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Systematic Identification of Proteins Binding to Chromatin-Embedded Ubiquitylated H2B Reveals Recruitment of SWI/SNF to Regulate Transcription

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SUMMARY

Chromatin posttranslational modifications (PTMs), including monoubiquitylation of histone H2B on lysine 120 (H2Bub1), play a major role in regulating genome functions. To elucidate the molecular mechanisms of H2Bub1 activity, a chromatin template uniformly containing H2Bub1 was used as an affinity matrix to identify preferentially interacting human proteins. Over 90 such factors were found, including proteins and protein complexes associated with transcription, RNA posttranscriptional modifications, and DNA replication and repair. Notably, we found that the SWI/SNF chromatin remodeling complex associates preferentially with H2Bub1-rich chromatin. Moreover, SWI/SNF is required for optimal transcription of a subset of genes that are selectively dependent on H2Bub1. Our findings substantially expand the known H2Bub1 interactome and provide insights into the functions of this PTM in mammalian gene regulation.

INTRODUCTION

Posttranslational modifications (PTMs) of core histones play important roles in regulating gene expression, DNA replication, DNA repair, and other DNA-templated biological processes (Berger, 2007; Weake and Workman, 2008; Zhou et al., 2011). Whereas most PTMs involve covalent attachment of small chemical moieties (e.g., acetylation and methylation), monoubiquitylation comprises conjugation of the bulky 76-amino-acid ubiquitin protein to the ε-amino group of lysine. A well-studied example is monoubiquitylation of histone H2B on lysine 123 in yeast or lysine 120 in mammals (H2BK120ub1, abbreviated here as H2Bub1), carried out primarily by the yeast E3 ubiquitin ligase BRE1 (Hwang et al., 2003) and the orthologous heteromeric hBRE1(RNF20)/RNF40 mammalian complex (Kim et al., 2005; Zhu et al., 2005).

Recent studies, relying mainly on manipulation of RNF20/RNF40, have provided insights into the compound roles of H2Bub1 in mammalian cells. Partial depletion of RNF20, which markedly reduces H2Bub1 levels, exerts opposite transcriptional effects on different gene subsets: H2Bub1 suppresses the transcriptional elongation of a gene subset that includes several proto-oncogenes, partly through inhibiting the recruitment of the elongation factor TFIIS, while positively regulating the transcription of other genes, including the p53 tumor suppressor (Shema et al., 2008). Such seemingly opposing functions of H2Bub1 in transcriptional regulation are supported by yeast studies (Chandrasekharan et al., 2009; Osley et al., 2006; Wyce et al., 2007). Additional work in mammalian cells revealed crucial roles for H2Bub1 in maintaining chromatin boundaries (Ma et al., 2011), promoting DNA damage repair (Kari et al., 2011; Moyal et al., 2011; Nakamura et al., 2011), histone messenger RNA 3′ end processing (Pirngruber et al., 2009), and stem cell differentiation (Fuchs et al., 2012; Karpiuk et al., 2012).

How H2Bub1 exerts its effects is largely unknown. H2Bub1 can impact directly the physical properties of chromatin, in particular its arrangement into higher-order structures (Fierz et al., 2011). H2Bub1 has also been implicated in enhancing nucleosome stability and mediating transcriptional repression or activation, depending on the context and localization of the modification (Batta et al., 2011; Chandrasekharan et al., 2009). Other studies suggest that H2Bub1 may also serve as a signaling platform. Thus, yCps35/hWDR82 associates with chromatin in an H2Bub1-dependent manner, enhancing histone H3 lysine 4 methylation by COMPASS-type complexes (Lee et al., 2007; Wu et al., 2008). Furthermore, H2Bub1 stimulates H3 lysine 79 methylation by the DOT1 histone methyltransferase (Lee et al., 2007; McGinty et al., 2008). H2Bub1 can also affect nonhistone

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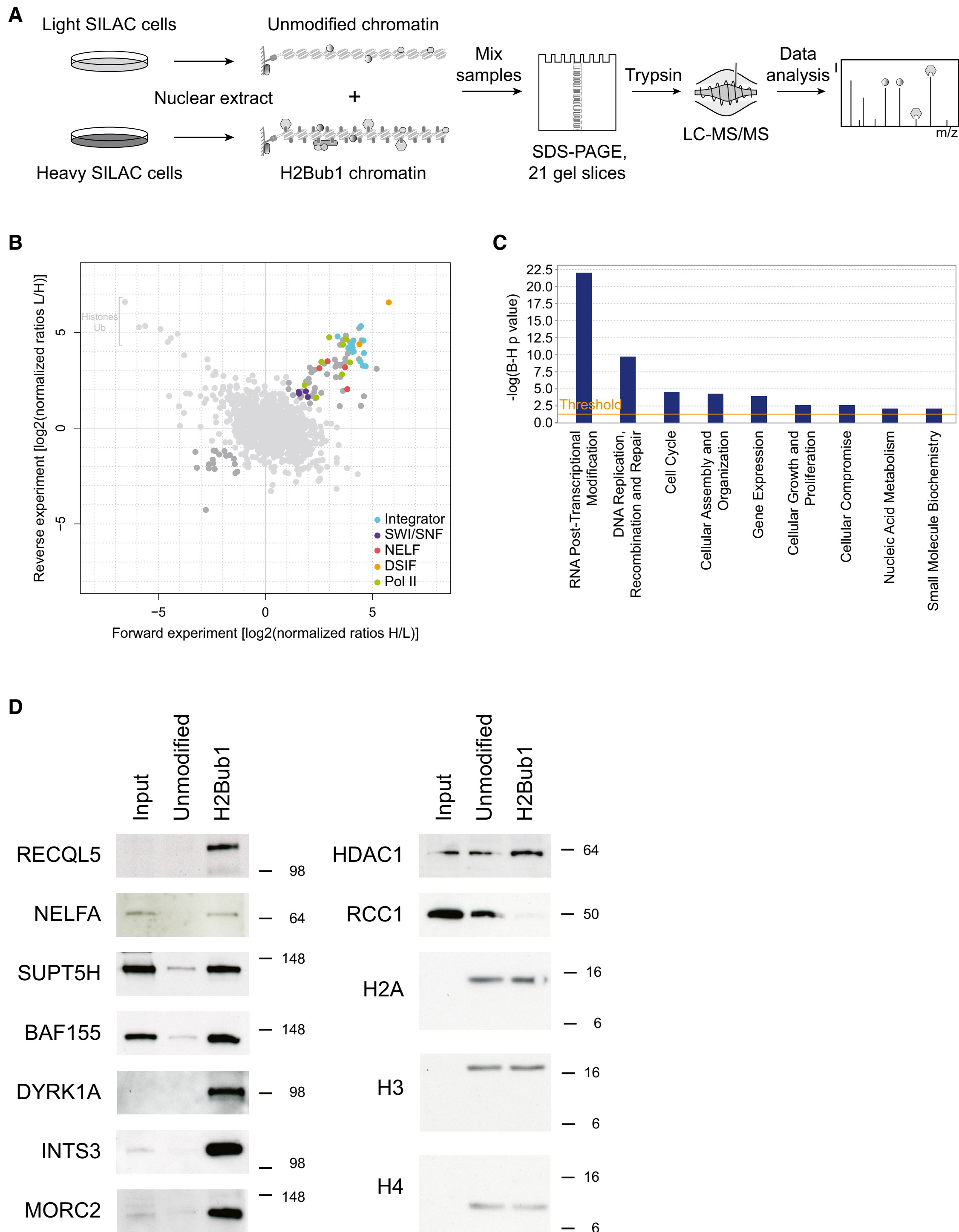

