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LETTERS

# A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling

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**Lysine methylation of histones is recognized as an important component of an epigenetic indexing system demarcating transcriptionally active and inactive chromatin domains. Trimethylation of histone H3 lysine 4 (H3K4me3) marks transcription start sites of virtually all active genes[1–4]. Recently, we reported that the WD40-repeat protein WDR5 is important for global levels of H3K4me3 and control of *HOX* gene expression[5]. Here we show that a plant homeodomain (PHD) finger of nucleosome remodelling factor (NURF), an ISWI-containing ATP-dependent chromatin-remodelling complex, mediates a direct preferential association with H3K4me3 tails. Depletion of H3K4me3 causes partial release of the NURF subunit, BPTF (bromodomain and PHD finger transcription factor), from chromatin and defective recruitment of the associated ATPase, SNF2L (also known as ISWI and SMARCA1), to the *HOXC8* promoter. Loss of BPTF in *Xenopus* embryos mimics WDR5 loss-of-function phenotypes, and compromises spatial control of *Hox* gene expression. These results strongly suggest that WDR5 and NURF function in a common biological pathway *in vivo*, and that NURF-mediated ATP-dependent chromatin remodelling is directly coupled to H3K4 trimethylation to maintain *Hox* gene expression patterns during development. We also identify a previously unknown function for the PHD finger as a highly specialized methyl-lysine-binding domain.**

Recent genomic-scale analyses of histone modifications indicate that H3K4 trimethylation (H3K4me3) is preferentially associated with the transcription start sites of active genes[2–4]. Several factors have been proposed to recognize H3K4 methylated tails and mediate transcriptional activation (for example, CHD1 (refs 6, 7), Isw1 (ref. 8) and WDR5 (ref. 5)). However, these factors do not distinguish between di- and tri-methylated H3K4. In a previous study, we postulated that an effector recognizing H3K4me3 should act downstream of WDR5 (ref. 5).

To identify proteins that preferentially bind H3K4me3, nuclear extracts from HEK293 cells were incubated with unmodified and modified histone H3 peptides. This analysis revealed that a polypeptide >300 kDa specifically bound to the H3K4me3 peptide (Fig. 1A). This protein was identified by mass spectrometry to be BPTF. BPTF is the largest subunit of NURF[9,10], a chromatin-remodelling complex closely involved in transcription[9–17] (see Supplementary Fig. S1). To confirm the specificity of the above interaction, nuclear extracts from a cell line expressing Flag-tagged human BPTF or from mouse NIH3T3 cells were used in peptide pull-down assays with a series of H3 peptides. As shown in Fig. 1B, human (Fig. 1B, panel a) and mouse (Fig. 1B, panel b) BPTF specifically associates with H3K4me3 peptide, but not H3K4me2 peptide or H3 peptides trimethylated at other residues (for example, K9 and K27).

