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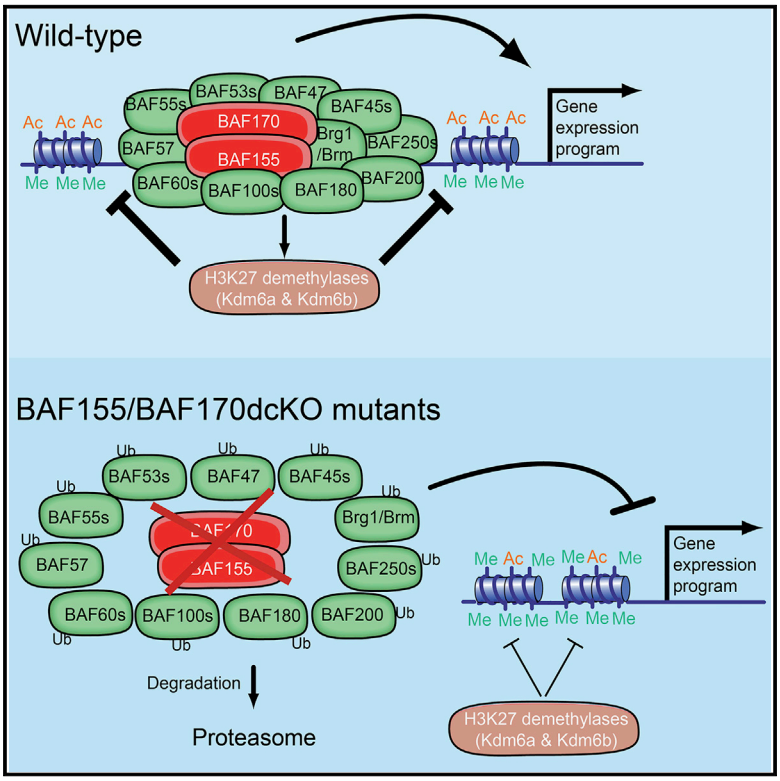
Loss of BAF (mSWI/SNF) Complexes Causes Global Transcriptional and Chromatin State Changes in Forebrain Development

Graphical Abstract



Highlights

- The BAF complexes are lost in BAF155/BAF170 dcKO mutants
- BAF complexes control chromatin state and global gene expression programs
- BAF complexes potentiate the activity of H3K27 demethylases
- BAF complexes are indispensable for forebrain development

Authors

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In Brief

Narayanan et al. show that elimination of both core BAF155 and BAF170 subunits causes degradation of the BAF complexes and impairment of gene expression program in forebrain development. Mechanistically, BAF complexes control key chromatin modifications (H3K27Me2/3) by modulating the activity of H3K27 demethylases JMJD3/UTX.

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Loss of BAF (mSWI/SNF) Complexes Causes Global Transcriptional and Chromatin State Changes in Forebrain Development

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SUMMARY

BAF (Brg/Brm-associated factors) complexes play important roles in development and are linked to chromatin plasticity at selected genomic loci. Nevertheless, a full understanding of their role in development and chromatin remodeling has been hindered by the absence of mutants completely lacking BAF complexes. Here, we report that the loss of BAF155/BAF170 in double-conditional knockout (dcKO) mice eliminates all known BAF subunits, resulting in an overall reduction in active chromatin marks (H3K9Ac), a global increase in repressive marks (H3K27me2/3), and downregulation of gene expression. We demonstrate that BAF complexes interact with H3K27 demethylases (JMJD3 and UTX) and potentiate their activity. Importantly, BAF complexes are indispensable for forebrain development, including proliferation, differentiation, and cell survival of neural progenitor cells. Our findings reveal a molecular mechanism mediated by BAF complexes that controls the global transcriptional program and chromatin state in development.

INTRODUCTION

Chromatin remodeling is an important regulator of developmental gene expression. Vertebrate mSWI/SNF, also known as the Brg1/Brm-associated factor (BAF) complexes, contains at least 15 different subunits, including two interchangeable core ATPase subunits (Brg1 or Brm), invariant core (BAF47, BAF155, and BAF170), and a variety of lineage-restricted subunits (Ho and Crabtree, 2010). Together with core components, Brg1 or Brm can remodel nucleosomes in vitro at a rate comparable to that of the entire complex (Phelan et al., 1999). However, hundreds of distinct BAF complexes are predicted to form in vivo through combinatorial assembly (Ho and Crabtree, 2010). Integrated BAF complex subunits, which interact through composite surfaces with transcription factors, co-activators, co-repressors, and signaling pathways, are essential for genome targeting and, thus, are responsible for conferring functional specificity (Ho and Crabtree, 2010; Narayanan and Tuoc, 2014).

Understanding the roles of BAF complexes in murine neural development so far has been based on data from phenotypic analyses of neural cell-type-specific mouse mutants of genes encoding the ATPase subunit Brg1 and some other BAF subunits (Bischof et al., 2015; Lessard et al., 2007; Matsumoto et al., 2006; Ninkovic et al., 2013; Tuoc et al., 2013a; Vogel-Ciernia et al., 2013; Weider et al., 2012; Wu et al., 2007; Yu et al., 2013). In ATPase Brg1-deficient neural cells, other BAF subunits are expressed at normal levels and are incorporated into ATPase Brm-based complexes (Lessard et al., 2007). In addition, the loss of both Brg1 and Brm subunits does not affect the expression of other BAF subunits in cancer cell lines (Strobeck et al., 2002). Because most BAF subunits have chromatin-, DNA-, and protein-binding capacity, BAF complexes lacking ATPase subunits likely have additional ATPase-independent roles in development (Chaiyachati et al., 2013). Thus, previous studies using knockout mouse models of individual BAF subunits possibly did not provide a complete view of BAF complex function.

We previously demonstrated that a dynamic competition between BAF155 and BAF170 subunits that determines the mammalian body and cortical size takes place during embryogenesis and corticogenesis (Tuoc et al., 2013a, 2013b). Here we present evidence that elimination of both core BAF155 and BAF170 subunits causes proteasome-mediated degradation of

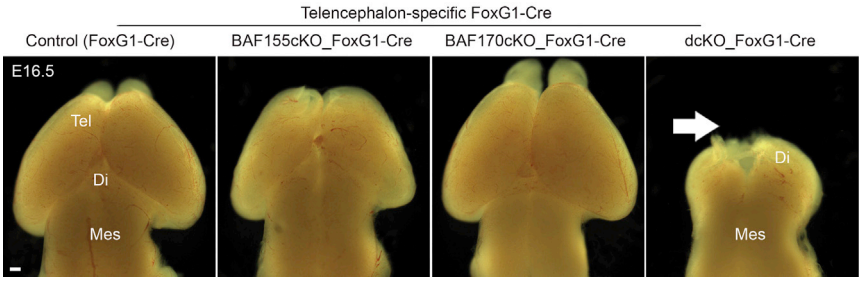

Figure 1. The Expression of BAF155 and BAF170 Is Indispensable for Telencephalon Development

The telencephalon-specific *dcKO_FoxG1*-Cre mutants lack the telencephalon at E16.5. Tel, telencephalon; Di, diencephalon; Mes, mesencephalon. Scale bar, 100 μm.

the entire BAF complexes and impairs the global epigenetic and gene expression program of forebrain development. We found that BAF complexes themselves do not possess H3K27-methylating activity; however, they inhibit transcriptional repression in neural cells by interacting with H3K27 demethylases and modulating their activity.

