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Supplemental Data

Recognition of Trimethylated Histone H3 Lysine 4 Facilitates the Recruitment of Transcription Postinitiation Factors and Pre-mRNA Splicing

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Supplemental Experimental Procedures

Protein identification by mass spectrometry

Gel-resolved proteins were digested with trypsin, the mixtures fractionated on a Poros 50 R2 RP micro-tip, and resulting peptide pools analyzed by matrix-assisted laser-desorption / ionization reflectron time-of-flight (MALDI-reTOF) MS using a BRUKER UltraFlex TOF/TOF instrument (Bruker; Bremen, Germany), as described (Erdjument-Bromage et al., 1998; Sebastiaan Winkler et al., 2002). Selected experimental masses (*m/z*) were taken to search the human segment of a non-redundant protein database ('NR'; ~134,604 entries; National Center for Biotechnology Information; Bethesda, MD), utilizing the PeptideSearch algorithm (Dr. Matthias Mann), with a mass accuracy restriction better than 40 ppm, and maximum one missed cleavage site allowed per peptide. Mass spectrometric sequencing of selected peptides was done by MALDI-TOF/TOF (MS/MS) analysis on the same prepared samples, using the UltraFlex instrument in 'LIFT' mode. Fragment ion spectra were taken to search NR using the MASCOT MS/MS Ion Search program, version 2.0.04 for Windows (Matrix Science Ltd.; London, UK). Any identification thus obtained was verified by comparing the computer-generated fragment ion series of the predicted tryptic peptide with the experimental MS/MS data.

Co-immunoprecipitation, western blotting, and antibodies

Co-immunoprecipitation assays were conducted using 0.5-2 ug IgG per reaction using the H3K4me3 elution material as input. Western blotting was performed using standard methods. Antibodies used for western and ChIP analyses include: SNF2h and Rsf-1 (Loyola et al., 2003), CHD1 (kind gift of R. P. Perry)(Stokes and Perry, 1995), CHD3 (Zhang et al., 1998), hPaf1 (Zhu et al., 2005), hCdc73 and hLeo1 (Bethyl Laboratories), SF3A1, SF3A2, SF3A3 (kind gift from A. Kramer, University of Geneva)(Kramer et al., 1995), SF3B3 (Abcam), U2-B'' (kind gift from R. Reed), U2AF56 (kind gift from M. Carmo-Fonseca), BAF170 (Santa Cruz), H3K4me2 (Upstate), H3K4me3 (Sarma et al., 2004), PRPF8 (Abcam), RNAPII (Santa Cruz), Histone H3 (Cell Signalling), H4-Acetyl (Upstate), TBP, SPT16 (Orphanides et al., 1999), SSRP1 (kind gift from S. C. Lee, National Taiwan University) (Tan and Lee, 2004), U1-70K (a kind gift from Dr. W. van Venrooij) (Shin and Manley, 2002), and mAb104 (anti-SR) (Zahler et al., 1992). DNase and RNase treatment was carried out according to the instructions of the manufacturer (Promega). Prior to DNase and RNase treatment, fractions were dialyzed to remove excess salt.

RNA analyses and in vitro splicing

Extracts (1-10 µl) were digested with proteinase K, and RNA was extracted with phenol/chloroform (1:1) and precipitated by adding 0.25 volumes 10M ammonium acetate, and 2.5 volumes ethanol. ^{32}P-labeled β-globin pre-mRNA substrates were prepared as described previously (Tacke et al., 1998). In vitro splicing reactions were performed in 12.5-µl reaction mixtures, which contained 2- 6-µl nuclear extract and 1-2 ng labeled β-globin substrate. The final concentrations of buffer components were 12 mM HEPES (pH 7.9), 60 mM KCl, 0.12 mM

EDTA, 0.30 mM DTT, 12% glycerol, 3 mM $MgCl_2$, 20 mM creatine phosphate (di-Tris), 1 mM ATP, 3% polyvinyl alcohol (PVA), and 0.5 U of RNasin (Promega). Reactions were incubated at 30^{o}C for 2 hrs unless indicated otherwise. ΔIgG, ΔCHD1, and untreated nuclear extracts contain near equal total protein concentration as determined by protein assays (BioRAD). Reactions supplemented with U2 snRNP contain 200 ng immunopurified 17S U2 snRNP (Will et al., 2002). Quantitation of gel images were performed using ImageJ software. RNA immunoprecipitation experiments were performed essentially as described (Martianov et al., 2007). The primers used for RT-PCR are: cyclin D1 (5': AAGCTGTGCATCTACACCGA-CAACTCCA; 3': TGCGCGTGTTTGCGGATGATCTGTTTGT) and GAPDH (5': ACATCAAGAAGGTGGTGAAGCAGGCGT; 3': AGCTTGACAAAGTGGTCGTTGAGG-GCAA).