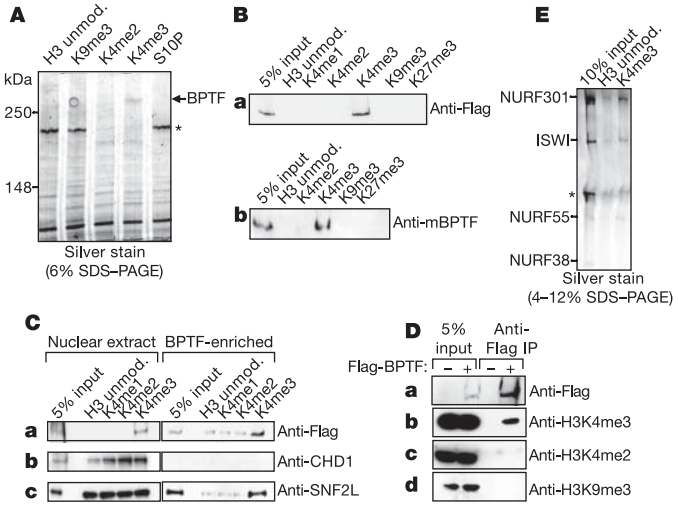


**Figure 1 | Human and *Drosophila* NURF specifically associate with histone H3 trimethylated at K4. A**, Peptide pull-down assays were performed using HEK293 cell nuclear extracts and unmodified (unmod.), methylated or phosphorylated H3 peptides as indicated and visualized by silver staining. A polypeptide specifically enriched in the H3K4me3 pull down was analysed by mass spectrometry and identified as BPTF (arrow). The polypeptide indicated with an asterisk corresponds to Mi-2, a subunit of the NuRD complex previously reported to associate with unmodified, but not K4 methylated, H3 tail[27,28]. S10P, H3 peptide phosphorylated at serine 10. **B**, Human and mouse BPTF specifically associate with H3K4me3 peptide. Nuclear extracts from HEK293 cells stably expressing Flag-tagged human BPTF (**a**), or from NIH3T3 cells (**b**), were used in peptide pull-down assays and analysed by immunoblotting with anti-Flag (**a**) or anti-mouse BPTF (mBPTF; **b**) antibodies. **C**, BPTF targets SNF2L to H3K4me3 in a CHD1-independent manner. Pull-down assays were performed using Flag–BPTF HEK293 nuclear extract or the same enriched for BPTF by Flag immunoprecipitation. Results were analysed by immunoblotting with the indicated antibodies (**a**–**c**). **D**, BPTF associates with H3K4me3 nucleosomes. Mononucleosomal fractions were purified from mock-transfected or Flag–BPTF HEK293 cell lines and used for immunoprecipitation (IP) with anti-Flag antibody; immunoprecipitates were analysed by immunoblotting with the indicated antibodies (**a**–**d**). **E**, *Drosophila* NURF recognizes H3K4me3. Reconstituted, gradient-purified *Drosophila* NURF was used in a histone-tail peptide pull-down assay. Input and bound proteins were resolved by SDS–PAGE and visualized by silver staining. NURF subunits are indicated on the left. The asterisk indicates a NURF301 degradation product, which is not enriched in the H3K4me3 pull down.

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Next, we verified that this association is independent of CHD1 (ref. 7), and also targets the SNF2L/ISWI ATPase to the H3K4 trimethylated tail. Immunoblot analysis indicated that BPTF preferentially associated with H3K4me3 in both nuclear extracts and BPTF-enriched material (Fig. 1C, panel a). In contrast, CHD1 bound to all forms of methylated H3K4 (with a preference for di- and tri-methyl), but was not detected in the BPTF-enriched input or peptide elutions (Fig. 1C, panel b). SNF2L/ISWI associated with the H3 tail in a methylation-independent manner (Fig. 1C, panel c), but in BPTF-enriched material SNF2L/ISWI was specifically enriched in the H3K4me3 pull down. These results indicate that (1) BPTF associates with H3K4me3 independently of CHD1, (2) BPTF can target SNF2L/ISWI to H3K4me3, and (3) the majority of SNF2L/ISWI is not associated with BPTF and does not bind preferentially to H3K4me3. Furthermore, tail peptide pull-down assays using recombinant *Drosophila* NURF complex indicates that the association between NURF and H3K4me3 is conserved (Fig. 1E).

To confirm that these *in vitro* interactions occur in the context of chromatin *in vivo*, we tested association of BPTF with H3K4 methylated nucleosomes. Chromatin was isolated from either Flag–BPTF-expressing (+) or non-expressing (−) HEK293 cells and solubilized with micrococcal nuclease. Mononucleosome fractions were pooled and immunoprecipitated with anti-Flag antibody, followed by Flag peptide elution and immunoblotting with antibodies against (1) Flag (Fig. 1D, panel a), (2) H3K4me3 (Fig. 1D, panel b), (3) H3K4me2 (Fig. 1D, panel c) or (4) H3K9me3 (Fig. 1D, panel d). BPTF specifically co-immunoprecipitated with the H3K4 trimethylated mononucleosomes (compare panels b–d of Fig. 1D), suggesting that NURF binds H3K4me3 in a nucleosomal context. Antibodies suitable for *in situ* analyses for NURF301 (the *Drosophila* orthologue of BPTF; also known as E(bx) (enhancer of bithorax)) do not exist, but double immunostaining of salivary gland polytene chromosomes showed some overlap between sites of ISWI binding and sites of H3K4me3 (data not shown), consistent with the possibility that the NURF complex associates with H3K4me3.

