Supplementary Detailed Materials and Methods

Chromatin immunoprecipitation.

ChIP analyses were performed essentially as described (Nelson *et al*, 2006) using the antibodies and dilutions listed in supplementary Table S1. Briefly, medium was removed from the cell culture plates and DNA binding complexes were cross-linked by adding PBS containing 1.42% formaldehyde to the cells for 15 min at room temperature. Cross-linking was quenched by adding 1.25 M glycine to a final concentration of 125 mM and incubated at room temperature for an additional 5 min. Cross-linked cells were then washed twice with ice-cold PBS prior to adding 1 ml IP Buffer (150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5% (v/v) NP-40, 1.0% (v/v) Triton X-100 containing 1 mM NEM, 10 mM BGP and 1X PIC). Cells were scraped, transferred to 1.5 ml tubes and pelleted by centrifugation (12,000 X g) at 4 °C. The nuclear pellets were washed an additional time with IP buffer before resuspending in 300 µl IP buffer. Samples were sonicated using a Bioruptor (Diagenode, Liège, Belgium) on "H" setting 3 times for 10 minutes each with alternating pulses and pauses for 10 s each. Sonicated chromatin was centrifuged at full speed and pre-cleared with 100 µl of 50% Sepharose 4B (GE Healthcare, Piscataway, NJ) slurry in IP Buffer for 1 h at 4 °C. After centrifugation, pre-cleared chromatin was diluted, aliquoted, shock frozen in liquid nitrogen, and stored at -80 °C. For ChIP analysis, 50 µl of chromatin was diluted to a final volume of 500 µl in IP buffer containing NEM, BGP and PIC and incubated overnight with the indicated amounts of antibody (see supplementary Table S1). Chromatin complexes were captured by adding 30 µl of a 50% Protein-A or Protein-G Sepharose slurry (GE Healthcare) and incubated for an additional 2 h with tumbling at 4 °C. Samples were centrifuged at 2000 X g for 2 min at 4 °C and washed 6 times with IP buffer. The immunoprecipitated chromatin complexes were reverse cross-linked by adding 100 µl 10% (w/v) Chelex 100 (BioRad, Hercules, CA) slurry and heating at 95 °C for 10 min. Two µl proteinase K (20 µg/µl, Invitrogen) was added to each sample and incubated at 55 °C for 30 min. Proteinase K was then inactivated by incubating at 95 °C for 10 min. Finally, samples were centrifuged at 12,000 X g for 1 min and the supernatant was transferred to a new tube. For normalization, input samples were prepared by adding 1 µl GlycoBlue (15 µg/µl, Applied Biosystems, Austin, TX) to 50 µl of chromatin extract and precipitated by adding 2.5-3 volumes of ethanol and incubating overnight at -20 °C. Input samples were then centrifuged at

12,000 X g and washed twice with 70% ethanol before preparing DNA by the Chelex method described above.

Gene expression analysis and real-time PCR

RNA was harvested using the TRIZOL method (Invitrogen) according to the manufacturer's instructions. Reverse transcriptions were performed using either oligo-dT ($T_{23}VN$) or random nonamer primers as indicated below or in the respective figure legends. One µg total RNA was mixed with 2 µl random nonamer (15 µM) or oligo-dT (50 µM) primers (Metabion, Martinsried, Germany), 4 µl dNTPs (2.5 mM, Promega), and brought to a volume of 16 µl in RNAse-free water. Samples were heated at 70 °C for 5 minutes and placed on ice. A total volume of 4 µl containing 2 µl 10X reaction buffer, 0.25 µl (10 U) RNAse inhibitor (New England Biolabs, Ipswich, MA), and 0.125 µl (25 U) M-MuLV reverse transcriptase (New England Biolabs) was added to each sample and incubated at 42 °C for 1 h. Finally, the reverse transcriptase was inactivated by heating at 95 °C for 5 minutes and samples were diluted to 50 µl with water.