Figure 1. Identification and Validation of H2Bub1-Interacting Proteins

(A) Experimental workflow of the chromatin affinity purification.

(B) Scatterplot representing normalized enrichment ratios of proteins from two label swap chromatin affinity purification experiments. Proteins with log2-transformed ratios above 1.0 in each experiment and above 1.5 average are in dark gray. Upper right quadrant and lower left quadrant display proteins enriched with H2Bub1 chromatin or unmodified chromatin, respectively; protein identities are listed in Table S1. Factors belonging to five indicated complexes are color-coded. Histones and ubiquitin, identified only in light form as part of the affinity matrix, are displayed in the upper left quadrant.

(C) Significantly enriched biological functions (Ingenuity Pathway Analysis, B-H corrected p value < 0.05) associated with proteins selectively bound to H2Bub1-rich chromatin. See Table S2 for a detailed list of the biological functions, canonical pathways, and individual proteins with associated p values.

(legend continued on next page)

proteins, as demonstrated by its requirement for methylation of Dam1 at the kinetochore (Latham et al., 2011). We now report the systematic identification of proteins that bind favorably to H2Bub1-rich chromatin and show that preferential binding of the SWI/SNF chromatin remodeling complex to specific genes via H2Bub1 is required for optimal transcription of those genes.

RESULTS

Systematic Identification of H2Bub1-Binding Proteins

To obtain insights into the diverse functions of H2Bub1, we utilized an approach we (Nikolov et al., 2011) and others (Bartke et al., 2010) previously applied for identification of specific histone PTM-binding proteins in the context of chromatin. Semisynthetic H2Bub1 (Haj-Yahya et al., 2012) (see also Figure S1A) was used in reconstitution of recombinant chromatin on a biotinylated DNA template (''H2Bub1-rich chromatin''). The resulting nucleosomal array, as well as one with nonmodified H2B, served as affinity matrices for enrichment of factors from SILAC-labeled (Ong and Mann, 2007) nuclear extracts (Figure 1A). We compared the proteins recruited to unmodified chromatin versus H2Bub1-rich chromatin using quantitative mass spectrometry. To minimize false-positive hits, two independent SILAC label-swap experiments were performed (Figures 1B and S1B). The list of factors preferentially associated with H2Bub1-rich chromatin (average log2 enrichment ratio > 1.5) contains more than 90 proteins, greatly expanding the known H2Bub1 interactome (Table S1). Of these, WDR82 is known to associate with chromatin in a H2Bub1-dependent manner (Wu et al., 2008), whereas chromodomain helicase DNA binding protein 1 (CHD1) is crucial for maintaining high H2Bub1 levels (Lee et al., 2012).

Remarkably, the enriched set includes multiple components of several protein complexes (examples are color coded in Figure 1B), deduced from interaction relationships based on experimental or curated database evidence (Figure S1C). Ingenuity Pathway Analysis (Ingenuity Systems, http://ingenuity.com) revealed a number of highly enriched (B-H corrected p value < 0.05) biological functions, including RNA posttranscriptional modification, gene expression, and DNA replication, recombination, and repair (Figure 1C; Table S2). Notably, control affinity purification using biotinylated recombinant ubiquitin as bait failed to yield similar binding partners (Figure S1D).

We repeated the affinity purification and analyzed the enriched material by western blotting (Figure 1D), verifying specific enrichment on H2Bub1-rich chromatin of NELFA, a subunit of the NELF negative elongation complex; SUPT5H, a subunit of the DSIF elongation complex; BAF155, a subunit of the SWI/SNF complex; and INTS3, a subunit of the Integrator (INT) complex (Baillat et al., 2005). Remarkably, all other known subunits of the NELF and DSIF complexes and several other subunits of the INT and SWI/SNF complexes were also identified as preferential binders to H2Bub1-rich chromatin (Figure 1B; Table S1). Likewise, we validated modification-dependent enrichment of the DNA helicase RECQL5, a putative tumor suppressor implicated in maintaining genome stability (Islam et al., 2010); the DYRK1A kinase, implicated in neuronal development and neurodegenerative diseases (Wegiel et al., 2011); and the MORC2 CW zinc finger protein, involved in histone deacetylation (Shao et al., 2010) (Figure 1D). Conversely, a small group of proteins, including Regulator of Chromosome Condensation 1 (RCC1), bound preferentially the nonmodified template (Figure 1B, lower left quadrant; Figure 1D). In contrast, HDAC1 was not found enriched on either template by mass spectrometry (MS) or western blot. Importantly, analysis of histones H2A, H3, and H4 confirmed equal amounts of chromatin template in all experiments.

H2Bub1-Dependent Differential Interactions within Cells

The two subunits of the DSIF complex, SUPT4H and SUPT5H, were among the most highly enriched binders of H2Bub1-rich chromatin (Figure 1B). Notably, a functional link between H2Bub1 and Spt5, the ortholog of SUPT5H, has recently been described in fission yeast (Sansó et al., 2012).

To examine this interaction in vivo, we transiently expressed in HeLa cells either FLAG-tagged wild-type (WT) H2B or H2B mutated at lysines 120 and 125 (H2B2KR), rendering it ubiquitylation resistant (Minsky and Oren, 2004). Cell extracts were subjected to coimmunoprecipitation (coIP) with antibodies against FLAG followed by western blot analysis with antibodies against SUPT5H. As seen in Figure 2A, more SUPT5H associated with WT than with mutant H2B, confirming that this interaction is favored by H2Bub1.

We then asked whether transient reduction of H2Bub1 levels, via small interfering RNA (siRNA)-mediated RNF20 depletion, decreases the association of SUPT5H with chromatin. However, despite reduced H2Bub1 levels, significant changes in the association of SUPT5H could not be detected (Figure S2). Conceivably, the affinity of SUPT5H to H2Bub1 might be so strong that a 2- to 3-fold decrease in H2Bub1 may not suffice to reduce substantially its recruitment to chromatin. In such cases, lower-affinity interactions are expected to be more sensitive to a modest decrease in H2Bub1. Indeed WDR82, a moderate preferential binder in our MS screen, was shown to associate with chromatin in an H2Bub1-dependent manner (Wu et al., 2008).