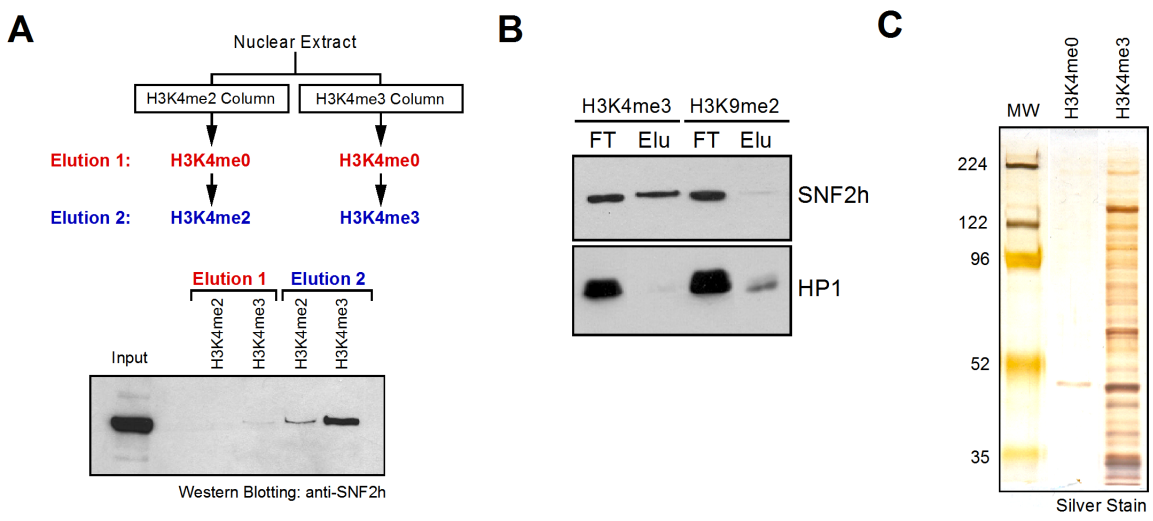

Figure S1. A) Schematic of the binding assay to methyl-H3K4 (Top). (Bottom) Western blot of eluate derived from di- and tri-methyl H3K4 columns. B) Western blot of the flow thru (FT) or elution (Elu) from di-methyl H3K9 and tri-methyl H3K4 columns. The antibodies used are indicated to the right of the panels. C) Silver stain of eluted proteins from unmodified or tri-methyl H3K4 columns.