RESULTS

Expression of BAF155 and BAF170 Is Required for Forebrain Development

To examine the effects of loss of both BAF155 and BAF170 in neural development, we crossed mice harboring floxed alleles of *BAF155* (Choi et al., 2012) and *BAF170* (Tuoc et al., 2013a) (*BAF155*$^{fl/fl}$ and *BAF170*$^{fl/fl}$) with different Cre lines, including tamoxifen (TAM)-inducible ubiquitous *CAG*-Cre (Hayashi and McMahon, 2002), telencephalon-specific *FoxG1*-Cre (Hébert and McConnell, 2000), cortical progenitor-specific *Emx1*-Cre (Gorski et al., 2002), and projection neuron-specific *Nex*-Cre (Goebbels et al., 2006), generating the corresponding dcKO mutants dcKO_*CAG*-Cre, dcKO_*FoxG1*-Cre, dcKO_*Emx1*-Cre, and dcKO_*Nex*-Cre. The *dc*KO_*CAG*-Cre mutants died between embryonic days 14.5 and 15.5 (E14.5–E15.5) with severe developmental retardation upon TAM treatment at E10.5 (data not shown). The telencephalon-specific dcKO_*FoxG1*-Cre embryos, in which Cre was active from very early forebrain development (E8.5) in the entire telencephalon, exhibited a complete loss of the telencephalon structure (Figure 1, arrow), making these animals unsuitable for further study.

At E15.5, the cortex in dcKO_*Emx1*-Cre mutants was thinner compared to that in the control brain, but still allowed the study of important characteristics of corticogenesis in the absence of both BAF155 and BAF170 (Figure 2; Figure S1). Double immunolabeling with Pax6, a marker of radial glial progenitors (RGs) in the ventricular zone, and Tuj1, a marker for postmitotic neurons in the cortical plate, revealed dramatically thinner proliferative and mantle zones of the cortex in dcKO_*Emx1-Cre* mutants (Figure 2A). The number of phosphorylated histone H3-positive (pHH3+) dividing cortical progenitors in M phase was reduced at E12.5, and these cells were almost absent at E15.5 in mutants compared to controls (Figures 2B and 2C). To determine whether the loss of both *BAF155* and *BAF170* influenced differentiation and cell-cycle progression of progenitors, we performed 24-hr CldU and 1-hr IdU pulse labeling of cycling cortical progenitor cells in vivo. Triple-labeling immunohistochemistry (IHC) with antibodies for CldU, a marker for both exited and cycling progenitors, IdU, an S phase progenitor marker, and Ki67, a marker for proliferating progenitors in all phases of the cell cycle (except late G2), revealed no CldU+/Ki67−/IdU− progenitors exiting from the cell cycle in the dcKO-*Emx1-Cre* cortex, which was kept exclusively in S phase (Ki67+/CldU+/IdU+) at E15.5 (Figures 2D and 2E; Figure S2A). We examined apoptosis in different dcKO tissues and cultured cells by performing IHC and western blot (WB) with cleaved Casp3 (Figure 2F; Figures S2B and S2C). In cortex-specific dcKO_*Emx1*-Cre mutants, expression of BAF155 and BAF170 was completely lost from E12.5. However, a significant number of dead cells were found only after E14.5 in Pax6+ cortical progenitors, but not in Tuj+ cortical neurons. Our findings suggest the possibility that mutant progenitors, which were exposed for a long period to a defective cell-cycle progression, undergo apoptosis. Accordingly, we detected no significant number of dead cells in neuron-specific dcKO_*Nex*-Cre at post-natal day 1 (P1) (Figure 2F) or cultured dcKO_CAG-Cre after 6 days of TAM treatment (data not shown).

To study cortical layer formation, we analyzed the expression of neuronal layer-specific markers at E16.5. With the exception of Rorβ, expression of other indicated cortical layer markers was largely lost in cortices of dcKO mutants (Figures 2G and 2H). Taken together, these findings indicate that expression of BAF155 and BAF170 is vital for embryogenesis and forebrain formation. During corticogenesis, loss of both BAF155 and BAF170 in RGs causes multiple defects in proliferation and differentiation capacities of cortical progenitors and promotes cell death.

BAF155 and BAF170 Control the Stability of BAF Complexes

Previous in vitro studies have shown that BAF155 and BAF170 directly interact with certain BAF subunits, including Brg1, BAF60a, BAF57, and BAF47, to protect them from proteasome-mediated degradation (Chen and Archer, 2005; Sohn et al., 2007). However, our in vivo experiments indicated that, upon loss of either BAF155 or BAF170 in the cortex of *BAF155cKO_Emx1*-Cre and *BAF170cKO_Emx1*-Cre mice (Tuoc et al., 2013a), the expression of other BAF subunits was largely preserved. This prompted us to investigate whether the expression of both BAF155 and BAF170 subunits is required for the stability of the known components of different BAF complexes. Strikingly, we found that the expression of all tested BAF subunits was reduced at E12.5 (Figure S3A) and largely absent at E16.5 (Figures 3A and 3B; Figures S3B–S3G) in cortical tissue of dcKO_*Emx1*-Cre mutants. A similar effect was observed in WB analyses of lysates of primary neurons from forebrains of E13.5 dcKO_*CAG*-Cre embryos (Figures S4A and S4B) and of embryonic stem cells (ESCs) from E3.5 dcKO_*CAG*-Cre blastocysts

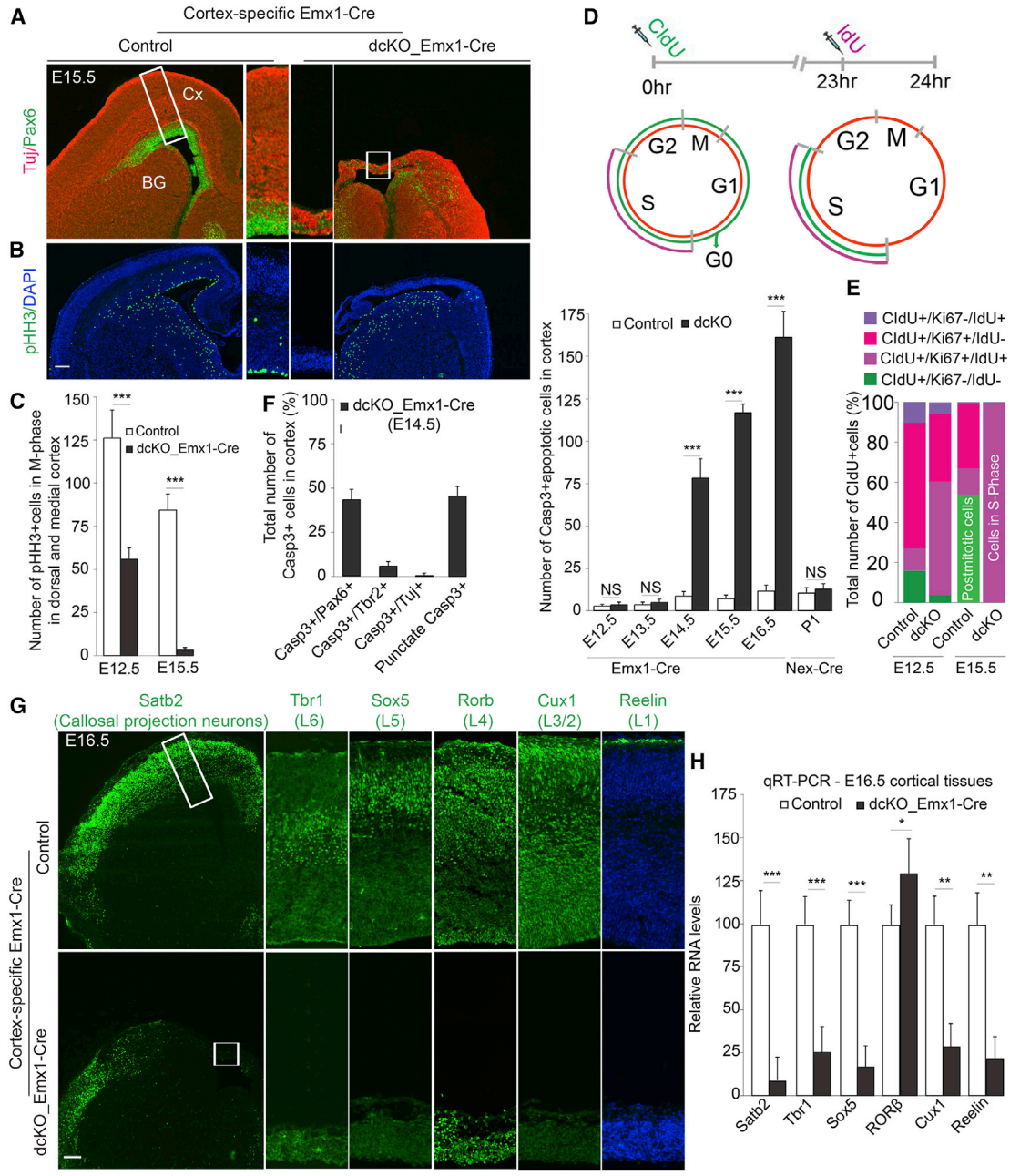

Figure 2. BAF155 and BAF170 Expression Is Essential for Cerebral Cortical Development

(A) Double IHC analyses using antibodies that specifically label RGs (Pax6) or neurons (Tuj1) at E15.5. Middle panels are higher magnification images of fields in the cortex indicated by white frames.