SWI/SNF Associates Preferentially with H2Bub1-Rich Chromatin

The group of moderate preferential binders to H2Bub1-rich chromatin comprised several subunits of the SWI/SNF chromatin remodeling complex (BRG1, BRM, BAF155, and BAF170; Figures 1B and S1C), suggesting that H2B ubiquitylation may help recruit this complex to chromatin. Mammalian SWI/SNF complexes contain multiple subunits: one of the two mutually exclusive catalytic ATPase subunits BRM and BRG1; several highly

(D) Affinity purification of HeLa extracts on chromatin templates, reconstituted with either unmodified H2B or H2Bub1, was performed as in (A), except that extracts were not stable-isotope labeled and bound proteins were analyzed by western blot with the indicated antibodies. Left: proteins binding preferentially to the H2Bub1-rich chromatin template according to MS. Right: HDAC1 (identified in background by MS), RCC1 (found preferentially on the unmodified template by MS), and histone proteins from chromatin templates.
See also Figure S1.

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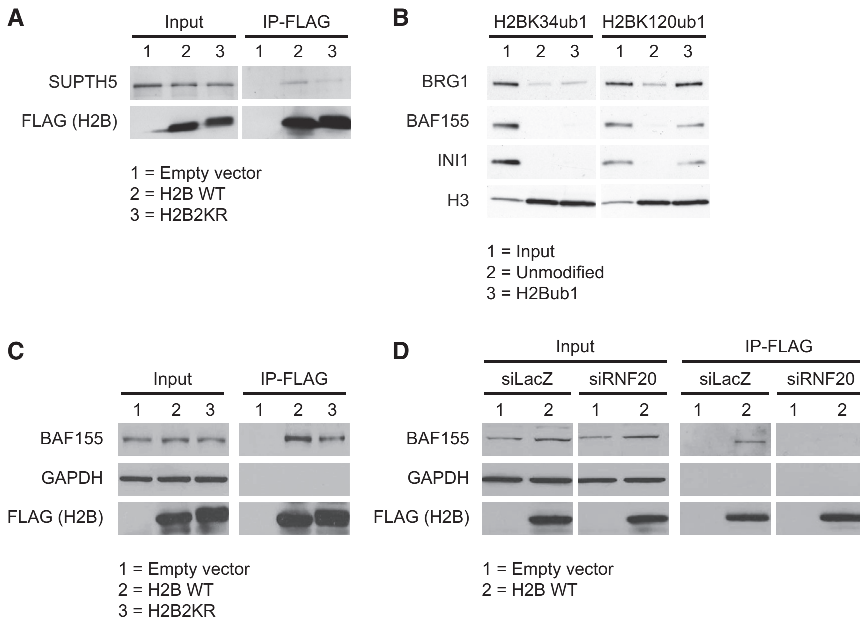

Figure 2. The SWI/SNF Complex and SUPT5H Associate Preferentially with H2Bub1

(A) HeLa cells were transiently transfected with control empty vector (1) or plasmids encoding wild-type FLAG-tagged H2B (2) or FLAG-tagged H2B2KR, carrying lysine-to-arginine substitutions at positions 120 and 125 and therefore incapable of undergoing ubiquitylation (3). Then, 24 hr later, cells were harvested and extracts subjected to immunoprecipitation (IP) with antibodies against FLAG followed by western blot analysis for SUPT5H.

(B) Affinity purification of HeLa extracts on chromatin templates, reconstituted with unmodified H2B, H2BK120ub1, or K2BK34ub1, was performed as described in Figure 1D and analyzed by western blot with the indicated antibodies.

(C) HeLa cells were transfected as in (A). IP was performed 24 hr later with antibodies against FLAG followed by western blot analysis for BAF155.

(D) HeLa cells were transfected with siRNA oligonucleotides directed against RNF20 (siRNF20) or LacZ (siLacZ), and cells were transfected 24 hr later with a plasmid encoding WT H2B or with control vector. 24 hr posttransfection, cells were harvested, extracted, and subjected to IP as in (A), followed by western blot analysis for BAF155.

See also Figure S2.

conserved core subunits including INI1 (SNF5/SMARCB1), BAF155 (SMARCC1), and BAF170; and various associated polypeptides. SWI/SNF complexes mobilize nucleosomes, as well as evict H2A/H2B dimers and entire histone octamers, and thereby can either enhance or suppress transcription (Wilson and Roberts, 2011; Wu et al., 2009).

SWI/SNF complex components were specifically recruited to chromatin containing H2BK120ub1 but not H2BK34ub1, another histone ubiquitylation recently identified (Siman et al., 2013; Wu et al., 2011) (Figure 2B). To validate the preferential interaction of SWI/SNF with H2Bub1-rich chromatin in vivo, we performed coIP experiments with FLAG-tagged WT H2B or H2B2KR after transient overexpression in HeLa cells. As seen in Figure 2C, endogenous BAF155 was preferentially recruited by WT H2B as compared to the H2B2KR mutant (IP-FLAG; compare lanes 2 and 3). Furthermore, RNF20 depletion reduced markedly the association of H2B with BAF155 (Figures 2D and S2C, siRNF20).

SWI/SNF Is Required for Optimal Transcription of RNF20-Dependent Genes

Preferential recruitment of SWI/SNF to H2Bub1-rich chromatin might facilitate the transcription of RNF20-dependent genes, thereby explaining their dependence. We therefore monitored the expression of representative genes upon knockdown of several SWI/SNF subunits (Figure 3A). Depletion of RNF20, but not BRG1, BAF155, or BRM, downregulated global H2Bub1 levels (Figure 3B). As reported (Shema et al., 2008), expression of the RNF20-dependent genes *p53*, *H2BD*, *HOX1A*, *GDF15*, *APITD1*, *TSN*, and *CD46* decreased significantly (Figure 3C, siRNF20). Importantly, a comparable decrease was observed also upon knockdown of BRG1 and, to a lesser extent, BAF155. In contrast, BRM depletion had little effect. Depletion of BRG1, BRM, or BAF155 did not affect the RNF20-independent genes *GAPDH* and *NDUFA6* or the RNF20-suppressed genes *MYC* and *FOS* (Figure 3D). Thus, SWI/SNF is selectively involved in the positive regulation of genes whose transcription is dependent on RNF20 and H2Bub1.

