferred to polyvinylidene difluoride membrane, and the membrane corresponding to the mobility of phosphorylated H2AX was excised, and PAA using two-dimensional electrophoresis on thin layer cellulose plates was performed as described.[29]

Whole-cell extracts, immunoprecipitation and cell fractionation. Yeast whole-cell extracts were prepared either by breaking the cells with glass beads in PBS or boiling the cells in denaturing buffer (2% SDS with 30 mM dithiothreitol) for 10 min. To immunoprecipitate the Flag-tagged proteins under denaturing conditions, whole-cell extracts were prepared as noted above in denaturing buffer, and the SDS concentration was adjusted to 0.1% by adding dilution buffer (150 mM NaCl, 1% Triton X-100, 20 mM Tris pH 8.0), then immunoprecipitated overnight using anti-Flag (M2-Sigma) conjugated to agarose beads, and washed five times with dilution buffer. Bound proteins were eluted with 100 μg ml⁻¹ 3× Flag peptides in Tris-buffered saline for 20 min at 37°C twice and then eluted overnight with 5 μg of M2 antibody conjugated to magnetic beads. The beads were washed three times with the same lysis buffer, and the proteins bound to the beads were analysed by mass spectrometry or by immunoblotting.

Mass spectrometry. Protein samples were prepared as described.[29] In brief, the protein samples were diluted in TNE buffer (50 mM Tris pH 8.0, 100 mM KCl, 1 mM EDTA). RapiGest SF reagent (Waters Corp.) was added to the mix to a final concentration of 0.1% and samples were boiled for 5 min. TCEP (Tris (2-carboxyethyl) phosphine) was added to a final concentration of 1 mM and the samples were incubated for 30 min at 37°C. Subsequently, the samples were carboxymethylated by 500 μg ml⁻¹ of iodination reagent for 30 min at 37°C followed by reduction with 2 μl of M2 Flag antibody conjugated to magnetic beads. The beads were washed three times with the same lysis buffer, and the proteins bound to the beads were analysed by mass spectrometry or by immunoblotting.
was then added to a new tube and the peptides were extracted and desalted using C18 desalting columns (Thermo Scientific).

Trypsin-digested peptides were analysed by ultra-high-pressure liquid chromatography (UPLC) coupled with tandem mass spectroscopy (MS/MS) using nano-spray ionization as described29. The nano-spray ionization experiments were performed using a TripleTof 5600 hybrid mass spectrometer (ABSCIEX) interfaced with nanoscale reversed-phase UPLC (Waters corporation nano ACQUITY) using a 20-cm, 75-micrometer inside diameter glass capillary tube packed with 2.5-μm C18 (130) CSH beads (Waters corporation). Peptides were eluted from the C18 column into the mass spectrometer using a linear gradient (5–80%) of ACN (acetonitrile) at a flow rate of 250 μl per minute for 1 h. The buffers used to create the ACN gradient were: buffer A (98% H2O, 2% ACN, 0.1% formic acid, and 0.005% TFA (trifluoroacetic acid)) and buffer B (100% ACN, 0.1% formic acid, and 0.005% TFA). MS/MS data were acquired in a data-dependent manner in which the MS1 (initial mass-to-charge-ratio (m/z) spectrum) data were acquired for 250 ms at an m/z of 400 to 1,250 Da and the MS2 (MS/MS or tandem MS) data were acquired from an m/z of 50 to 2,000 Da. The independent data acquisition parameters were as follows: MS1-TOF (time-of-flight) acquisition time of 250 ms, followed by 50 MS2 events of 48 ms acquisition time for each event. The threshold to trigger an MS2 event was set to 150 counts when the ion had the charge state +2, +3 or +4. The ion exclusion time was set to 4 s. Finally, the collected data were analysed using Protein Pilot 4.5 (ABSCIEX) for peptide identifications.

Yeast strains and yeast plasmids used in the study. Yeast strains and yeast plasmids used in the study are described in Supplementary Table 4.