(B and C) IHC (B) and quantitative analyses (C) showed the number of pHH3+ cortical progenitors in M phase in dcKO_*Emx1*-Cre mutants was diminished at E12.5 and lost at E15.5.

(D and E) Experimental paradigm (D) for determining cell-cycle parameters and color scheme for immunolabeling of micrographs (in Figure S2A) are shown. (E) Cell-cycle distribution of control and mutant CldU-labeled cells determined by IHC (see also Figure S2A).

(F) (Right) Quantitative analyses showed that cortical tissues of mutants had a low number of Casp3+ apoptotic cells at P1 (dcKO-*Nex*_Cre) and E12.5–E13.5 (dcKO-*Emx1*_Cre) and a high number at E14.5–E16.5 (dcKO-*Emx1*_Cre), as determined by IHC (see also Figure S2B). (Left) Quantitative analyses revealed that the majority of Casp3+ apoptotic cells were Pax6+ progenitors and cells with punctate forms (at the late phase of apoptosis) (see also Figure S2C for IHC images).

(G and H) IHC (G) and qRT-PCR (H) analyses of cortical phenotype at E16.5 with layer markers in an entire hemisphere (for Satb2 marker) and in a comparable dorsal area (for Tbr1, Sox5, RORβ, Cux1, and Reelin). Values are presented as means ± SEMs (n = 6; *p < 0.05, **p < 0.01, and ***p < 0.005). Cx, cortex; BG, basal ganglion; L, layer. Scale bars, 100 μm.

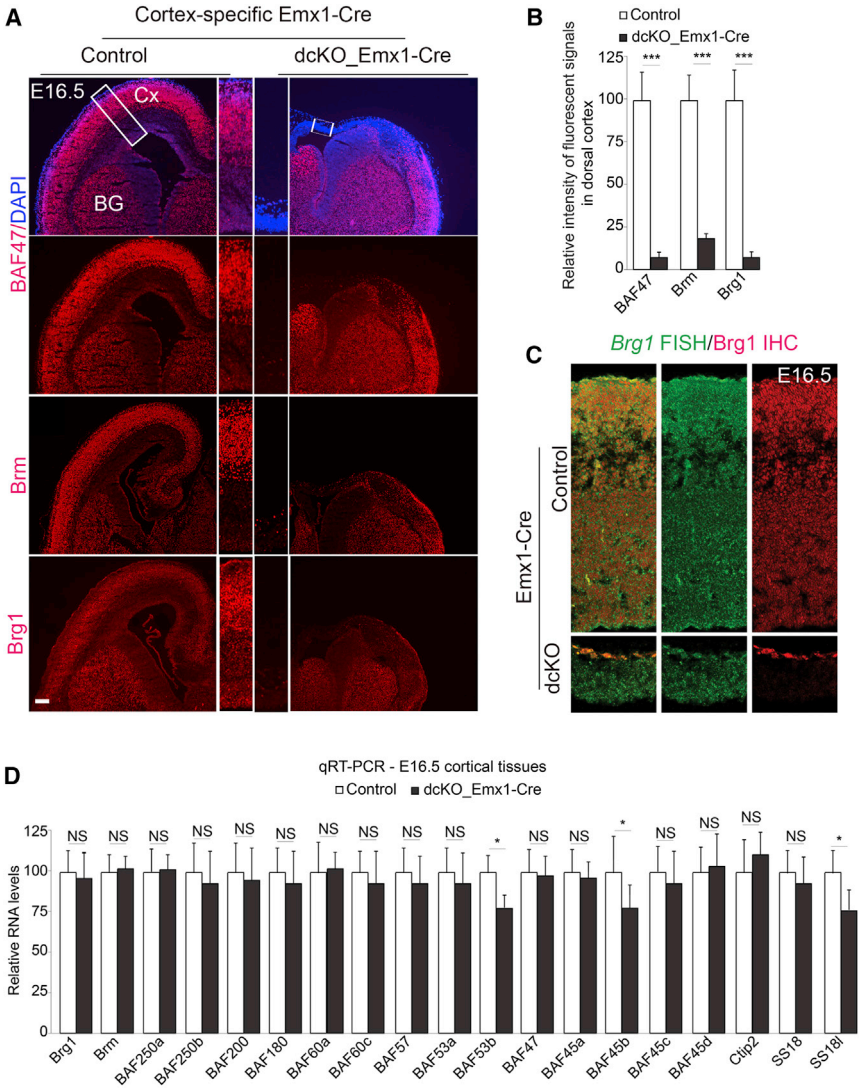

Figure 3. Expression of BAF Subunits at Protein Level Is Lost in dcKO Mutants

(A and B) Analysis of three core BAF subunits (Brg1, Brm, and BAF47) in the dcKO_*Emx1*-Cre cortex at E16.5 (see also Figure S3). (A) IHC of coronal sections is shown. (B) Quantification of fluorescence signals in (A) is shown.

(C) Loss of Brg1 protein (IHC), but not *Brg1* transcript (FISH), in dcKO_*Emx1*-Cre cortex at E16.5 is shown (see also Figure S5).

(D) The qRT-PCR analysis of BAF subunit transcripts in the cortex of dcKO_*Emx1*-Cre mutants. Values are presented as means ± SEMs (n = 6; *p < 0.05, **p < 0.01, and ***p < 0.005; NS, not significant). Cx, cortex; BG, basal ganglion. Scale bar, 100 μm.

(Figure S4D) after treating with TAM for 4 days, which caused efficient deletion of both BAF155 and BAF170 within 48 hr. Collectively, these results indicate that the loss of *BAF155* and *BAF170* in dcKO mutants eliminates all tested subunits of BAF complexes in ESCs and cortical cells both in vitro and in vivo.

Interestingly, application of double fluorescence in situ hybridization and IHC (FISH/IHC) revealed that Brg1, BAF60a, and BAF250a proteins were lost in cortical tissue of dcKO_*Emx1*-Cre mutants, whereas their transcripts were largely preserved (Figure 3C; Figure S5). Using qRT-PCR to compare the expression of BAF subunits at the transcript level in cortical tissue from dcKO_*Emx1*-Cre embryos and dcKO_*CAG*-Cre primary neurons and ESCs with corresponding controls, we similarly found that the loss of both BAF155 and BAF170 did not affect the transcription of most BAF subunits. Notably, only transcripts encoding the unique neuron (n)BAF complex subunits, BAF53b, BAF45b, and SS18l, were slightly downregulated in dcKO_*Emx1*-Cre cortices (Figure 3D). This is possibly attributable to neuronal differentiation defects, since no such difference at the transcript level was found between dcKO_*CAG*-Cre and control primary neurons (Figure S4E).