H2Bub1 Modulates Gene Expression by Affecting SWI/SNF Recruitment

To test whether H2Bub1 is involved in recruiting SWI/SNF to specific loci, we performed chromatin immunoprecipitation (ChIP) analysis to examine the association of BRG1 with the transcription start site (TSS) or the 5′ regions of representative genes. As seen in Figure 4A, RNF20 knockdown reduced the association of BRG1 with genes positively regulated by RNF20 and H2Bub1, but not with the RNF20-suppressed *FOS* or the RNF20-independent *GAPDH*, *p21*, and *NDUFA6* genes, although H2Bub1 levels were reduced at all tested sites (Figure 4B). Notably, RNA polymerase II (Pol II) occupancy on the TSS and 5′ regions of *p53* and *H2BD* did not decrease (Figure S3A), indicating that the reduction in BRG1 is specific and not merely correlated to Pol II levels in these regions. This suggests that BRG1, and presumably the SWI/SNF complex as a whole, is recruited in an H2Bub1-dependent manner to a specific subset of genes, selectively facilitating their transcription.

We next asked whether BRG1 overexpression (Figures S3B and S3C) could rescue the transcription of RNF20-dependent genes in RNF20-depleted cells. RNF20 knockdown reduced the expression of all RNF20-dependent genes tested (Figure 4C, first and third bars). Notably, BRG1 overexpression partially rescued this defect (fourth and third bars), while not effecting siLacZ control cells (second and first bars). This suggests that H2Bub1 is selectively required to recruit to those

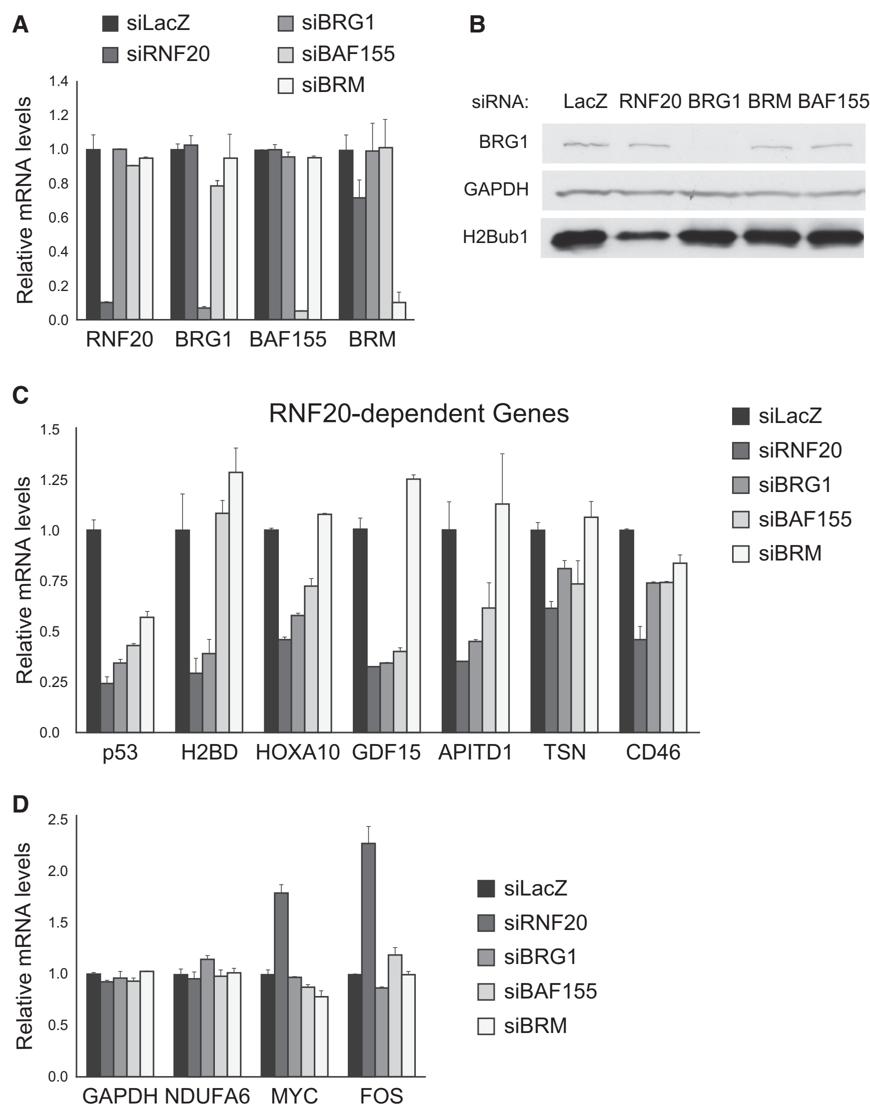

Figure 3. SWI/SNF Is Selectively Required for Optimal Transcription of RNF20-Dependent Genes

(A) HeLa cells were transiently transfected with siRNA oligonucleotides targeting LacZ (control), RNF20, or each of the following subunits of the SWI/SNF complex: BRG1, BAF155, and BRM. Cells were harvested 48 hr later and RNA was extracted and analyzed by quantitative RT-PCR, employing primers specific for the indicated genes. For each gene, messenger RNA (mRNA) levels were first normalized to *GAPDH* mRNA in the same sample and then divided by the normalized levels of the same transcript in the control siLacZ sample, set as 1. Error bars represent the SD of duplicate qPCR reactions. Similar data were obtained in three independent experiments.

(B) HeLa cells were transfected as in (A), and cells were harvested 48 hr later and subjected to western blot analysis. GAPDH served as loading control.

(C) HeLa cells were transfected and analyzed as in (A), employing primers specific for RNF20-dependent genes.

(D) HeLa cells were transfected and analyzed as in (A), employing primers specific for the RNF20-independent *GAPDH* and *NDUFA6* and the RNF20-suppressed *MYC* and *FOS* genes. *GAPDH* mRNA levels in each sample, relative to the siLacZ culture, are also shown.

genes limiting amounts of SWI/SNF needed for their optimal transcription.

DISCUSSION

We show that H2Bub1-reconstituted chromatin selectively associates with particular proteins, indicating that this PTM serves as recruiting signal within chromatin. Like antibodies against H2Bub1 (Minsky et al., 2008), some factors may recognize directly a unique structure at the H2B-ubiquitin junction. Alternatively, recognition may encompass cooperativity where ubiquitin together with other chromatin components (histones and DNA) need to be bound simultaneously. Since H2Bub1 alters higher-order chromatin folding (Fierz et al., 2011), some of the preferentially interacting complexes may recognize different chromatin conformational states in an indirect binding mode rather than interact directly with H2Bub1. So far, we have been unable to demonstrate a direct interaction between H2Bub1-rich chromatin and recombinant SWI/SNF subunits. We attempted to purify SWI/SNF complex via INI1-FLAG immunoprecipitation; however, only weak preferential binding to H2Bub1-rich chromatin was observed (data not shown). Hence, the selective interaction observed in nuclear extracts may rely on a composite signal (i.e., containing multiple direct and indirect components). This notion is supported by the differential interaction of SWI/SNF with chromatin containing H2BK120ub1 or H2BK34ub1. The exact nature and mechanism of SWI/SNF binding to H2BK120ub1-rich chromatin requires further investigation.