We next verified that the large subunit of NURF dictates H3K4me3-binding by testing the individual binding specificities of BPTF, NURF301 or other subunits of the *Drosophila* and human NURF complexes. Haemagglutinin (HA)- or Flag-tagged components of NURF were individually expressed in SF9 cells, and extracts used in histone tail peptide pull-down assays. NURF301 and BPTF were enriched in the H3K4me3 pull down, similar to the gradient-purified, reconstituted NURF complex (Fig. 2A, panels a–c). Human SNF2L/ISWI bound irrespective of the peptide H3K4 methylation status (Fig. 2A, panel d), whereas *Drosophila* NURF55 and its human orthologue RbAp48 (also known as RBBP4) preferentially bound to the unmodified H3 peptide (Fig. 2A, panels e and f). Recombinant NURF38 did not bind any of the H3 peptides tested (data not shown).

BPTF and NURF301 are large, multidomain proteins. To identify the region responsible for H3K4me3-binding, glutathione *S*-transferase (GST) fusions that span NURF301 (ref. 15) were expressed in *Escherichia coli*, purified and tested in peptide pull-down assays. Only the most carboxy-terminal region (residues 1,999–2,669; see schematics in Fig. 2B), specifically bound to H3K4me3 (Fig. 2B, panel a; data not shown). Further deletion narrowed the region of interaction to the second (bromodomain-proximal) PHD finger (PHD2), which is conserved in human BPTF (Fig. 2B, panels e–g).

We next confirmed that the PHD finger is obligatory—in the context of the full-length protein—for H3K4me3-binding. Cells were transfected with either Flag-tagged wild-type (WT) human BPTF, or Flag-tagged BPTF containing a W32E substitution in the PHD finger (PHD-W32E; see ref. 18), and extracts were subjected to a peptide pull-down assay. The W32E substitution abolished recognition of