We then examined whether BAF subunits in dcKO mutants are degraded by the ubiquitin-proteasome system (UPS), focusing on two ATPase subunits, Brg1 and Brm. Protein ubiquitination (Ub) and stability were analyzed in cultured primary neurons derived from E13.5 dcKO_*CAG*-Cre embryo forebrain tissue using different biochemical assays (Tuoc and Stoykova, 2008b). First, we determined whether Brg1 and Brm had undergone ubiquitination in *dcKO_CAG*-Cre primary neurons, which had been treated with TAM for 24 hr, compared to the untreated controls. Both proteins were immunoprecipitated from cell lysates with anti-Brg1 and anti-Brm antibodies, and the immunoprecipitates were analyzed for the appearance of poly(Ub)-conjugated forms of Brg1 and Brm (Figure 4A). In WBs of immunoprecipitates with an anti-polyubiquitin (Ub) antibody, a large amount of ubiquitinated Brg1 and Brm proteins were detected in TAM-treated cells (Figure 4A, smear bands), suggesting that loss of BAF155 and BAF170 induces ubiquitination of intracellular Brg1 and Brm proteins. Next we applied a pulse-chase approach using the protein biosynthesis inhibitor cycloheximide to compare the half-life of BAF subunits in TAM-treated dcKO_*CAG*-Cre neurons (Figure 4B). In the absence of the proteasome inhibitor MG132, 24-hr treatment with cycloheximide led to a reduction in Brg1 and Brm protein levels. This loss of Brg1 and Brm protein was substantially eliminated in the presence of MG132 (Figures 4B and 4C). Collectively, these results indicated that Brg1 and Brm are degraded by the UPS in the absence of BAF155 and BAF170.

Free subunits of multi-subunit complexes tend to be degraded by protein quality control systems if not assembled into a complex, a regulatory mechanism that serves to ensure proper stoichiometry (Goldberg, 2003). We hypothesized that, in the absence of BAF155/BAF170, BAF subunits dissociate from the

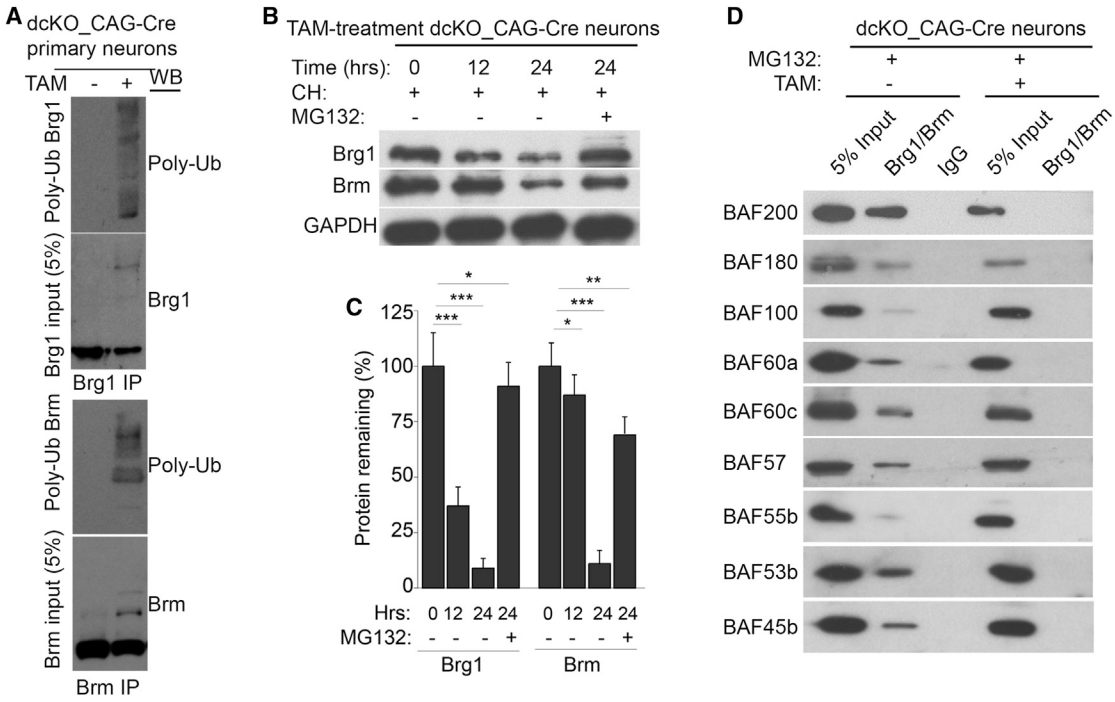

Figure 4. BAF155 and BAF170 Are Required for the Integrity of BAF Complexes

(A) Ubiquitination assays were performed in dcKO_*CAG*-Cre primary neurons. WB analysis with an anti-polyubiquitin (Ub) antibody and with eluted proteins after immunoprecipitation (IP) with Brg and Brm antibodies revealed a strong smeared band of ubiquitinated Brg1 and Brm in the absence of BAF155 and BAF170 (TAM+) compared with untreated controls (TAM−). Blots (against anti-Brg1 and Brm antibodies) showed the inputs of corresponding IP experiments.

(B) Pulse-chase assay of protein stability in dcKO_*CAG*-Cre primary neurons using the protein synthesis inhibitor cycloheximide (CH) in the presence or absence of the proteasome inhibitor MG132. A representative immunoblot is shown.

(C) Quantitative densitometry of immunoblots revealed proteasome-mediated degradation of Brg1 and Brm.

(D) Protein complex stability assay. Brg1 and Brm interactions with other BAF subunits in the presence (TAM−) or absence (TAM+) of BAF155/BAF170 were assessed by CoIP and WB analysis of MG132-treated dcKO_*CAG*-Cre primary neurons. Values are presented as means ± SEMs (n = 3; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$).

BAF complex prior to their degradation. To test this, we investigated the interaction of BAF subunits with their core subunits Brg1/Brm by performing co-immunoprecipitation (CoIP) experiments with antibodies against both Brg1 and Brm using MG132-treated dcKO_*CAG*-Cre neurons with and without TAM administration (Figure 4D). We found that, under stringent conditions in the presence of BAF155 and BAF170 (i.e., TAM−), all tested nBAF subunits physically associated with Brg1/Brm, as previously reported (Lessard et al., 2007). In contrast, these interactions were largely abolished in the absence of BAF155/BAF170 (TAM+). Thus, BAF155 and BAF170 subunits are required for the integrity of BAF complexes.

Global Impairment of Epigenetic and Gene Expression Programs in dcKO Mutants

Previous reports have indicated that the loss or overexpression of individual BAF subunits only influences local chromatin marks without causing global effects (Ho et al., 2011; Singhal et al., 2010; Tuoc et al., 2013a). The dcKO mutant mice generated here allowed us to re-examine whether loss of the entire BAF complexes has a global effect on epigenetics. To examine repressive and active chromatin marks, we performed IHC on cortical tissues from dcKO_*Emx1*-Cre mutants at E16.5 (Figures 5A and 5B) and WB analyses of lysates of TAM-treated dcKO_*CAG*-Cre primary neurons (Figure S4C) using antibodies against H3K27me2, H3K27me3, and H3K9Ac. Strikingly, the loss of BAF complexes resulted in a global reduction in active marks (H3K9Ac) with a concurrent global increase in repressive marks (H3K27me2 and H3K27me3) (Figures 5A and 5B; Figure S4C). The increase in H3K27me3 over gene bodies in the murine embryonic cortex resulting from the loss of BAF complexes was confirmed by chromatin immunoprecipitation sequencing (ChIP-seq) assays (Figures 5C–5E). The dcKO_*Emx1*-Cre embryos manifested a clear overall increase in H3K27me3 levels over gene bodies in E13.5 cortices (Figures 5C and 5E). The trend also was clear in P1 cortices obtained from projection neuron-specific dcKO_*Nex*-Cre mutants (Figure 5D). This diminished methylation appeared to be specific for H3K27, because methylation on other lysines of H3, such as H3K4 and H3K9, was not altered in the BAF155/BAF170-deficient cortex or primary neurons (Figures S6A and S6B). These findings suggest that the presence of BAF complexes is essential for the balance between global repressive and active epigenetic programs in neural development.