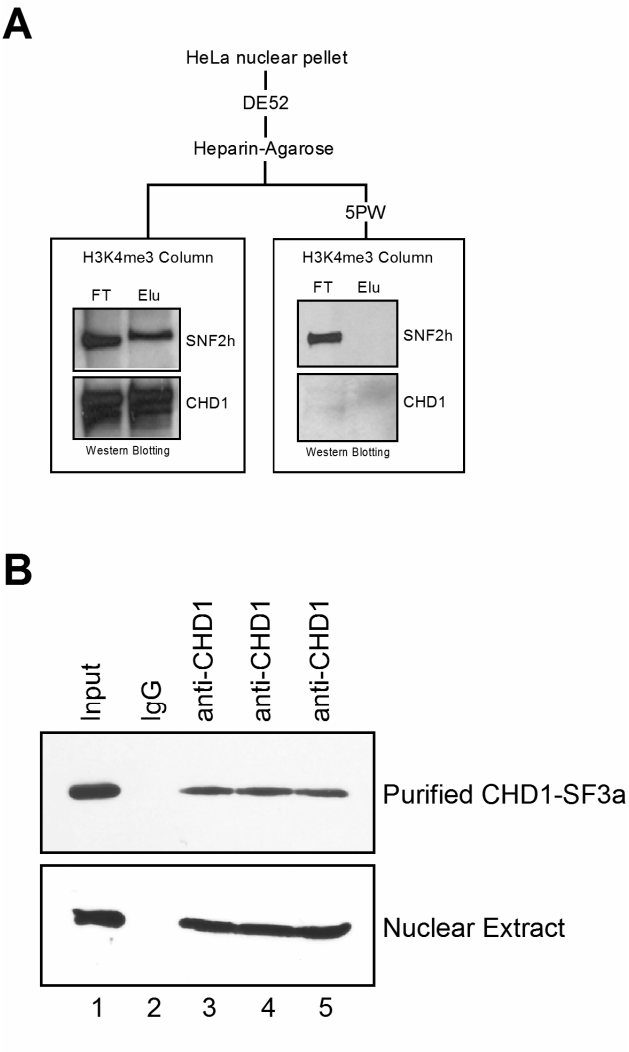

Figure S2. A) Western blot of the flow through (FT) or elution (Elu) from H3K4me3 affinity columns. The antibodies used for immunoblotting are indicated (right of panel). B) Western blotting of co-immunoprecipitation experiments probing with SF3A2 antibodies. Purified fractions and nuclear extracts containing CHD1-SF3a complexes were treated with DNase (lane 4) or RNase (lane5) and then immunoprecipitated using control or anti-CHD1 antibodies.