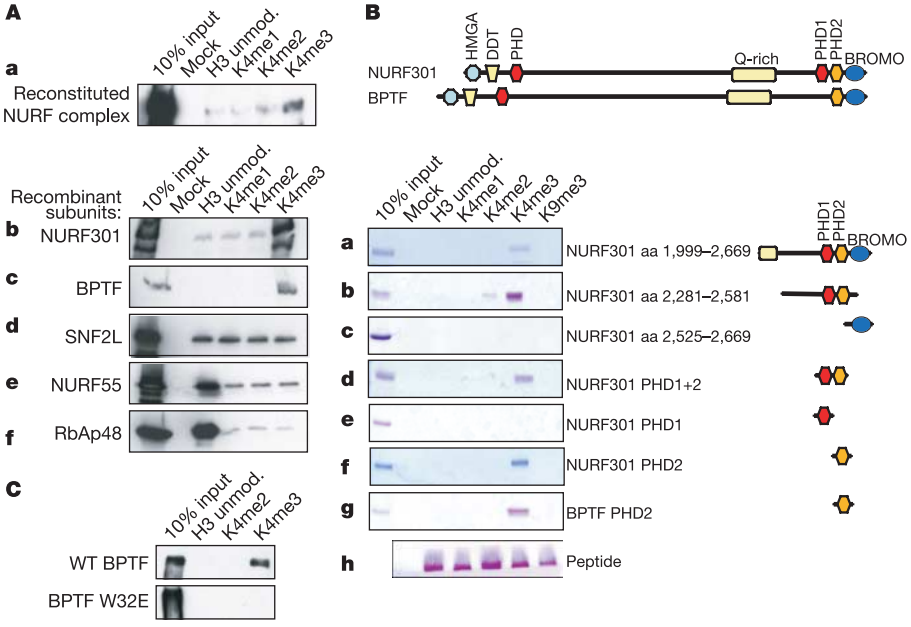


**Figure 2 | The bromodomain-proximal PHD finger of BPTF/NURF301 binds to H3 K4me3. A**, BPTF/NURF301 confers H3K4me3 recognition. Reconstituted, gradient-purified *Drosophila* NURF (**a**), extracts from SF9 cells expressing individual tagged components of *Drosophila* NURF (**b**, **e**), or human NURF components (**c**, **d**, **f**), were used in pull-down assays followed by immunoblotting. **B**, The PHD finger of BPTF/NURF301 is sufficient for association with H3K4me3. Top, schematics show *Drosophila* NURF301 and human BPTF. HMGA, high-mobility group box A domain; DDT, DNA-binding homeobox and different transcription factors domain; Q-rich, glutamine-rich domain; BROMO, bromodomain. Bottom, peptide pull-down assays using recombinant GST–NURF301 (**a**–**f**) and GST–BPTF (**g**) fusion proteins containing different regions of the NURF301/BPTF C terminus. H3 peptides used in the assays are indicated. Bound proteins were analysed by Coomassie-blue staining. Equivalent peptide loading was ensured by silver staining following SDS–PAGE (**h**). aa, amino acids. **C**, The PHD finger of BPTF is necessary for H3K4me3-binding. Nuclear extracts from cells transfected with a construct encoding Flag-tagged wild-type (WT) human BPTF, or BPTF containing a W32E substitution in the PHD finger, were used in pull-down assays with unmodified and H3K4me3 peptides. Bound proteins were probed with an anti-Flag antibody.

H3K4me3 by BPTF (Fig. 2C). Taken together, these results indicate that the bromodomain-proximal PHD finger is both necessary and sufficient for H3K4me3 association.

Although NURF is known to be directly recruited by site-specific transcription factors (for example, in *Drosophila*, GAGA factor[15] and the ecdysone receptor[17]), its association with chromatin is likely to be stabilized by H3K4me3 (see Supplementary Fig. S3A). This model predicts that (1) loss of BPTF, unlike loss of WDR5, should not affect H3K4me3, (2) loss of H3K4me3 should reduce chromatin association of BPTF, and (3) WDR5 and BPTF function in a common biological pathway.

To determine whether BPTF directly controls H3K4 methylation, HEK293 cells transfected with *BPTF* short interfering RNA (siRNA) were compared with cells transfected with non-coding and *WDR5* siRNA controls. *BPTF* siRNA transfection caused efficient knockdown of BPTF (Fig. 3A, panel a), but did not affect either WDR5 levels (Fig. 3A, panel b) or global levels of H3K4me (Fig. 3A, panels c and d).

To test the second prediction, HEK293 cells stably expressing Flag–BPTF were transfected with either control or *WDR5* siRNA, and then fractionated to separate subcellular fractions[19]. *WDR5* siRNA treatment ablated WDR5 from all fractions and reduced H3K4me3 levels (Fig. 3B, panels b and c). WDR5 knockdown caused the partial loss of BPTF from chromatin (~40–50% loss), and the appearance of BPTF in the soluble cytosolic/nuclear fraction. Partial loss of BPTF–chromatin association may be due to incomplete loss of H3K4me3 and/or redundancy in other modes of chromatin association. Nevertheless, our results suggest that H3K4me3 stabilizes association of BPTF/NURF with chromatin (see the model in Supplementary Fig. S3B). This conclusion is additionally supported by chromatin immunoprecipitation (ChIP) analysis of the *HOXC8* locus in control versus