Because H3K27me2/3 marks are chromatin modifications associated with the repression of gene expression (Cao et al.,

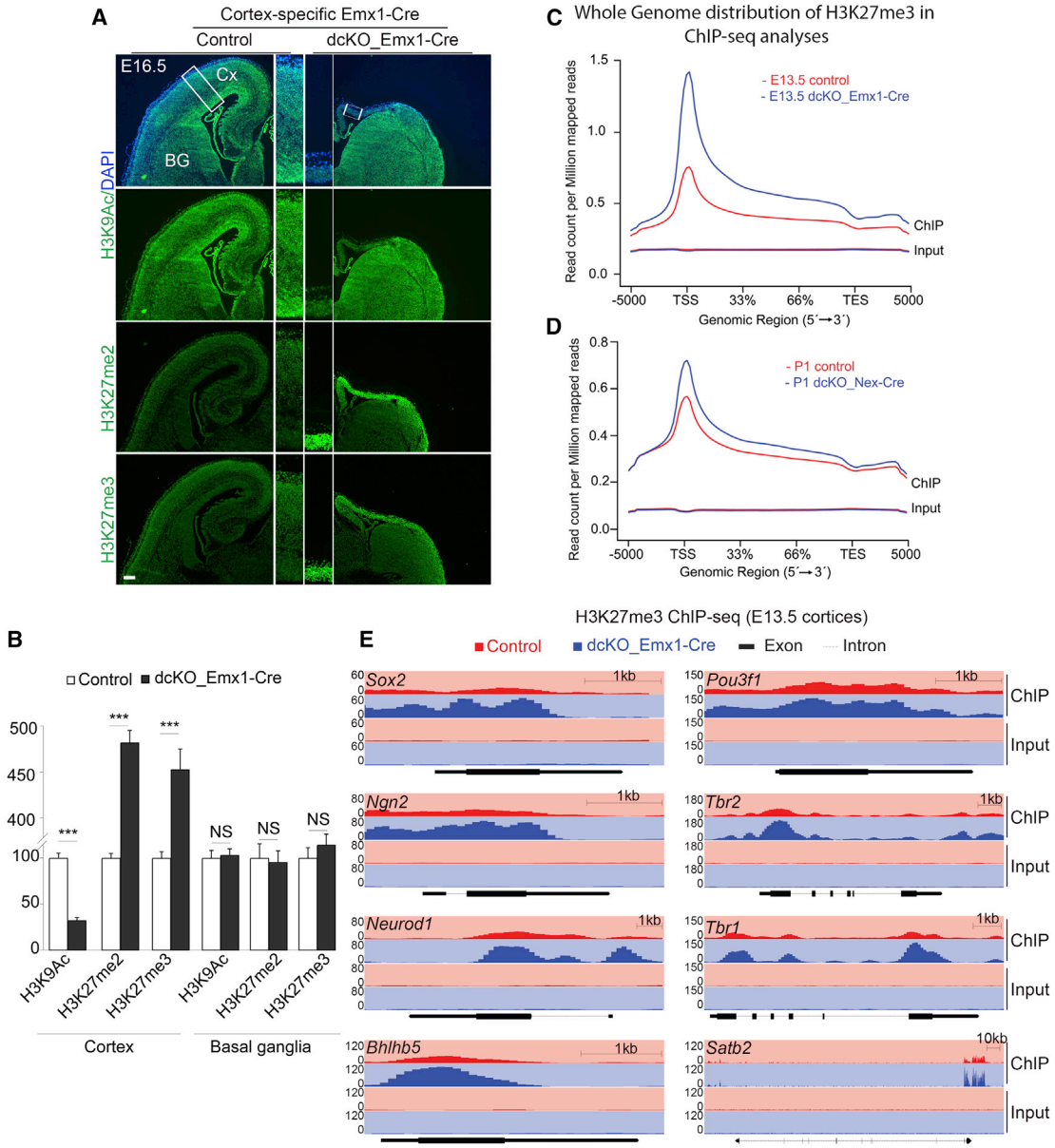

Figure 5. BAF Complexes Control Global Epigenetic Programs in Cortical Development

(A and B) Global changes in active (H3K9Ac) and repressive (H3K27me2/3) chromatin marks in the dcKO_*Emx1*-Cre cortex. (A) IHC of coronal sections is shown. (B) Quantification of fluorescence signals in (A) is shown.

(C and D) Genome-wide increases in H3K27me3 upon deletion of BAF complexes in E13.5 dcKO_*Emx1*-Cre (C) and P1 dcKO_*Nex*-Cre (D) cortices are shown, as determined by ChIP-seq analysis of mutant and control chromatin (paired Student's t test, $p < 0.0001$).

(E) Distribution of H3K27me3 along the bodies of representative genes that are important for cortical development in E13.5 control (red) and dcKO_*Emx1*-Cre (blue) cortices. Depicted are distributions after IP (upper two rows) and in the inputs (bottom two rows). Values are presented as means ± SEMs (n = 6; ***$p < 0.005$). Cx, cortex; BG, basal ganglion; TSS, transcription start site; TES, transcription end site. Scale bar, 100 μm.

2002; Pereira et al., 2010), the massive enhancement of H3K27me2/3 in dcKO mutants would be expected to result in marked downregulation of gene expression. To test this, we first performed gene expression profiling of the dcKO null cortex during the early stages of cortical neurogenesis (E12.5). This analysis revealed that 1,723 transcripts were downregulated, whereas only 102 transcripts were upregulated (Figures 6A–6C; Table S1). Many of the downregulated genes are known to play essential roles in brain development (Figures 6B and 6C; Figure S6C; Table S2). We then compared the intersection between the 1,723 genes downregulated following the deletion of BAF complexes (Figure 6A) with previously reported data on 1,080 genes upregulated in the *Ezh2cKO_Emx1*-Cre cortex at E12.5 in which H3K27me2/3 marks were removed (Pereira

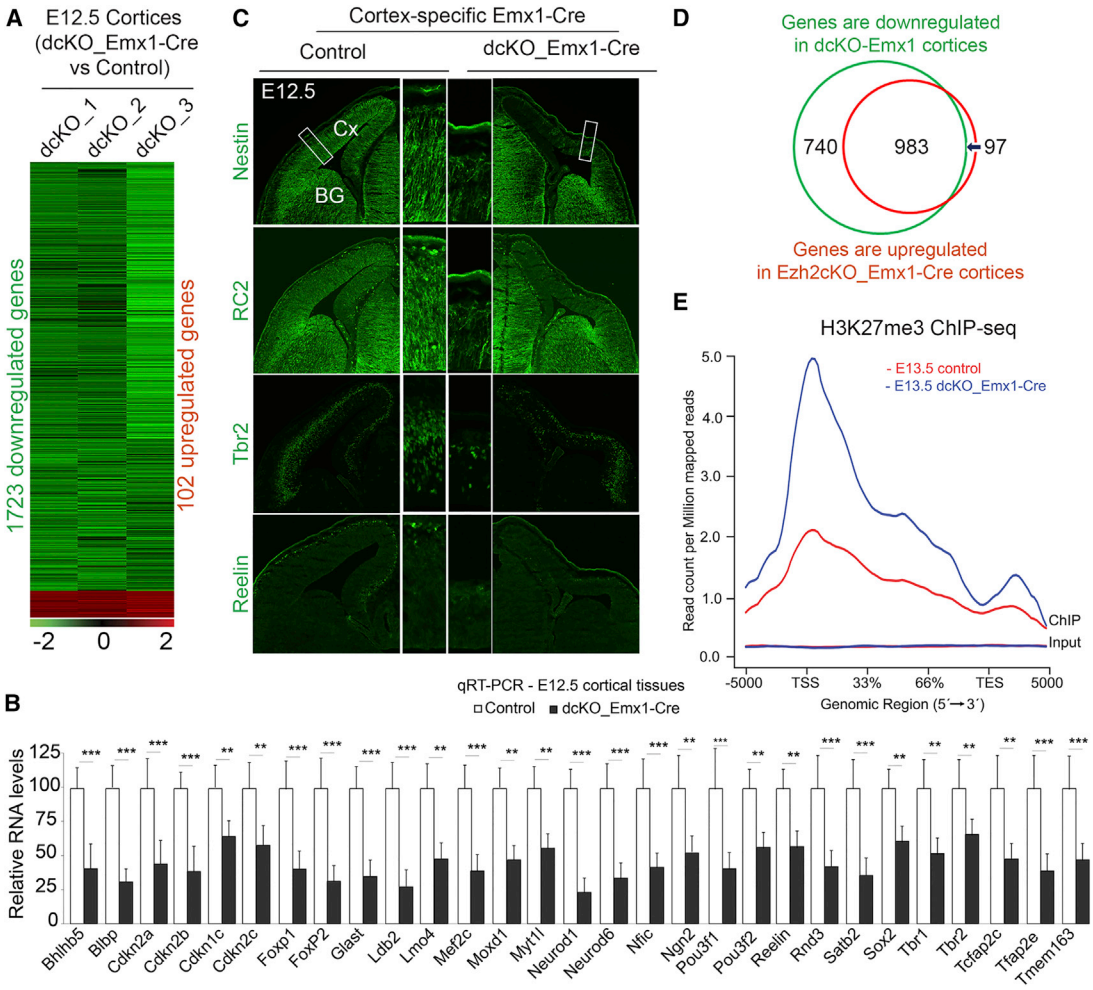

Figure 6. Loss of BAF Complexes Results Predominantly in Downregulation of Gene Expression

(A) Cluster diagram shows statistically significant changes in gene expression in microarray analyses of three cortices of E12.5 dcKO_*Emx1*-Cre littermates.