Two µl of each DNA (ChIP or cDNA) sample were used for quantitative real-time PCR analysis in a final volume of 25 µl. Real-time PCR analysis was performed using the iQ SYBR Green Supermix (BioRad) with the primers listed in supplementary Tables S2 and S3. A two-step protocol (10 min at 95 °C; followed by 40 cycles of: 95 °C for 15 s and 60 °C for 1 min) followed by a melting curve analysis was used for each primer pair and performed on a Chromo4 Real-Time PCR System (BioRad). ChIP and input samples were quantified using a standard curve made from ChIP input DNA. ChIP samples were normalized to their appropriate input samples and expressed as "% input". Experimental background was determined by performing a control ChIP with either a non-specific IgG (see supplementary Table S1 for specific information) and/or no primary antibody (i.e., Protein A-sepharose alone). The average of the background signals for all primer pairs were similar and are graphically represented by the dotted line in Fig 1C-E, Fig 4A-E and Supplemental Fig S2D. RT-PCR analysis was quantified using a mixture of cDNA (for p21 gene expression analysis) or a genomic DNA dilution curve (for determining the precise "% of total transcript" for processed and read-through HIST1H2BD transcripts). The percent of total transcript for the processed and read-through HIST1H2BD transcripts shown in Fig 3C were determined by RT-PCR using random primed cDNA from control or CDK9 siRNA transfected cells and dividing the signal for the "read through"

transcript by the total amount of transcript (for the "% total transcript" for the read-through transcript) and the remaining fraction of total transcript was interpreted as the processed fraction. A schematic showing the position of the amplicons investigated is displayed in Fig 3A. Polyadenylated HIST1H2BD and HIST1H2AC were analyzed by oligo-dT primed reverse transcription followed by quantitative real-time PCR analysis using primers that lie in the first exon of the respective gene. All qRT-PCRs were normalized to 36B4 or MTRNR2 prior to additional normalization and statistical analyses.

Supplementary Table S1 Antibodies used for ChIP and western blot analyses and the respective dilutions.

Target Protein	Clone	Cat. Nr.	ChIP	WB	Source
CBP80	-	-	1 µl	-	(Izaurralde *et al*, 1994)
CDK9	C-20	sc-484	1 µg	1:100	Santa Cruz
Histone H2B	-	07-731	-	1:3,000	Upstate
Histone H2Bub1	-	-	75 µl[†]	1:1,000[†]	(Minsky *et al*, 2008)
Hsc70	B6	sc-7298	-	1:25,000	Santa Cruz
IgG (Non-specific)	-	ab46540	1 µg	-	Abcam
Lsm11	-	-	2 µl	-	(Pillai *et al*, 2003)
NELF-E	KM2479	-	1 µl	-	(Narita *et al*, 2007)
PAF1	-	pAb-015-050	1 µg	-	Diagenode
RNAPII (N-Term)	N-20	sc-899	1 µg	-	Santa Cruz
RNAPII (P-Ser2-CTD)	H5	MMS-129R	2 µl	-	Covance
RNAPII (P-Ser2-CTD)	3E10	-	-	1:10[†]	(Chapman *et al*, 2007)
RNAPII (P-Ser5-CTD)	H14	MMS-134R	1 µl	1:10,000	Covance
RNF20	-	NB100-2242	1 µg	1:2,000	Novus Biologicals
RNF20	-	ab32629	-	-	Abcam
RNF40	-	ab26082	1 µg	1:1,000	Abcam
SLBP	-	-	2 µl	-	(Arnold *et al*, 2008)
TBP	SI-1	sc-273	0.5 µg	-	Santa Cruz
Goat Anti-Mouse IgM	-	M 8644	3 µg	-	Sigma
Donkey Anti-Mouse IgG-HRP	-	715-036-150	-	1:10,000	Jackson ImmunoResearch
Donkey Anti-Mouse IgM-HRP	-	115-035-044	-	1:3,000	Jackson ImmunoResearch
Donkey Anti-Rabbit IgG-HRP	-	711-036-152	-	1:10,000	Jackson ImmunoResearch
Goat Anti-Rat IgG + IgM-HRP	-	112-035-068	-	1:10,000	Jackson ImmunoResearch

[†] From hybridoma supernatant.

Supplementary Table S2 Primers utilized in ChIP in 5' to 3' orientation. Primers not obtained from other previous studies were designed using the Primer3 program (http://frodo.wi.mit.edu/).