Besides locally opening chromatin structure to increase accessibility to repair proteins (Moyal et al., 2011; Nakamura et al., 2011), H2Bub1 may play a more direct role in double-strand break (DSB) repair. We found that several proteins recruited to DSBs to mediate DNA repair are enriched on H2Bub1, including subunits of the BRCA1-A complex (NBA1, RAP80, BRE, CCDC98, and BRCC3), the E3 ubiquitin ligase RNF168, and the DNA helicase RECQL5 (Doil et al., 2009; Popuri et al., 2012). Likewise, enrichment for ERCC1 and ERCC4(XPF) suggests a role for H2Bub1 in nucleotide excision repair (Fagbemi et al., 2011).

H2Bub1 exerts opposing transcriptional effects on different classes of genes (Chandrasekharan et al., 2009; Osley et al., 2006; Wyce et al., 2007). In mammalian cells, loci suppressed

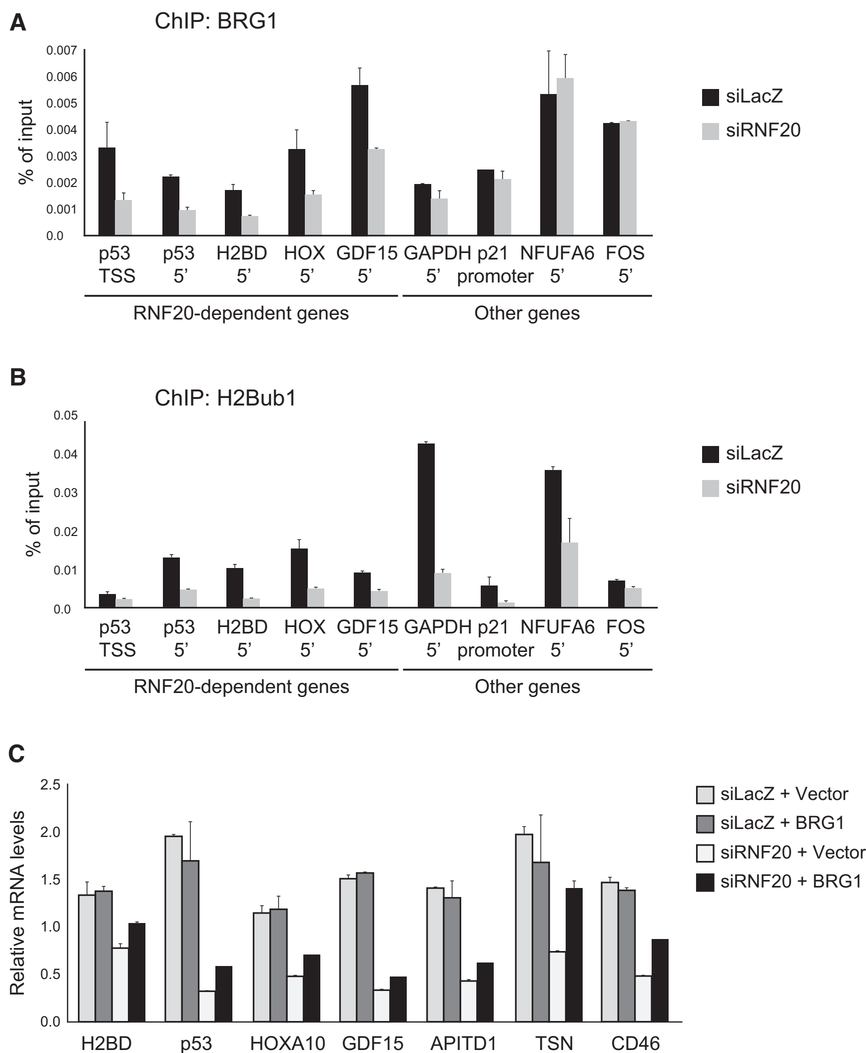

Figure 4. H2Bub1 Recruits SWI/SNF Complexes in Order to Modulate Transcription

(A and B) Chromatin immunoprecipitation analysis of BRG1 (A) and H2Bub1 (B) in HeLa cells transiently transfected with RNF20 siRNA (siRNF20) or LacZ siRNA (siLacZ). Immunoprecipitated DNA and input DNA were quantified by quantiative PCR with primers specific for the promoter, TSS, or 5′ transcribed regions of the indicated genes. Results are presented as percentage of input. Error bars represent the SD of duplicate qPCR reactions. Similar data were obtained in three independent experiments.

(C) HeLa cells were transfected with RNF20 siRNA (siRNF20) or LacZ siRNA (siLacZ), and cells were transfected 24 hr later with either empty vector or a plasmid encoding BRG1. After an additional 24 hr, cells were harvested, RNA was extracted, and expression of RNF20-dependent genes was analyzed by quantitative RT-PCR. Relative expression values and error bars were calculated as in Figure 3A.

See also Figure S3.

by high RNF20/H2Bub1 reside within relatively closed chromatin and are excessively loaded with stalled or paused Pol II (Shema et al., 2008). Their expression depends on the transcription elongation factor TFIIS, which is required for overcoming Pol II pausing and whose recruitment to chromatin is inhibited by H2Bub1 (Shema et al., 2011). We found that H2Bub1-rich chromatin favors the binding of two negative elongation complexes, DSIF and NELF, as well as several subunits of Pol II (Figure 1B). The ability of NELF to inhibit binding of TFIIS to Pol II (Palangat et al., 2005) might explain the inhibitory effect of H2Bub1 on RNF20/H2Bub1-suppressed genes.

While mammalian H2Bub1 is mostly implicated in transcriptional elongation (Minsky et al., 2008), SWI/SNF regulates both initiation and elongation (Batsché et al., 2006; Corey et al., 2003; Euskirchen et al., 2011; Schwabish and Struhl, 2007). SWI/SNF is mostly associated with the promoter, TSS, and 5′ region (Euskirchen et al., 2011), but is also present throughout the coding regions of some genes (Corey et al., 2003; Schwabish and Struhl, 2007). Different histone PTMs have been implicated in locally recruiting/retaining and modulating the activity of chromatin remodeling complexes. Thus, acetylated histones promote retention of SWI/SNF on gene promoters (Hassan et al., 2001). Likewise, histone H3 methylation on lysine 4, favored by H2Bub1 (Lee et al., 2007), mediates the association of SNF2H (subunit of the Isw1 remodeling complex) with chromatin in vivo (Nakamura et al., 2011; Santos-Rosa et al., 2003). Recently, H3K56ac was shown to regulate the histone exchange activity of SWR-C (Watanabe et al., 2013). We found that genes sensitive to RNF20 depletion are generally more dependent on BRG1 and presumably SWI/SNF for efficient transcription. Why this particular set of genes is so dependent on SWI/SNF remains an open question. Perhaps optimal expression of such genes is negatively regulated by particularly stable nucleosome(s), which need to be remodeled in an ATP-dependent manner in order to allow efficient transcription. Alternatively, other transcription-related functions distinct from nucleosome remodeling may be involved.