(B and C) Validations of gene expression profile data by IHC (B) and qRT-PCR (C) are shown.

(D) Overlap between downregulated genes in microarray studies of E12.5 dcKO_*Emx1*-Cre embryos and upregulated genes in similar experiments on E12.5 *Ezh2_Emx1*-Cre embryos (Pereira et al., 2010) is shown.

(E) Comparison of average H3K27me3 tag density at loci of downregulated genes from gene expression profiling in (A), between control and dcKO_*Emx1*-Cre cortices from E13.5 (paired Student's t test $p < 0.0001$). Values are presented as means ± SEMs (n = 6; $^{**}p < 0.01$ and $^{***}p < 0.005$). Cx, cortex; BG, basal ganglion; TSS, transcription start site; TES, transcription end site. Scale bars, 100 μm.

et al., 2010). Remarkably, BAF complexes positively regulated the majority of genes repressed by the H3K27 methyltransferase Ezh2 (Figure 6D). In addition, genes that were downregulated in the E12.5 cortex of dcKO_*Emx1*-Cre embryo showed an increase in H3K27me3 levels (Figure 6E). These data suggest that the regulation of H3K27me2/3 is crucial for normal gene expression programs in cortical development.

BAF Complexes Modulate the Activity of H3K27 Demethylases

To investigate the mechanisms underlying BAF155/BAF170-mediated global control over the epigenetic program during neurogenesis, we asked whether BAF complexes interact with chromatin-modifying enzymes and modulate their enzymatic activity. To search for BAF155/BAF170-interacting proteins, we performed CoIP on cell lysates from forebrain tissue of E13.5 or E17.5 wild-type (WT) mice and cultured neural stem cells (NSCs; NS-5 line) using anti-BAF155 and anti-BAF170 antibodies, followed by mass spectrometry (MS). The results indicated that BAF155/BAF170 subunits interact with numerous key factors involved in neural development. Interestingly, BAF155 and BAF170 bound to UTX/Kdm6a and JMJD3/Kdm6b (Figure S7A), the only enzymes known to specifically demethylate H3K27me2/3 (Agger et al., 2007; De Santa et al., 2007; Hong et al., 2007; Jepsen et al., 2007; Lan et al., 2007; Lee et al., 2007; Shpargel et al., 2014; Xiang et al., 2007). The interaction of BAF155 and BAF170 with these H3K27 demethylases was confirmed by WB analysis of BAF155 and

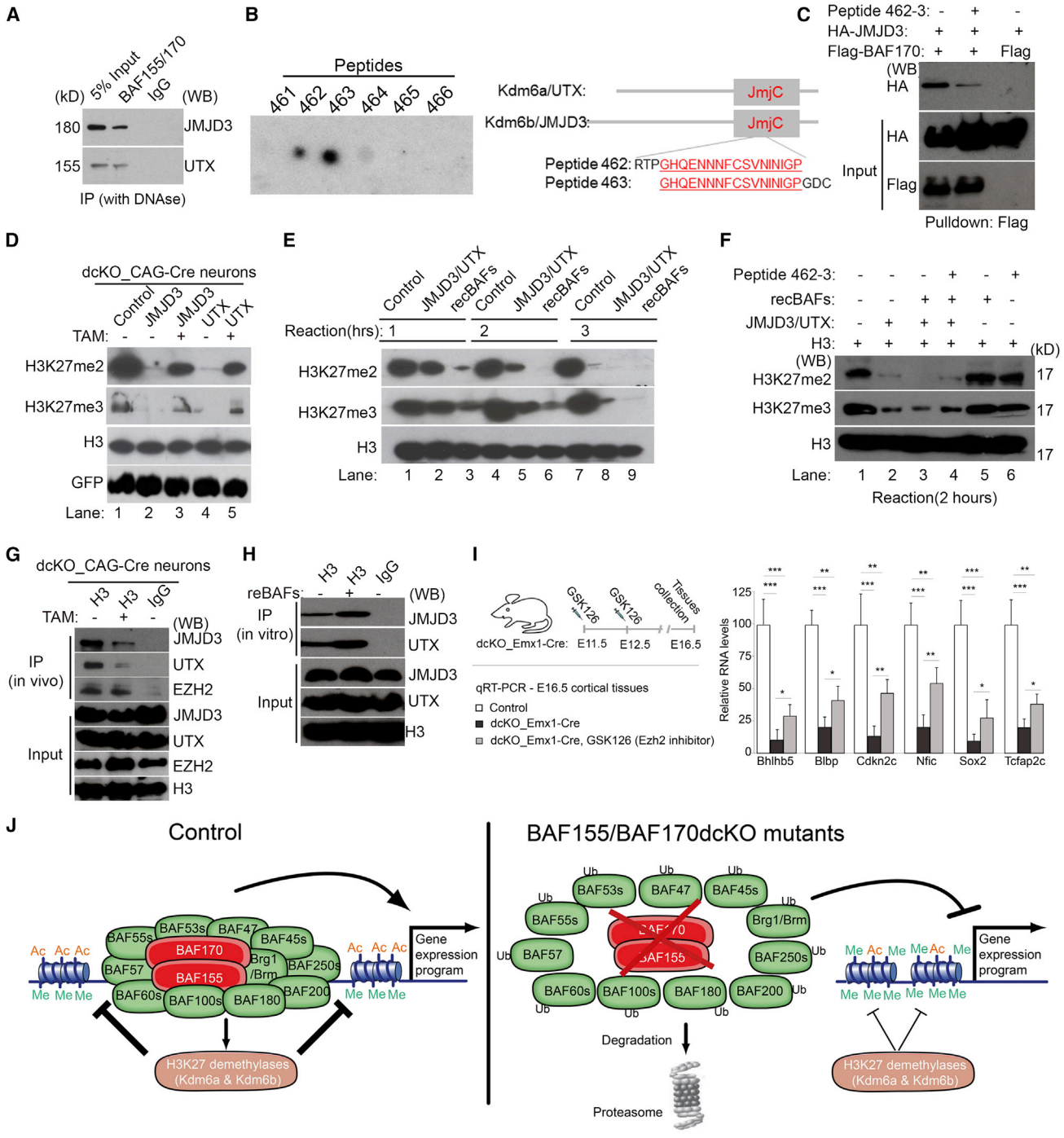

Figure 7. BAF Complexes Potentiate the H3K27 Demethylase Activity of UTX/Kdm6a and JMJD3/Kdm6b

(A) Interactions of BAF155 and BAF170 with UTX/Kdm6a and JMJD3/Kdm6b were found in CoIP/MS (see in Figure S7A) and confirmed by CoIP and WB analysis within E15.5 forebrain tissues.

(B) (Left) Binding of a mixture of purified Flag-tagged BAF155 and BAF170 protein to a Kdm6b/JMJD3 peptide array is shown. (Right) Peptides bound by BAF155/BAF170 are shown. Peptides numbered 462 and 463 are found in the conserved catalytic JmjC domain of the H3K27 demethylases UTX/Kdm6a and JMJD3/Kdm6b (see also Figure S7B).