ChIP Primers		**Source**
HIST1H2BD TSS F	ACCTCATTTGAATACCGCATCT	This study
HIST1H2BD TSS R	TGGTAGGTTCAGGCATCGTAG	This study
HIST1H2BD +0.5kb F	TCTTTTAAGAGCCACGCATGT	This study
HIST1H2BD +0.5kb R	CGTAACATTAAAGATTGGTACTGTGG	This study
HIST1H2BD +13kb F	TGCCATGGACTACCTTTGCT	This study
HIST1H2BD +13kb R	AGGTTTGGATCAACAATGAAGG	This study
HIST1H2AC TSS F	AAAAGCGGCCATGTTTTACA	This study
HIST1H2AC TSS R	AAAAATCACCAAAACCAGCG	This study
HIST1H2AC +15kb F	AGATTTCCCAAATTCCAGCC	This study
HIST1H2AC +15kb R	AAGGAAGAAAGGAAATGGCAA	This study
p21 TSS F	GGGGCGGTTGTATATCAGG	This study
p21 TSS R	GGCTCCACAAGGAACTGACTTC	(Gomes *et al*, 2006)
p21 5.3kb F	CCAGGGCCTTCCTTGTATCTCT	(Gomes *et al*, 2006)
p21 5.3kb R	ACATCCCCAGCCGGTTCT	(Gomes *et al*, 2006)

Supplementary Table S3 Primers utilized in RT-PCR in 5' to 3' orientation. Primers not obtained from other previous studies were designed using the Primer3 program (http://frodo.wi.mit.edu/).

RT-PCR Primers		**Source**
36B4 F	GATTGGCTACCCAACTGTTG	(Fritah *et al*, 2005)
36B4 R	CAGGGGCAGCAGCCACAAA	(Fritah *et al*, 2005)
HIST1H2BD Total F	ACGATGCCTGAACCTACCAA	This study
HIST1H2BD Total R	AGCCTTAGTCACCGCCTTCT	This study
HIST1H2BD PolyA F	CCAACTCATCCTGGTTTGCT	This study
HIST1H2BD PolyA R	TCCCCTCGGTAACCTTCTTT	This study
HIST2H2AA Total / PolyA F	AAGGCAAAGGGCAAGTGAG	This study
HIST2H2AA Total R	AAGAGCCTTTTGAGTTCACAGGT	This study
HIST2H2AA PolyA R	TGAAAACGTGGGTGGCTCT	This study
HIST1H2AC Total F	GACGAGGAGCTCAACAAACTG	This study
HIST1H2AC Total R	ACCTGTCAAATCACTTGCCC	This study
HIST1H2AC PolyA F	CCTGTCCACTGTTGGTAGGC	This study
HIST1H2AC PolyA R	TTCACTTACCACCATTCCAGC	This study
MTRNR2 F	CATAAGCCTGCGTCAGATCA	This study
MTRNR2 R	CCTGTGTTGGGTTGACAGTG	This study
p21 F	CCAGCTGGGCTCTGCAATT	(Gomes *et al*, 2006)
p21 R	GCTGAGAGGGTACTGAAGGGAAA	(Gomes *et al*, 2006)

Supplementary Table S4 siRNAs utilized in a 5' to 3' orientation.

Target Gene	siRNA Target Sequence	Source	Reference
CDK9 (#1)	UAG GGA CAU GAA GGC UGC UAA	Qiagen	
CDK9 (#2)	GUC AAC UUG AUU GAG AUU UGU CGA A	Invitrogen	
CDK9 (#3)	GCA AGG GUA GUA UAU ACC UGG UGU U	Invitrogen	
CTR9 (#1)	UUC CGU UAC CAC GUC AUA UAA	Qiagen	
CTR9 (#2)	UAG CAA CCC UAU GGU AUU GAA	Qiagen	
CyclinT1	AAT CCC TTC CTG ATA CTA GAA	Qiagen	(Chiu *et al*, 2004)
NELF-E	AAG UCA ACA UAG CCC GAA AUU	Ambion	
RNF20	SmartPool® containing: CCA AUG AAA UCA AGU CUA AUU, UAA GGA AAC UCC AGA AUA UUU, GCA AAU GUC CCA AGU GUA AUU, AGA AGA AGC UAC AUG AUU UUU	Dharmacon	
RNF20 #1	CAG CGA CUC AAC CGA CAC UUA	Ambion	
RNF20 #2	AGC UCU UAU CCC GGA AGC UAA	Ambion	
RNF20 #3	CAG GUC CGC AAG GAG UAU GAA	Ambion	
RNF40	SmartPool® containing: GAG AUG CGC CAC CUG AUU AUU, GAU GCC AAC UUU AAG CUA AUU, GAU CAA GGC CAA CCA GAU UUU, CAA CGA GUC UCU GCA AGU GUU	Dharmacon	
RNF40 #1	AGA GAU GGA UGU GAC AGG UCA	Ambion	
RNF40 #2	TGA GGA CAU GCA GGA ACA GAA	Ambion	
RNF40 #3	AGG UAC UAC AGU UCA AGA ACA	Ambion	
SUPT5H	AAC UGG GCG AGU AUU ACA UGA	Qiagen	(Ping *et al*, 2004)