H2Bub1 deregulation has been extensively linked to cancer (reviewed in Johnsen, 2012). We expand this link, as many preferential H2Bub1 interactors harbor tumor-suppressive properties. Notably, mutations in genes encoding SWI/SNF subunits were found in tumors (Hargreaves and Crabtree, 2011; Wilson and Roberts, 2011). By recruiting protein complexes involved in DNA repair and transcriptional control, H2Bub1 may help to maintain cellular homeostasis and genome stability. Deregulation of H2Bub1 may compromise these processes, eventually promoting cancer.

EXPERIMENTAL PROCEDURES

Chromatin Affinity Purification

Chromatin affinity purification, MS, and data analysis were carried out as described using an immobilized H2Bub1 containing 12-mer oligonucleosomal array (Nikolov et al., 2011). Synthesis and characterization of H2BK34ub1 is described in Siman et al. (2013).

RNA Purification and Quantitative Real-Time PCR

Total RNA was extracted with the NucleoSpin kit (Macherey Nagel). A total of 2–5 μg of each RNA sample was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Promega) and random hexamer primers (Applied Biosystems). Real-time PCR was performed in a StepOne real-time PCR instrument (Applied Biosystems) with SYBR Green PCR Supermix (Invitrogen). The primers used are listed in Table S3.

Chromatin Immunoprecipitation

ChIP analysis was performed as described elsewhere (Nelson et al., 2006). The primers used are listed in Table S3.

For further details, please refer to Extended Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, three figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.07.014.

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Supplemental Information

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EXTENDED EXPERIMENTAL PROCEDURES

Chromatin Affinity Purification

Metabolic labeling and nuclear extract preparation of HeLa S3 cells was carried out as described (Nikolov et al., 2011) using light or heavy (D_4-lysine and C_6-arginine, EurisoTop) amino acids. Synthesis of H2Bub1 was previously described (Haj-Yahya et al., 2012). Recombinant chromatin was assembled on biotinylated 12x601x200 DNA template and immobilized essentially as described (Nikolov et al., 2011). For proteomics analysis duplicate label swap experiments using 5 mg of heavy and light SILAC extracts and 50 μg of recombinant chromatin were performed. Chromatin affinity purification with western blot detection was performed using 8-10 μg chromatin.

Mass Spectrometry and Data Analysis

Quantitative proteomics sample preparation and analysis was done as described (Nikolov et al., 2011). MS data were analyzed using MaxQuant (Cox and Mann, 2008) (version 1.2.2.5) and International Protein Index (IPI) human database (version 3.87). Results were analyzed and plotted using R (R Foundation for Statistical Computing, Vienna, Austria) as described (Nikolov et al., 2011). Biological functions and canonical pathways enrichment analysis was performed using Ingenuity Pathways Analysis software (Ingenuity Systems, http://ingenuity.com). UniProtKB accession numbers were mapped to the Ingenuity database and statistically enriched (Benjamini-Hochberg corrected p value < 0.05) terms were plotted as bar chart. Known protein-protein interactions between the enriched proteins were obtained from the STRING database (http://string-db.org/) and plotted using Cytoscape (http://www.cytoscape.org/).

Cell Culture and Transfections

Human cervical carcinoma HeLa cells were grown in DMEM with 10% bovine serum supplemented with antibiotics. SMARTpool oligonucleotides for siRNA transfection were purchased from Dharmacon. Transfections were carried out with Dharmafect 1 reagent (Dharmacon) according to the manufacturer's protocol. Transfection of expression plasmids was carried out using the jetPEI DNA transfection reagent (101-10, Polyplus Transfection), according to the manufacturer's protocol.

Antibodies

Primary antibodies used were: BRG1 (Santa Cruz Biotechnology), BRM (Abcam), BAF155 (Abcam), H3 (Abcam), DYRK1A (Cell Signaling), INTS3 (Abcam), MORC2 (Bethyl Laboratories), RCC1 (Abcam), RECQL5 (Abcam). SUPT5H and NELFA antibodies are from the lab of Yuki Yamaguchi. INI1 antibody is from the lab of Christian Muchardt.

Coimmunoprecipitation Analysis

HeLa cells were harvested, washed with ice-cold PBS and lysed on ice in NET lysis buffer (50mM Tris-HCl, pH7.4, 150mM NaCl, 1mM EDTA 0.1% NP40) supplemented with protease inhibitor mix (Sigma). Cells were sonicated in a Bioruptor sonicator (Diagenode) for a total of 10 min, including intervals of 30 s on and 1 min off, and then centrifuged at 13000 RPM for 10 min at 4°C. Total protein content was determined with the BCA protein assay kit (PIERCE, #23227). Anti-FLAG antibodies (A2220, Sigma-Aldrich), covalently attached to beads, were washed once with ice-cold PBS and twice with NET buffer. Equal amounts of protein from each sample were incubated with the antibody beads at 4°C overnight. Next, beads were washed three times with NET buffer, and elution was carried out using FLAG peptide (F3290, Sigma-Aldrich) in PBS. Samples were resolved by SDS-PAGE.

SUPPLEMENTAL REFERENCE

Cox, J., and Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat. Biotechnol. *26*, 1367–1372.