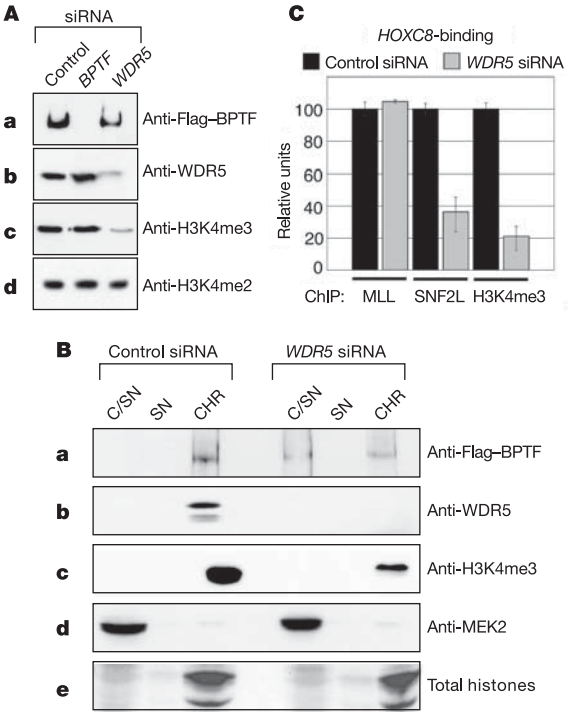


**Figure 3 | WDR5 regulates BPTF chromatin association. A**, BPTF knockdown does not affect H3K4 methylation. HEK293 Flag–BPTF cells were transfected with control siRNAs, a *BPTF* siRNA pool, or a *WDR5* siRNA pool and probed with the indicated antibodies (**a**–**d**). **B**, WDR5 knockdown results in partial release of BPTF from chromatin. Biochemical fractionation[19] was used to generate three fractions: C/SN (cytosolic proteins and some soluble nuclear components); SN (remaining soluble nuclear components); and, CHR (chromatin). Fractions were probed with the antibodies indicated (**a**–**d**) or stained with Coomassie blue for analysis of total histones (**e**). **C**, Association of SNF2L with the *HOXC8* locus requires WDR5. ChIP assays with anti-MLL, anti-SNF2L and anti-H3K4me3 antibodies were performed using HEK293 cells treated with control (black bars) and *WDR5* (grey bars) siRNAs. Signals were quantified relative to inputs (see Methods). Error bars indicate s.d.