Supplementary Figure Legends

Supplementary Figure 1. CDK9, RNF20 and RNF40 are essential for maintaining global levels of H2B monoubiquitination. **(A)** The CDK9 inhibitor II (CDK9iII) dose-dependently decreases P-Ser2-RNAPII-CTD and H2Bub1 levels with similar effective concentrations. Cells were treated with different concentrations of CDK9iII and analyzed by western blot for the presence of P-Ser2, H2Bub1 and Hsc70 (loading control). **(B)** RNF20 and -40 levels are unaffected by CDK9 inhibition. The same extracts from H1299 cells treated with CDK9 inhibitors in Fig 2A were analyzed by western blot for the expression of RNF20, RNFr0 and Hsc70. Note that RNF20 and RNF40 expression remain unaltered after 4 h treatment. **(C,D)** The effects of CDK9 knock-down are specific. H1299 cells were transfected as in Fig 2B with three individual siRNAs against hCDK9. (C) Both siRNAs which efficiently targeted CDK9 (#1,3) also efficiently decreased H2Bub1 levels. (D) Over-expression of mCdk9 rescues the H2Bub1 phenotype caused by hCDK9 knock-down. H1299 cells were co-transfected with different combinations of mCdk9 or control expression vector together with a control or hCDK9 specific siRNA as indicated. Hsc70 is shown as a loading control. **(E)** Densitometric analysis of western blot data from Fig 2D. The intensity of the specific H2Bub1 signal was normalized to its respective H2B control and displayed as "Normalized H2Bub1:H2B Ratio"; mean values, error bars show the range of the two values. **(F-H)** RNF20 and RNF40 are essential for H2B monoubiquitination. (F) SiRNA knockdown and rescue was performed as in (D) by transfecting different combinations of specific siRNAs against human RNF40 (or control siRNA) together with an HA-tagged mRnf40 or control expression plasmid. Three distinct siRNAs targeting RNF20 (G) or RNF40 (H) each result in dramatically decreased levels of H2Bub1 and the respective targeted protein. Hsc70 is shown as a loading control.

Supplementary Figure 2. CDK9, RNF20/40 and associated proteins and chromatin modifying enzymes specifically affect replication-dependent histone mRNA polyadenylation. **(A)** The cDNA samples analyzed in Fig 3 were additionally analyzed for the expression of total and polyadenylated HIST2H2AA. **(B)** The knockdown of CyclinT1, SUPT5H, NELF-E, or CTR9 all increase HIST1H2BD polyadenylation. cDNA from H1299 cells that were transfected as in Fig 3 with the indicated siRNAs were analyzed for total and

polyadenylated HIST1H2BD mRNA. The ratio of polyadenylated to total mRNA was normalized to the control and expressed as "relative mRNA expression"; mean values + s.d., n = 3. **(C)** PolyT reverse transcribed cDNA samples from B and Fig 3 were analyzed for the expression of polyadenylated 36B4 mRNA. In order to avoid any non-specific effects due to general effects, samples were not normalized to an internal control. Values are graphed relative to the control sample and are expressed as "relative mRNA expression"; mean values + s.d., n = 3. **(D)** Data for P-Ser2 and P-Ser5 shown in Fig 4B was subtracted from the background values and normalized to the amount of total RNAPII. The data is expressed as relative % of total RNAPII ("P-Ser5 / Total RNAPII"); mean values + s.d., n = 3. **(E)** CDK9 knockdown affects NELF-E recruitment in a similar manner as RNAPII and has no effect on CBP80 recruitment to the HIST1H2BD gene. ChIP extracts analyzed in Fig 4 were examined for the effects on NELF-E and CBP80 recruitment to the HIST1H2BD gene. Note that NELF-E recruitment essentially follows RNAPII (see Fig 4B), while CBP80 is unaffected. ChIP experiments are displayed as "% input" as in Fig 1 and 4; mean values + s.d., n = 3.