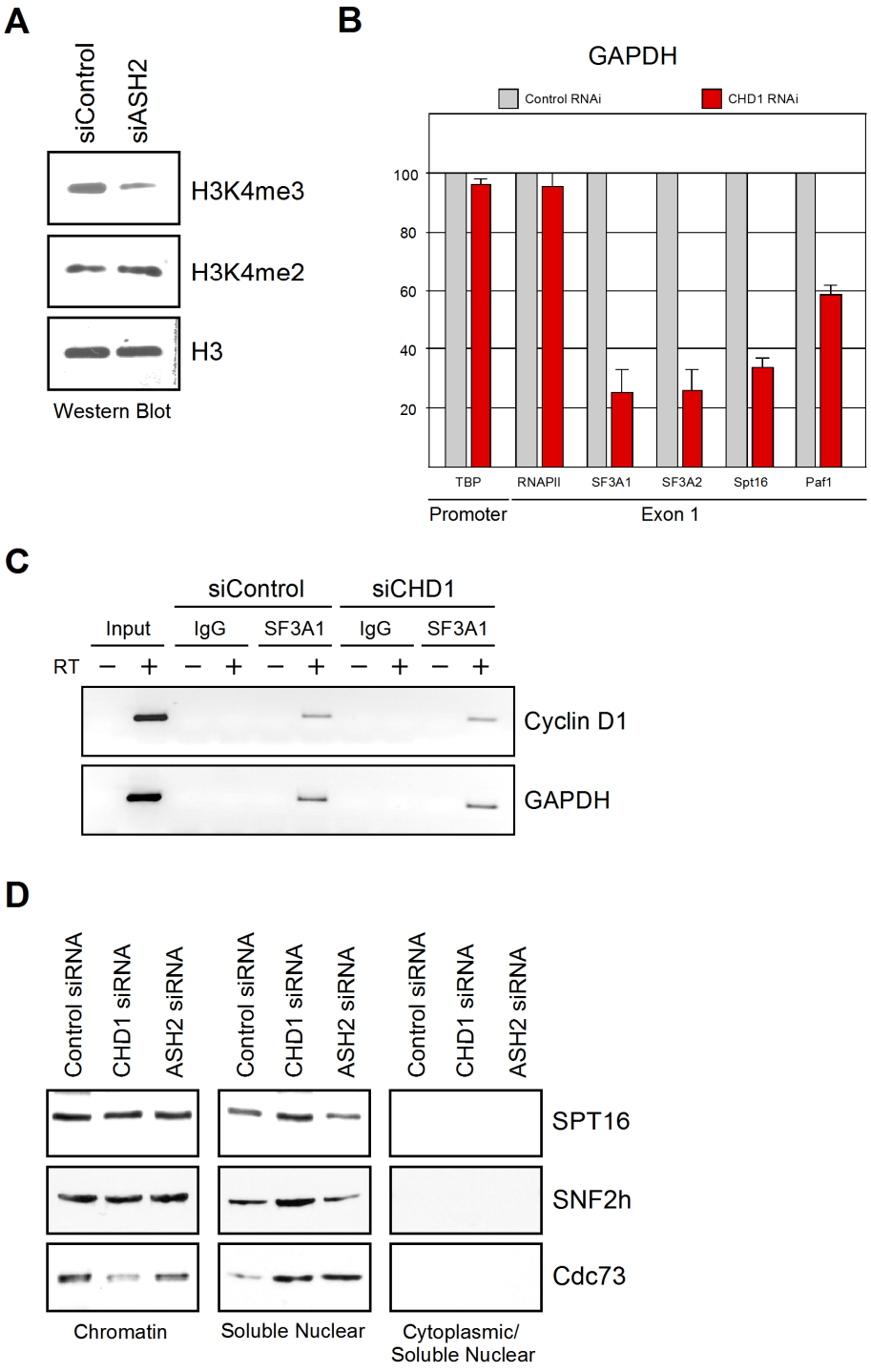

Figure S3. A) Western blotting of whole cell extracts derived from control or HeLa cells targeted by ASH2 siRNA. The antibodies used are indicated on the right. B) Percentage of TBP, RNAPII, SF3A1, SF3A2, Spt16 and hPaf1 detected by ChIP analyses from control or CHD1 siRNA-

treated cells on the GAPDH gene. The average and standard deviations of three independent experiments are shown. All primers sets are derived from the first exon, except for TBP, which was derived from the promoter region (-250 to -57). C) RT-PCR of mRNA products immunoprecipitated by IgG (control) or anti-SF3A1 antibodies from HeLa cells treated with control siRNA (siControl) or siRNA targeted against CHD1 (siCHD1). RT indicated the absence (-) or (+) inclusion of reverse transcriptase in the reaction. D) Sub-cellular fractionation from cells treated with the indicated siRNA (top). Western blotting was performed using the labeled antibodies (side).

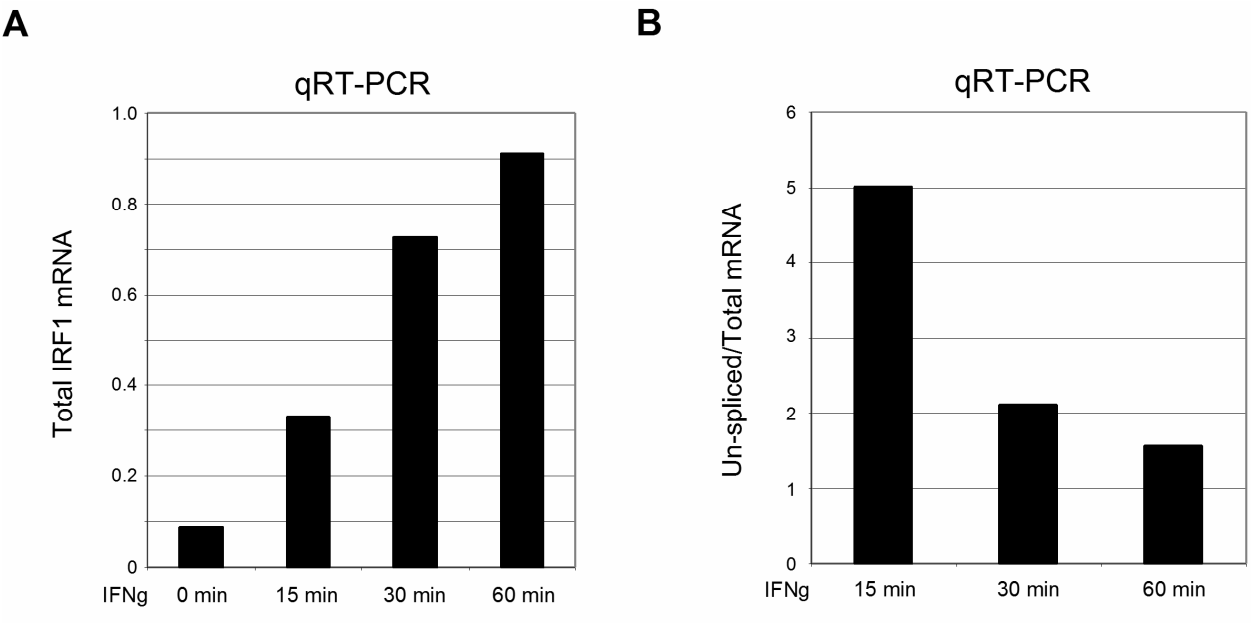

Figure S4. A) Real-time RT-PCR analyses detecting the total mRNA produced from the IRF1 gene after the indicated time points following treatment with IFN-γ. B) Real-time RT-PCR analyses monitoring the ratio of un-spliced to total mRNA of the IRF1 gene after the indicated time points following treatment with IFN-γ.