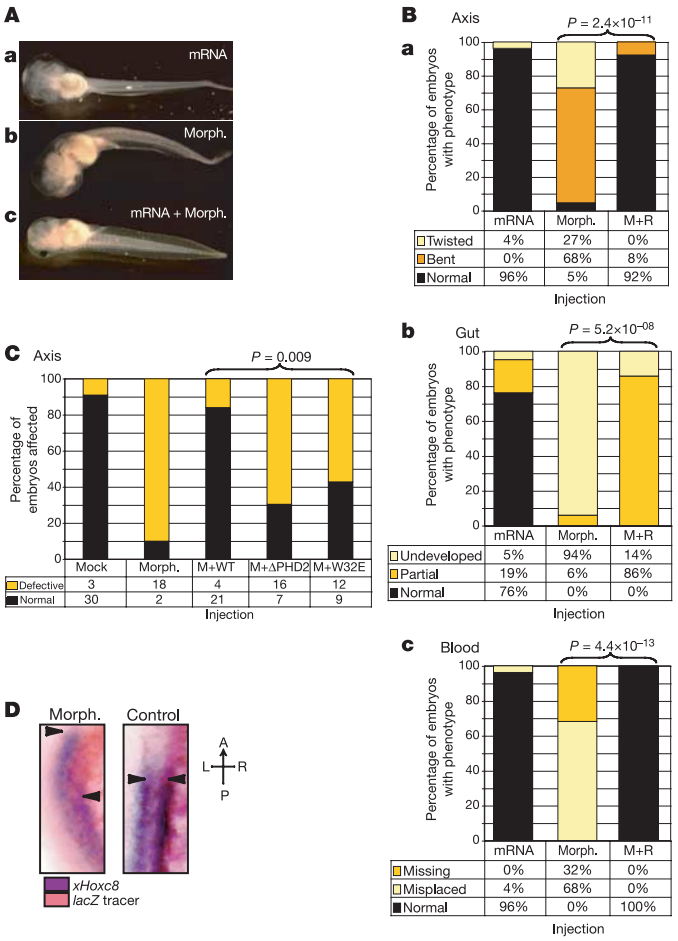


**Figure 4 | Loss of BPTF phenocopies loss of WDR5 in axial, gut and blood defects during *Xenopus* development. A**, Feeding-stage tadpoles, derived from two-cell-stage embryos that were injected in one blastomere with *xBPTF* morpholino (**b**), *hBPTF* mRNA (**a**), or *xBPTF* morpholino and *hBPTF* mRNA (**c**). Tadpoles derived from the morpholino-injected embryos show axial, blood and gut patterning defects (see the text) that are rescued by co-injection of *hBPTF* mRNA. **B**, Penetrance of phenotypes shown in **A**. The occurrence of axial deformities (**a**), gut patterning defects (**b**), and blood defects (**c**) were scored in morpholino-injected embryos ("Morph."), embryos co-injected with morpholino and *hBPTF* mRNA ("M+R"), or those injected with *hBPTF* mRNA alone ("mRNA"); *P*-values are indicated above each plot. **C**, Quantitation of rescue by WT BPTF or forms of BPTF defective in H3K4me3-binding. Axial deformities were scored in morpholino-injected embryos ("Morph."), and embryos co-injected with morpholino and mRNAs encoding either WT hBPTF ("M+WT"), hBPTF with C-terminal truncation ("M+ΔPHD2"), or hBPTF containing a point mutation in the PHD domain that abolishes H3K4me3 recognition ("M+W32E"). Amount of injected RNA was standardized between samples. Results were plotted as proportions in the total population counted; *P*-values are indicated above each plot, and the absolute number of embryos in each category are shown below. Similar analyses were done for blood and gut defects (Supplementary Fig. S4). **D**, Whole-mount *in situ* detection of the *xHoxc8* transcript. Dorsal view of the mid-trunk region of stage-30 tadpoles derived from embryos injected with *xBPTF* morpholino (left panel) or injected with a control (right panel). Purple stain indicates *xHoxc8* expression, which is most prominent in the neural tube, and red stain indicates the lineage tracer (*lacZ*). Arrows indicate misalignment of the *xHoxc8* expression domains between the left and right side of the neural tube in morpholino-injected, but not control, embryos. Images are shown in the same orientation. A–P, anterior–posterior axis; L–R, left–right axis.

*WDR5* siRNA-treated cells. As ChIP-grade BPTF antibodies are unavailable, we used antibodies against SNF2L/ISWI and determined that SNF2L/ISWI, but not MLL (mixed-lineage leukemia), binding to the *HOXC8* locus, was dependent on the presence of WDR5 and correlated with trimethylation of the locus at H3K4 (Fig. 3C). Furthermore, we demonstrated that binding of SNF2L/ISWI to the *Hoxc8* locus is reduced in $Mll^{-/-}$,compared with $Mll^{+/+}$, mouse embryonic fibroblasts (Supplementary Fig. S2C). Consistent with previous data[20], H3K4 methylation of the *Hoxc8* locus is dependent on MLL (Supplementary Fig. S2B). The correlation between binding of SNF2L/ISWI and H3K4me3 at *HOXC8* supports the hypothesis that H3K4me3 stabilizes BPTF–SNF2L complexes at target genes.

We next investigated whether loss of BPTF in *Xenopus* embryos phenocopies defects previously described for loss of WDR5 (ref. 5). One blastomere of the two-cell-stage *Xenopus laevis* embryo was injected with either a morpholino oligonucleotide targeting *Xenopus BPTF* (*xBPTF* morpholino), *xBPTF* morpholino and human *BPTF* messenger RNA (*hBPTF* mRNA), or *hBPTF* mRNA alone. Injected embryos were allowed to develop to the feeding tadpole stages. *xBPTF* morpholino-injected tadpoles showed axial defects on the injected side of the body resulting in a distinctive bend in the embryo (Fig. 4A, panel b), as well as gut and blood defects (see Fig. 4B, panels a–c, for penetrance analysis). All phenotypes were highly penetrant and were rescued by the co-injection of *hBPTF* mRNA along with the *xBPTF* morpholino (Fig. 4A, panel c; Fig. 4B). Injection of the *hBPTF* mRNA alone had no significant effect (Fig. 4A, panel a; Fig. 4B), and phenotype penetrance and severity was dose-dependent (data not shown). The mRNA-mediated rescue of these defects indicates that the phenotypes are due to BPTF protein knockdown and not to non-specific effects.