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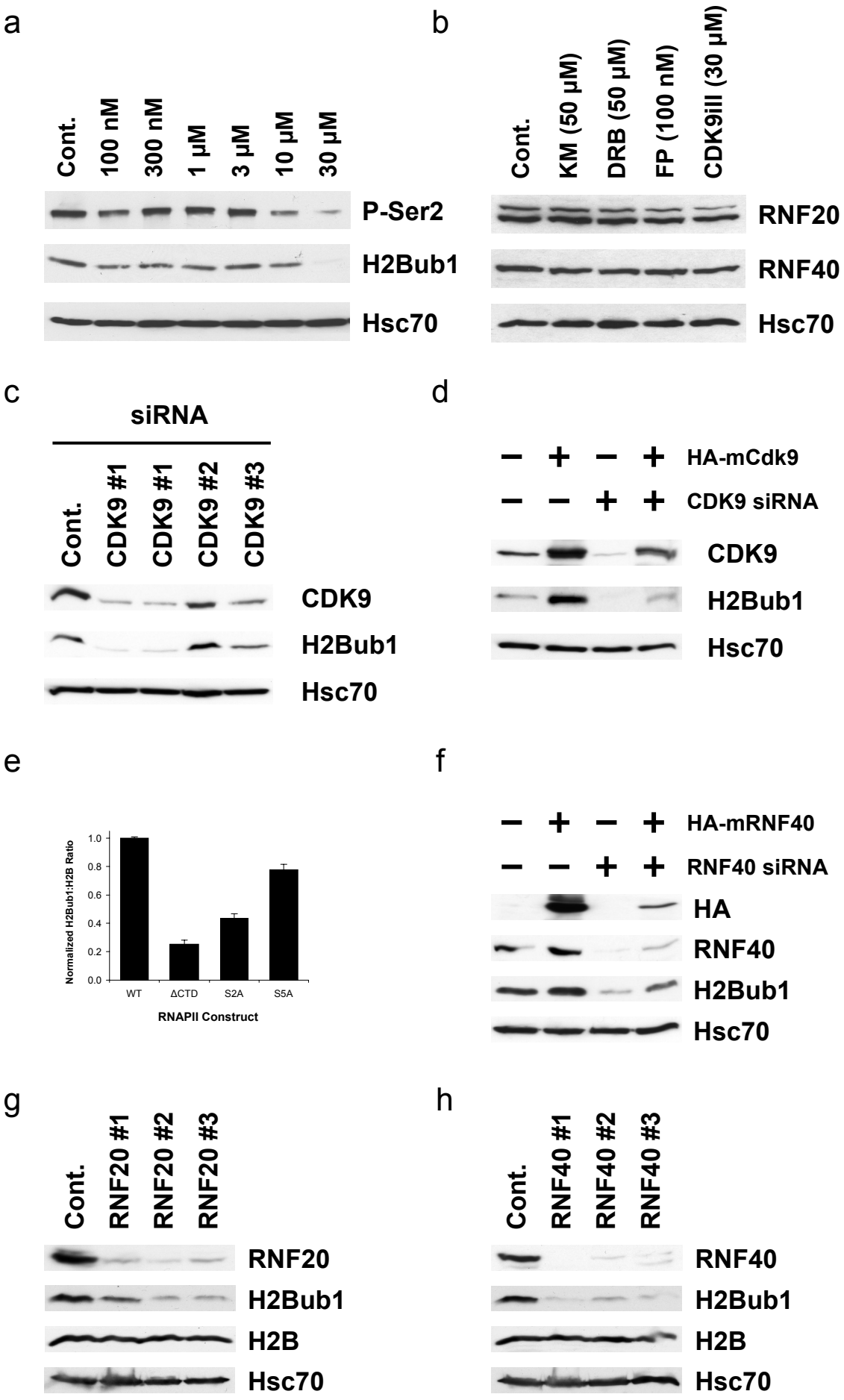
a
Cont.
100 nM
300 nM
1 μM
3 μM
10 μM
30 μM
P-Ser2
H2Bub1
Hsc70
b
Cont.
KM (50 μM)
DRB (50 μM)
FP (100 nM)
CDK9iII (30 μM)
RNF20
RNF40
Hsc70
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siRNA
Cont.
CDK9 #1
CDK9 #1
CDK9 #2
CDK9 #3
CDK9
H2Bub1
Hsc70
d
HA-mCdk9
CDK9 siRNA
CDK9
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Hsc70
e
Normalized H2Bub1:H2B Ratio
1.0
0.8
0.6
0.4
0.2
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WT
ΔCTD
S2A
S5A
RNAPII Construct
f
HA-mRNF40
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HA
RNF40
H2Bub1
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