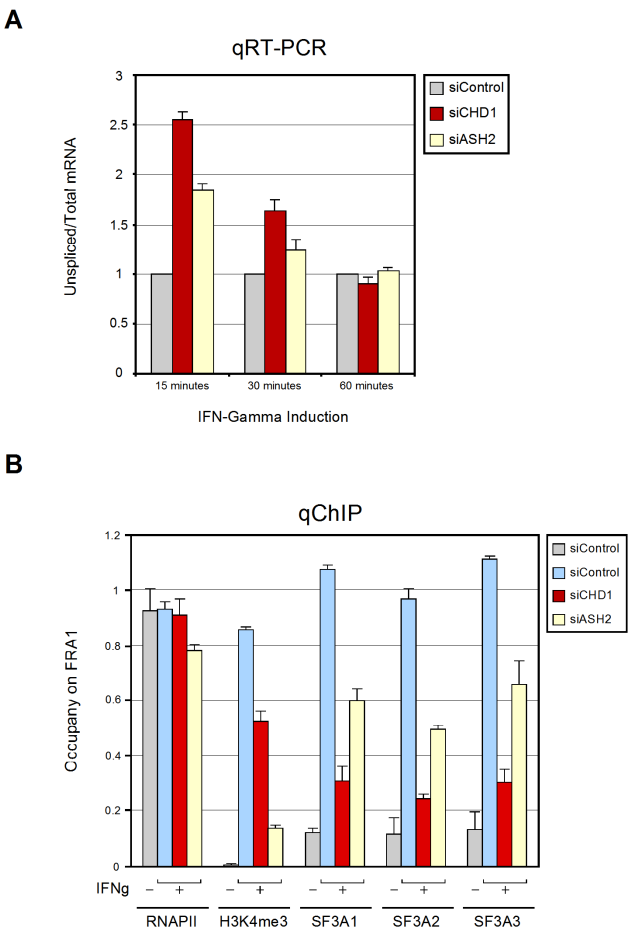

Figure S5. A) Real-time RT-PCR analyses detecting the ratio of un-spliced to total mRNA derived from the FRA1 gene after the indicated time points following treatment with IFN-γ. The control siRNA treated cells were compared to cells treated with siRNA targeted against CHD1 and ASH2. The specific siRNA treated cells are indicated in the panel on the upper right side of the graph. Results from three independent experiments are shown represented as the mean and standard error. B) Real-time PCR analyses of the FRA1 gene from ChIP-derived samples after 0 or 15 minutes of IFN-γ treatment (IFNg). The antibodies used for immunoprecipitation are shown on the bottom of the graph. The specific siRNA treated cells are indicated in the panel on the upper right side of the graph. Results from three independent experiments are shown represented as the mean and standard error.

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Table S1. Proteins identified by H3K4me3 affinity purification

Protein	Activities/comments
CHD1	Directly binds H3K4me3, chromatin remodeling and assembly, transcript elongation, termination
FACT	Facilitates transcript elongation on chromatin templates
SSRP1	
Spt16	
SNF2h	Chromatin remodeling
SPT6	Histone chaperone, transcript elongation, associates with exosome
PAF complex	3' end formation, modulates chromatin modification, associates with exosome
hCdc73	
hPaf1	
hLeo1	
ASH2	Associates with H3K4 methyltransferase activity and the PAF complex, among others; likely binds H3K4me3 directly via its PHD finger
WDR5	Associates with H3K4 methyltransferase activity
U2 snRNP	Core spliceosome component, constitutes pre-spliceosome (Complex A) along with U1 snRNP
SF3A1	
SF3A2	
SF3A3	
SF3B3	
SF3B4	
U2B"	
U2AF65	Associates with U2 snRNP, transcript elongation, export
ALY	Co-purifies with pre-spliceosome (Complex A) and is a component of the TREX complex (transcript elongation, splicing, export)
DDX9	Co-purifies with pre-spliceosome (Complex A), RNA helicase
hnRNP U	Co-purifies with pre-spliceosome (Complex A)
NF90	Co-purifies with pre-spliceosome (Complex A), export