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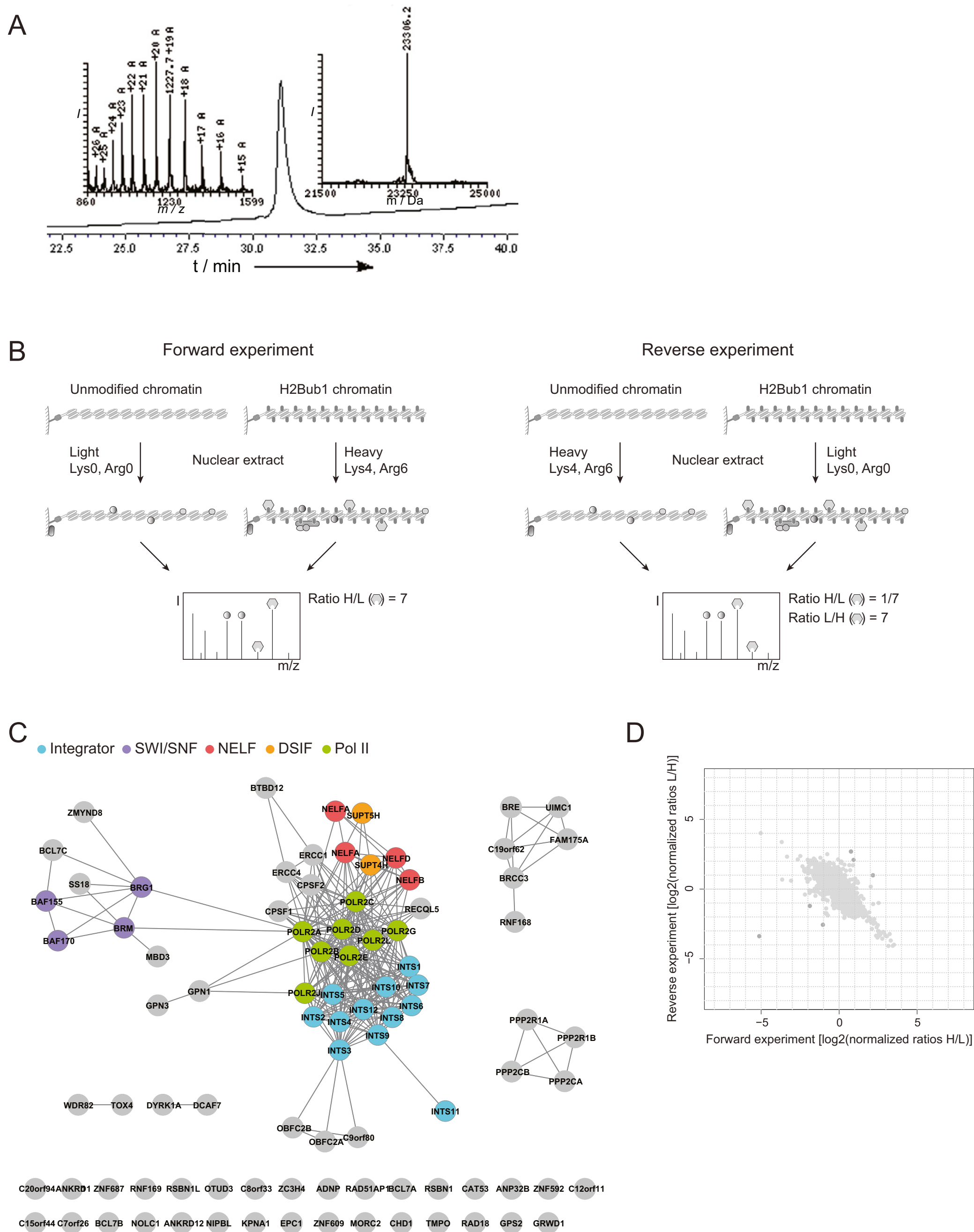

Figure S1. Validation of the Ubiquitylation of H2Bub1, Experimental Design of Label-Swap Experiments, Protein-Protein Interaction Network, and Ubiquitin Affinity Purification, Related to Figure 1

(A) Characterization of semi-synthetic H_6-H2Bub1. Analytical HPLC of the purified H_6-H2Bub1. The observed mass (23306.2 Da) corresponds to a product in which the three Met residues in H2B are oxidized (calculated 23305.8 Da).

(B) Experimental workflow of a duplicate label swap experiment. Specific interaction partners are expected to show opposite H/L enrichment ratios between the experiments (e.g., 7 and 1/7), while background or false positive hits would show similar H/L enrichment ratios. Related to Figure 1A.

(C) Interactome network of the proteins bound preferentially to H2Bub1-reconstituted chromatin. The identified enriched proteins (nodes) are connected based on known interactions (edges) from experimental and curated database evidence (STRING database). Nodes are labeled with official gene names. Proteins belonging to five complexes are color-coded as indicated at the top. Related to Figure 1B.

(D) Proteins bound preferentially by monoubiquitin. Scatter plot representing the normalized enrichment ratios from two label swap pull-down experiments using biotinylated purified recombinant ubiquitin as bait.

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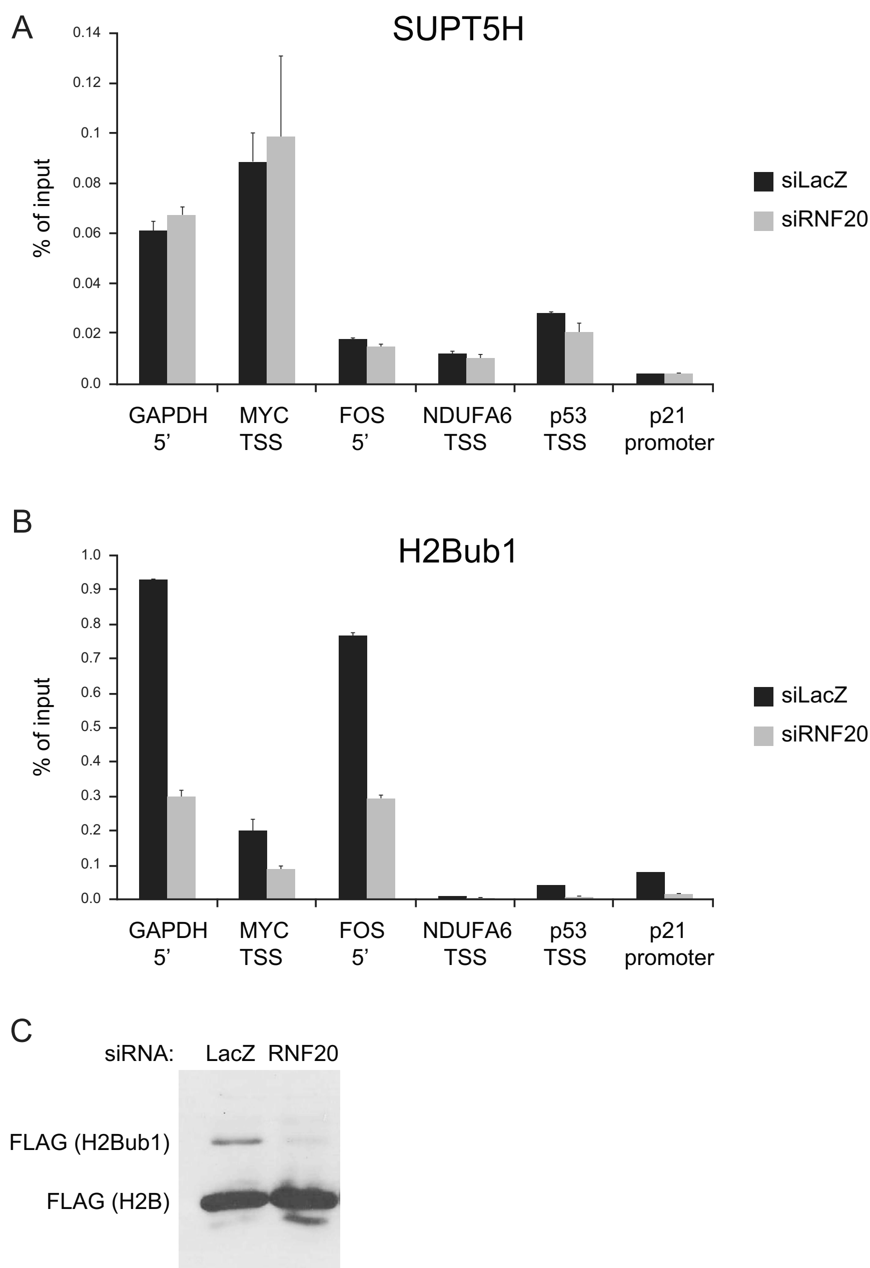