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Extended Data Figure 1 | The conserved Tyr 57 residue in H2A is phosphorylated. a, The Tyr 57 residue is conserved in all variants of H2A. Sequences of H2A variants surrounding the Tyr 57 residue (arrow) in mammals is shown, particular variant residues are highlighted in blue. b, The Y57F mutation in H2A does not affect the structural integrity of nucleosomes. Mononucleosomes containing Flag-tagged wild-type H2AX or H2AX(Y57F) were immunoprecipitated, and histones and DNA were visualized by Ponceau staining (right) and by ultraviolet light (left), respectively. c, The H2A Tyr 58 residue has overlapping functions with the H2AZ Tyr 65 residue in yeast. Fivefold serial dilutions of the indicated transformants were plated on SC–His–Ura–Trp for growth and 5-FOA for the loss of pJH33. d, e, H2A Tyr 57 is phosphorylated in 293T cells. d, Nuclear extracts from 293T cells were immunoblotted (IB) with anti-pTyr 57 H2A pre-incubated with indicated peptides, and re-probed with anti-H2A. e, Histone extracts from 293T cells were treated with calf intestinal phosphatase (CIP) for 1 h at 37 °C, and immunoblotted. f, The anti-pTyr 57 H2A antibody specifically recognizes the H2A peptide phosphorylated at Tyr 57 but not the non-phosphorylated peptide. Indicated peptides were spotted on nitrocellulose, and probed with anti-pTyr 57 H2A. Data represent three independent experiments.
Extended Data Figure 2 | CK2α phosphorylates Tyr 57 in H2A. An in vitro kinase reaction was performed using recombinant GST-CK2α, 10 μCi of [γ-32P]ATP supplemented with 10 μM cold ATP, and nucleosomes containing Flag-tagged wild-type H2AX or H2AX(Y57F) from 293T cells, and phosphoamino acid analysis of the phosphorylated Flag-tagged H2AX was performed. The red circle indicates pSer, the blue circle indicates pThr and the green circle indicates pTyr. Data represent two independent experiments.
Extended Data Figure 3 | H2A Tyr 57 phosphorylation regulates transcriptional elongation. a, H2A(Y58F) mutation does not affect several other histone marks. Whole-cell extracts from wild-type (WT) or H2A(Y58F) yeast cells were immunoblotted (IB). b, c, H2A(Y57F) mutation affects H2A ubiquitination in 293T cells. b, Flag-tagged wild-type H2A/H2AX and Y57F mutants were expressed in 293T cells, and mono-ubiquitination was assessed by immunoblotting with Flag antibody. c, The Flag-tagged H2A mutants were expressed in 293T cells, immunoprecipitated (IP) under denaturing conditions, and immunoblotted. d, H2A(Y58F) mutant cells are defective in transcriptional elongation. Fivefold serial dilutions of wild-type and H2A(Y58F) cells were plated on SC supplemented with NH₄OH (solvent) or 100 μg ml⁻¹ 6-azauracil (6-AU). e, Pol II protein level is comparable in wild-type and H2A(Y58F) yeast. Whole-cell extracts from wild-type or H2A(Y58F) yeast were immunoblotted. f, H2A(Y58F) mutation affects transcription. Wild-type H2A, H2A(Y58F), and H2A(Y58F) H2AZ(Y65F) yeast were grown at 30 °C or shifted to 37 °C for 10 min. RNA was extracted and transcript levels of the indicated genes were measured by reverse-transcription–qPCR, and normalized to SCR1, a Pol III transcript (n = 3, mean ± s.e.m., *P < 0.05, **P < 0.01). P values were calculated with two-tailed Student’s t-tests. g, H2AZ(Y65F) mutation alone in yeast does not affect transcription significantly. Wild type (HTZ1) transformed with vector, and htz1Δ strains transformed with vector (htz1Δ), HTZ1 (WT H2AZ) or HTZ1(Y65F) (H2AZ(Y65F)) were grown at 30 °C (blue bars) or shifted to 37 °C for 10 min. (orange bars), and transcript levels of the indicated genes were measured as in f (n = 3, mean ± s.e.m., *P < 0.05, **P < 0.01). P values were calculated with two-tailed Student’s t-tests. h, Tyr 57 in H2A is phosphorylated during transcriptional elongation. 293T cells were treated with vehicle (DMSO) or flavopiridol (FP) (1 μM) for 4.5 h, then flavopiridol was washed out (release). Cells were harvested at the indicated minute (,) after release, and the nuclear extracts were immunoblotted. Data represent two (a, d, h) or three (b, c, e–g) independent experiments.
Extended Data Figure 4 | H2A(Y58F) mutation enhances H2B deubiquitination. a–c, The H2A Tyr 58 mutation has moderate to no effect on the recruitment of the H2B ubiquitination machinery. Binding of (a) Rtf1-HA, (b) Paf1-myc, and (c) Rad6-HA was measured by ChIP-qPCR in the indicated genes in wild-type and H2A(Y58F) yeast. Whole-cell extracts from the yeast strains were immunoblotted (IB) to compare the protein levels. ORF of the genes, and the region amplified by the primer pairs are shown (n = 2, mean ± s.e.m.). d, UBP8 deletion does not rescue Pol II binding in the H2A(Y58F) mutant. Pol II binding in the indicated strains was measured by ChIP-qPCR. (n = 3, mean ± s.e.m., *P < 0.05, **P < 0.01). Pvalues were calculated with two-tailed Student’s t-tests. The ORF of the genes and the regions amplified by the primer pairs are shown. e, UBP8 deletion does not rescue the defect in transcriptional output in the H2A(Y58F) yeast. The mRNA levels of the indicated genes were determined by RT–qPCR and normalized to the SCR1 transcript. (n = 2, mean ± s.e.m.). f, UBP8 deletion does not rescue the growth defect in the H2A(Y58F) yeast. UBP8 and ubp8Δ strains expressing either wild-type (WT) H2A or H2A(Y58F) were plated at 2.5-fold serial dilutions on SC–His–Ura for growth and 5-FOA for the removal of pJH33. Data represent two (a–c, e, f) or three (d) independent experiments.
Extended Data Figure 5 | CK2 regulates transcriptional elongation. a, CK2 kinase activity is necessary for normal gene expression. LNCaP cells were treated with vehicle (DMSO) or TBBz (25 μM) for 60 min, and then treated with vehicle (ethanol) or 100 nM DHT for 90 min, and induction of the indicated androgen receptor (AR) target genes was measured by RT-qPCR (n = 3, mean ± s.e.m., *P < 0.05, **P < 0.01). P values were calculated with two-tailed Student’s t-tests. b, Nuclear extracts from 293T cells were immunoprecipitated (IP) using CK2α antibody and immunoblotted (IB). c, Enrichment of CK2α, H3K4me1 (ref. 18), H3K27ac (ref. 18) and androgen receptor genes18 at a representative androgen receptor enhancer (KLK3) is shown. d, Pol II tag density in cells treated with vehicle or TBBz at a representative RHOU enhancer is shown. Data represent two (b–d) or three (a) independent experiments; kb, kilobase.