(C) A pull-down experiment was performed with purified HA-JMJD3 and Flag-BAF170 protein, which was pre-incubated with the synthesized peptides 462–643. Note that the presence of the peptides substantially blocked interaction between BAF170 and JMJD3.

(legend continued on next page)

BAF170 immunoprecipitates of lysates from E15.5 cortical tissue (Figure 7A). Remarkably, CoIP/MS revealed that BAF155 and BAF170 bound to peptides within the JmjC domain, which is required for the H3K27me2/3 demethylase activity of UTX/Kdm6a and JMJD3/Kdm6b (Lan et al., 2007; Tsukada et al., 2006; Xiang et al., 2007). To validate the BAF155/BAF170-binding domains of JMJD3/Kdm6b, we applied a purified Flag-tagged BAF155 and BAF170 mixture to an array of JMJD3/Kdm6b peptides. The results confirmed that the BAF155/BAF170-binding domains lie within the conserved JmjC domain of both UTX/Kdm6a and JMJD3/Kdm6b (Figure 7B; Figure S7B, peptides 462–643). In addition, the specific interaction between BAF170 with JMJD3 via the JmjC domain was further confirmed by pre-incubation with purified BAF170 and the synthesized peptides 462–643 in the pull-down assay, as it was largely blocked (Figure 7C). Furthermore, IHC analyses revealed that BAF155 and BAF170 are co-expressed with UTX/Kdm6a and JMJD3/Kdm6b throughout the entire developing forebrain (Figure S7C).

Because the only enzymes known to methylate and demethylate H3K27 are Ezh1 and Ezh2 (or PRC2) (Cao et al., 2002; Pereira et al., 2010; Shen et al., 2008) and UTX/Kdm6a/JMJD3/Kdm6b (Agger et al., 2007; De Santa et al., 2007; Hong et al., 2007; Jepsen et al., 2007; Lan et al., 2007; Lee et al., 2007; Xiang et al., 2007), respectively, we examined the possibility that the increased level of H3K27me2/3 was attributable to altered expression of these enzymes in dcKO mutants. However, this proved not to be the case, as deletion of BAF155 and BAF170 in dcKO_*Emx1*-Cre cortices and dcKO_*CAG*-Cre primary neurons did not affect the level of these enzymes (Figures S6A and S6B). This suggests that BAF complexes control the methylation of H3K27 through mechanisms other than direct modulation of H3K27 demethylase and methyltransferase expression.

These findings prompted us to investigate the effects of endogenous BAF155 and BAF170 levels on the demethylase activity of UTX/Kdm6a and JMJD3/Kdm6b. Cultured dcKO_*CAG*-Cre neurons were nucleofected with mammalian expression vectors for either UTX/Kdm6a-IRES-GFP or JMJD3/Kdm6b-IRES-GFP in the presence of TAM (loss of both BAF155 and BAF170) or absence of TAM (control) (Figure 7D). After 2 days in vitro (2 DIV), GFP-positive (GFP+) cells were enriched by fluorescence-activated cell sorting (FACS) and analyzed for UTX/Kdm6a and JMJD3/Kdm6b demethylase activity by WB with antibodies against H3K27me2 and H3K27me3. Notably, we found that the H3K27 demethylase activities of both UTX/Kdm6a and JMJD3/Kdm6b were lower in neurons lacking BAF155 and BAF170 (TAM+) than in control (TAM−) neurons (Figure 7D, lanes 3 and 5). We then examined whether the BAF complex directly influenced the demethylase activity of UTX/Kdm6a and JMJD3/Kdm6b. In vitro histone demethylase assays using purified H3 and UTX/Kdm6a/JMJD3/Kdm6b proteins and reconstituted BAF complexes (recBAFs) (Phelan et al., 1999) containing five core subunits (Brg1, Brm, BAF155, BAF170, and BAF47) revealed that recBAFs dramatically increased the enzymatic activity of both UTX/Kdm6a and JMJD3/Kdm6b (Figure 7E, lanes 3, 6, and 9). Furthermore, the blockage of interaction between BAF155/BAF170 and JMJD3/UTX by the synthesized peptides 462–643 reduced H3K27me2/3 demethylation (Figure 7F, lane 4).

To understand a biochemical basis for the ability of BAF complexes to enhance H3K27 demethylase activity, we examined the accessibility of JMJD3, UTX, and EZH2 to H3 in the absence and presence of BAF complexes by performing a CoIP with an antibody against H3, using dcKO_*CAG*-Cre neurons with and without TAM administration (Figure 7G). We found that the absence of BAF155/BAF170 (TAM+) did not affect the interaction between H3 and EZH2, whereas the interactions between H3 and JMJD3/UTX were largely abolished. In addition, an H3 pull-down assay using purified H3, UTX, JMJD3, proteins, and recBAFs revealed that recBAFs substantially increased the binding between H3 and JMJD3/UTX (Figure 7H). Thus, our findings implicated that BAF complexes modulate H3K27 demethylase activity of JMJD3/UTX by regulating their accessibility to H3.

To determine more directly whether H3K27me2/3-dependent epigenetic regulation influences BAF complex-dependent gene expression, we used GSK126, an H3K27me2/3 methyltransferase EZH2 inhibitor (McCabe et al., 2012). GSK126 administration in dcKO_*Emx1*-Cre mutants at E11.5 and E12.5 significantly enhanced the expression of six of 22 genes, as assessed by qRT-PCR (Figure 7I). Taken together, these in vivo and in vitro findings demonstrate that the presence of BAF complexes is required for the full H3K27 demethylase activity of UTX/Kdm6a and JMJD3/Kdm6b and is essential for the proper gene expression program.

Collectively, our results suggest that the scaffolding subunits BAF155 and BAF170 are indispensable for forebrain development. More specifically, our data suggest that BAF155/BAF170-dependent chromatin remodeling facilitates global gene regulation through direct interactions of these subunits with H3K27 demethylases that potentiate their activity (Figure 7J).

DISCUSSION

We investigated the roles of the two paralogous subunits, BAF155 and BAF170, in different cell types and demonstrated that these two subunits are essential regulators of the stability

(D–F) Requirement of BAF complexes for the full H3K27 demethylase activity of UTX/Kdm6a and JMJD3/Kdm6b, demonstrated by in vivo (D) and in vitro (E) demethylase assays. WB with a GFP antibody indicated that an equal amount of electroporated cells was used in the assay (D). The blockade of interaction between BAF155/BAF170 and the demethylases by peptides 462–643 diminished H3K27me2/3 demethylation (F).

(G and H) BAF complexes control the accessibility of JMJD3/UTX to H3, as revealed by CoIP (G) and pull-down (H) experiments.

(I) The qRT-PCR analyses of dcKO_*Emx1*-Cre mutants at E16.5 show the effects of treatment with the H3K27me2/3 methyltransferase EZH2 inhibitor GSK126 on the expression of BAF complex target genes.

(J) A hypothetical model proposing how loss of BAF155 and BAF170 in dcKO mutants controls the stability of BAF complexes and the epigenetic and neural gene expression program. Values are presented as means ± SEMs (n = 6; *p < 0.05, **p < 0.01, and ***p < 0.005). Ac, H3K9Ac; Me, H3K27me2/3; Ub, ubiquitin.

and activity of BAF complexes. We showed that expression of BAF155 and BAF170 is critical for formation of the forebrain and cortex. Our data indicated that BAF155 and BAF170 subunits have an essential function in controlling global H3K27me2/3 levels and gene expression program by modulating the enzymatic activity of Kdm6a (UTX) and Kdm6b (JMJD3) toward histone 3.

BAF155 and BAF170 Control the Stability and Function of the BAF Complexes

The data presented here highlight the role of BAF155 and BAF170 subunits as essential regulators of BAF complex function(s). BAF155 is highly expressed in proliferating stem/progenitor cells in different tissues and cell lineages, whereas its expression generally is downregulated during differentiation (Ho et al., 2009; Tuoc et al., 2013a; Yan et al., 2008). In contrast, BAF170 is expressed at lower levels in stem/progenitor cells and at higher levels in differentiated cells (Ho et al., 2009; Tuoc et al., 2013a; Yan et al., 2008). Nevertheless, our data indicate that low levels of BAF155 and BAF170 in post-mitotic cells and dividing stem/progenitor cells, respectively, are required to maintain the stability of all distinct BAF complexes in cortex (Figure 3; Figures S3–S5), including npBAF (Lessard et al., 2007), nBAF (Wu et al., 2007), BAF-A, BAF-B, and PBAF (Yan et al., 2008).