Notably, the phenotypes observed in *xBPTF* morpholino-injected tadpoles are strikingly similar to those resulting from a *xWDR5* morpholino[5]. Like xWDR5 knockdown[5], xBPTF knockdown causes deregulation of *xHoxc8* expression. Two-cell-stage embryos were injected into one blastomere with *xBPTF* morpholino and *lacZ* mRNA as a lineage tracer. xBPTF knockdown resulted in the posteriorization of *xHoxc8* expression by several somite lengths, indicating that BPTF, like WDR5, is important for control of *Hox* gene expression (Fig. 4D). In support of this, *Drosophila* NURF301 (a Trithorax group member) is required for homeotic gene expression[16].

To address whether recognition of H3K4me3 is important for BPTF function *in vivo*, rescue experiments were performed in parallel using an mRNA encoding (1) WT hBPTF, (2) hBPTF with a C-terminal truncation that deletes the second (bromodomain-proximal) PHD finger along with the bromodomain (ΔPHD2), or (3) hBPTF containing a single point mutation resulting in loss of H3K4me3recognition (PHD-W32E; see Fig. 2C). The ΔPHD2 truncation and the PHD-W32E substitution decreased rescue of axial (Fig. 4C), blood and gut (Supplementary Fig. S4) phenotypes, as compared with WT mRNA. These results demonstrate that recognition of H3K4me3 is essential for NURF function *in vivo*.

Our work demonstrates that two conserved chromatin regulators—WDR5, an adaptor protein essential for H3K4 trimethylation, and NURF, an archetypal remodelling complex—function in a common biological pathway. As H3K4 trimethylation correlates with transcription start sites, we favour the view that NURF recruitment to promoters, mediated by sequence-specific transcription factors[9,17] and stabilized by binding to H3 tails trimethylated at K4, modulates transcription initiation through ATP-dependent nucleosome sliding.

Our study identifies the PHD finger as a trimethyl-lysine-binding domain, capable of highly specialized interpretation of the H3K4 methylation mark. The methyl-lysine association is likely to be a general function of the subset of PHD fingers containing the aromatic cluster (see ref. 18). In support of this, the PHD finger of the tumour suppressor protein ING2 also recognizes methylated H3K4 (see ref. 21). PHD fingers are present in multiple chromatin-associated proteins[22], often in conjunction with a bromodomain—a module that recognizes acetylated histone tails[23]. Moreover, PHD fingers and bromodomains have been shown to functionally cooperate[24,25]. Notably, the H3K4me3-binding PHD finger of BPTF/NURF301 is situated in close proximity to its extreme C-terminal bromodomain, suggesting the existence of a double-domain module, which would most likely recognize a trimethyl (K4)-acetyl pattern of modifications. The higher efficiency of rescue with the PHD-W32E point mutant, compared with the C-terminal truncation, suggests that the PHD finger and the bromodomain may cooperate in mediating BPTF function.

## METHODS

**Pull-down assays.** Pull-down assays with extracts and recombinant proteins were performed as described previously[5]. Nuclear extracts were prepared from HEK293 cells or NIH3T3 cells using the Dignam protocol[26]. Briefly, $10^8$ cells were used per pull-down assay. Salt and Triton-X100 concentrations were 150 mM and 0.2% (v/v), respectively. Nuclear extracts were precleared with avidin beads and incubated with peptide prebound to avidin beads for 3 h at 4 °C. Approximately 5 μg of peptide was used per pull down. Beads were washed eight times with 20 mM HEPES, pH 7.9, 250 mM KCl, 0.2% (v/v) Triton-X100, 1 mM phenylmethylsulphonyl fluoride (PMSF), and containing protease inhibitor cocktail (Roche). The final wash was performed with 4 mM HEPES, pH 7.9, 10 mM NaCl, 1 mM PMSF, and containing protease inhibitor cocktail. Bound proteins were eluted from the resin twice using 100 mM glycine, pH 2.8. Eluates were combined, neutralized using 0.1× volume of 1 M Tris-HCl, pH 8, and analysed by SDS–PAGE. The HEK293 Flag–BPTF cell line was prepared by co-transfection of pCMV-Flag–BPTF with a 20-fold excess of PAC vector, followed by puromycin selection at 4 mg $ml^{-1}$ and subcloning of individual colonies. All histone peptides (amino acids 1–20) were synthesized by the Rockefeller University Proteomics Resource Center.

Details of additional methods used in this study are included in Supplementary Information.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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