Figure S2. SUPT5H Association with Chromatin Is Unaffected by H2Bub1 Levels in the Tested Loci, Related to Figure 2

(A and B) ChIP analysis for SUPT5H (A) and H2Bub1 (B) in HeLa cells transiently transfected with RNF20 siRNA (siRNF20) or LacZ siRNA (siLacZ). ChIP was performed as previously described (Shema et al., 2008). Immunoprecipitated DNA and input DNA were quantified by qPCR with primers specific for the promoter regions, the transcription start sites (TSS), or the 5′ transcribed regions of the indicated genes; see Table S3 for primer sequences. Values are presented as percentage of input. Error bars represent the SD of duplicate qPCR reactions. Similar data were obtained in three independent experiments.

(C) The siLacZ and siRNF20 IP samples from the experiment presented in Figure 2D were analyzed by Western blot with α-FLAG antibodies. The higher band corresponds to FLAG-H2Bub1, which is significantly reduced upon RNF20 knockdown.

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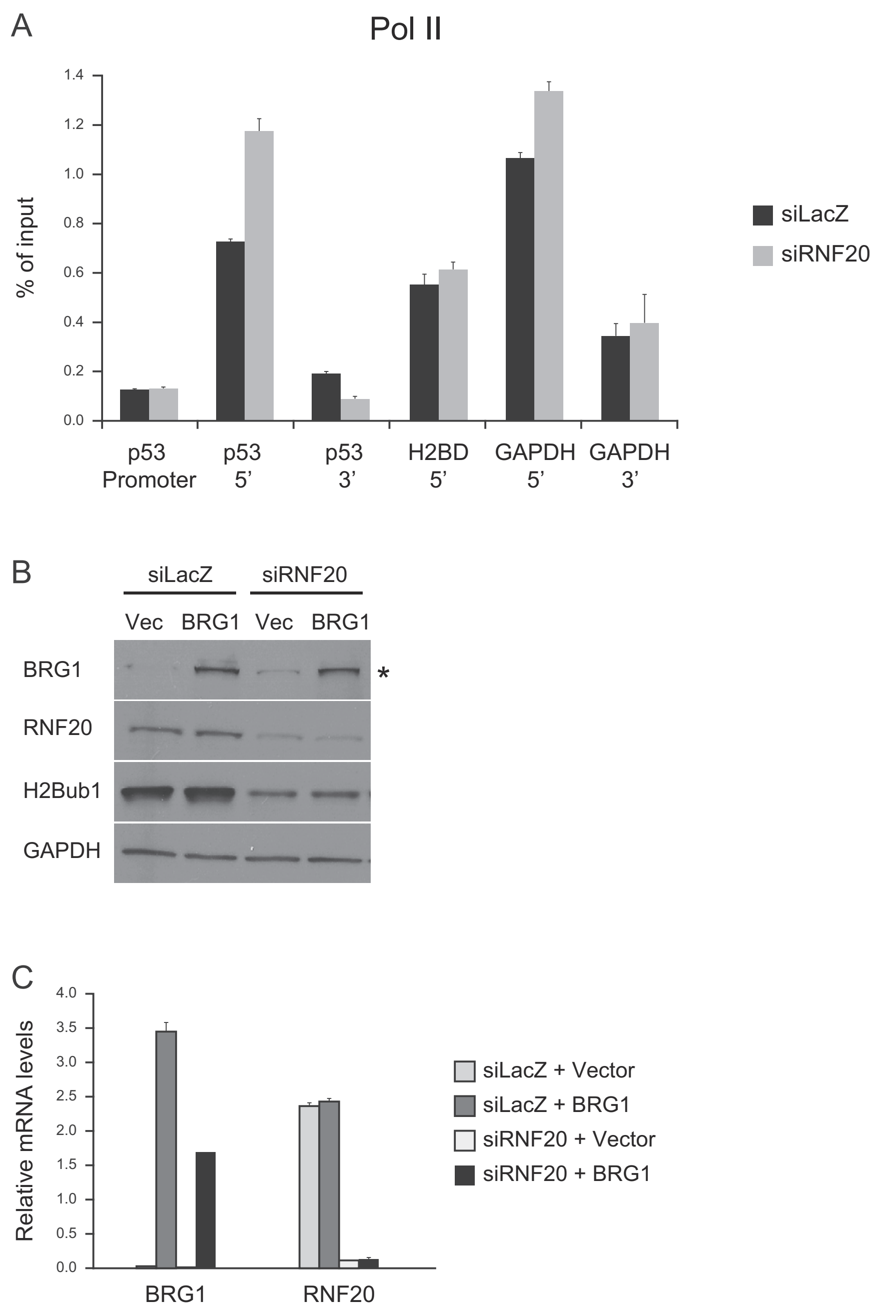

Figure S3. Pol II Association with Chromatin in RNF20-Depleted Cells and Levels of BRG1 Protein and mRNA upon Overexpression, Related to Figure 4

(A) Chromatin immunoprecipitation analysis of RNA Polymerase II in HeLa cells transiently transfected with RNF20 siRNA (siRNF20) or LacZ siRNA (siLacZ). Immunoprecipitated DNA and input DNA were quantified by qPCR with primers specific for the promoter, 5′ or 3′ transcribed regions of the indicated genes. Results are presented as percentage of input. Error bars represent the SD of duplicate qPCR reactions. Similar data were obtained in three independent experiments.

(B) HeLa cells were transfected with siRNA oligonucleotides and plasmids as in Figure 4. Cells were harvested, extracted and analyzed by western blot with the indicated antibodies. The BRG1 lane (asterisk) is derived from a different gel than the other 3 lanes; however, both gels were loaded with identical aliquots of the same samples.

(C) RNA from the same cultures as in (A) was quantified by qRT-PCR.