Previous studies have shown that ESCs, which contain distinctive esBAF complexes, express high levels of Brg1 and BAF155, but only low levels of Brm and BAF170 (Yan et al., 2008) or virtually none at all (Ho et al., 2009). However, a recent study reported that Brm catalyzes a minor fraction of esBAF complexes (Smith-Roe and Bultman, 2013), challenging the idea that Brg1 is the exclusive catalytic subunit of BAF complexes in ESCs. Although outside the focus of our study, we hypothesized that a low level of BAF170 expression together with BAF155 is required for the stability of the esBAF complex, as in late cortical progenitors (Figure 3A; Figure S3; Tuoc et al., 2013a). Therefore, we derived a dcKO_*CAG*-Cre ESC line from the corresponding transgenic blastocysts and examined protein levels of BAF subunits. We found that expression of all BAF subunits examined was almost completely abrogated in dcKO_*CAG*-Cre ESCs after 4 days of TAM treatment, as represented by Brg1 and Brm (Figure S4D). Taken together with our other findings, these results indicate that the loss of BAF155/BAF170 in dcKO mutants eliminates all known subunits of BAF complexes in cortical tissues, primary neurons, and ESC. Thus, dcKO mutants can be powerful tools for investigating the roles of the entire BAF complexes in development in vivo and in vitro.

It was recently reported that BAF subunits dissociate from each other in *BAF250a/b* double mutants, indicating that these subunits are important for the formation of BAF complexes (Helming et al., 2014). However, in contrast to our *BAF155/BAF170*-dcKO mutant, expression of many BAF subunits was only modestly reduced or even preserved in *BAF250a/b* double mutants. Similar to the case for other BAF subunits, expression of both BAF250a and BAF250b was lost in *BAF155/BAF170*-dcKO mutants, suggesting the possibility that BAF155 and BAF170 control the integrity of BAF complexes, at least in part, by regulating *BAF250a/b* expression. Still, why all BAF subunits became undetectable at the protein level in *BAF155/BAF170*-dcKO mutants, but not in *BAF250a/b* double mutants, requires further investigation. Interestingly, among BAF155- and BAF170-interacting proteins identified in our CoIP/MS analysis were numerous factors belonging to the UPS, such as ubiquitin ligases, deubiquitylases, and proteasome subunits. A separate, ongoing study is aimed at investigating the possibility that, in addition to their role in maintaining formation of BAF complexes, BAF155 and BAF170 might protect other BAF subunits by blocking their UPS-mediated proteolysis.

BAF Complexes Control Global Epigenetic and Gene Expression Programs in Cerebral Cortical Development

Developmental genes are poised to rapidly induce transcription and gene expression during neural development (Hirabayashi and Gotoh, 2010; Mohn et al., 2008; Tuoc et al., 2014). This poised state includes the simultaneous presence of histone modifications associated with transcriptional activation, such as H3K9Ac, and transcriptional repression, such as H3K27me2/3, which are established by the EZH2 methyltransferase of the polycomb repressive complex 2 (PRC2) and are removed by Kdm6a/b demethylases. Given that H3K27me2/3 marks are crucial for repression of many developmental genes in different tissues, the addition and removal of H3K27me3 in a cohort of tissue-specific genes are important steps in regulating proliferation and differentiation (Agger et al., 2007; Akizu et al., 2010; Bernstein et al., 2006; Boyer et al., 2006; Burgold et al., 2012; Cao et al., 2002; Estarás et al., 2012; Hirabayashi et al., 2009; Jepsen et al., 2007; Lee et al., 2006, 2012; Li et al., 2014; Miller et al., 2010; Pan et al., 2007; Pereira et al., 2010; Pirouz et al., 2013; Shpargel et al., 2014; Zhao et al., 2007). Accordingly, the loss of function of H3K27 methyltransferase EZH2 removes the H3K27me3 repressive mark in cortical progenitors, affecting the homeostasis of self-renewal and differentiation in the cerebral cortex (Pereira et al., 2010). Although a role for H3K27 demethylases in brain development is not known, the loss of both JMJD3 and Utx demonstrated significantly increased H3K27me3 levels with severe deficits of embryoid body (EB) differentiation in the double-mutant ESCs (Shpargel et al., 2014). Hence, the data implicated that H3K27me3 plays an important role in proliferation, differentiation, and cell-cycle progression.

An antagonistic relationship between PRC2 (H3K27 methyltransferase) and BAF complexes in modulating the level of H3K27me2/3 at target gene locations has been reported, although this remains a matter of considerable debate (Ho et al., 2011; Kia et al., 2008; Shao et al., 1999; Wilson et al., 2010). Loss of BAF47 in fibroblasts and cancer cells leads to elevated expression of the polycomb gene *EZH2* (Wilson et al., 2010), whereas deletion of Brg1 does not lead to upregulation of the PRC2 components Ezh2 or Suz12 in ESCs (Ho et al., 2011). Similar to the deletion of Brg1 in ESCs, the loss of both BAF155 and BAF170 does not affect the expression level of EZH2 or SUZ12 in cortical tissue or primary neurons (Figures S6A and S6B).

The removal of H3K27me2/3 marks is important for the robust induction of many cell-type-specific genes during differentiation (Burgold et al., 2008; Hirabayashi et al., 2009; Jacobs et al., 1999; Liu et al., 2009; Pereira et al., 2010).

The interaction between H3K27 demethylases (JMJD3 and UTX) and BAF subunits has been reported in Th1 cells (Miller et al., 2010) and lung tissues (Li et al., 2014), as well as in cardiac development (Lee et al., 2012). H3K27 demethylases and BAF complexes often are recruited to tissue-specific enhancers by key transcription factors, such as T-bet in Th1 cells (Miller et al., 2010); Nkx2.1 in lung development (Li et al., 2014); and SRF, Nkx2.5, Tbx5, and GATA4 in cardiac cells (Lee et al., 2012). These findings reveal a functional interaction between H3K27 demethylases and BAF complexes during the transition from poised to active chromatin that triggers tissue-specific chromatin changes during the development of many organs. Nevertheless, the loss of individual BAF subunits influences H3K27me2/3 levels only locally (Ho et al., 2011; Tuoc et al., 2013a). To date, no alteration in the bulk levels of H3K27me3 has been observed in BAF subunit mutants (Ho et al., 2011; Tuoc et al., 2013a). We showed in this study that BAF complexes themselves do not possess H3K27-methylating activity; however, they inhibit transcriptional repression in neural cells by potentiating Kdm6-mediated demethylation of H3K27me2/3.

To gain a genome-wide perspective on the role of BAF complexes in cortical development, we performed a transcriptome analysis of the dcKO cortex. These data suggest an important interaction between H3K27 methyltransferase and BAF complexes, as evidenced by the fact that ~90% of genes regulated by the H3K27me2/3 methyltransferase EZH2 are regulated by BAF complexes. Notably, these data also offer insights into the H3K27me2/3-independent roles of BAF complexes. Accordingly, our CoIP/MS data revealed that, in addition to H3K27 demethylases, BAF155 and BAF170 associate with many other factors, including signaling pathway, transcriptional, chromatin-remodeling, and epigenetic machineries. How the interaction between BAF complexes and these factors control gene expression programs at distinct neural developmental stages is an essential question that needs to be addressed in future studies.

Distinct BAF complexes have been shown to bind to and coordinate with tissue-specific transcription factors to regulate gene expression in different cell types, which explains the widespread role of BAF complexes in the development of numerous organs (Ho and Crabtree, 2010; Narayanan and Tuoc, 2014). Although BAF complexes per se have essential roles in development, analyses of mouse transgenic models in which individual subunits are deleted have yielded only a pinhole view of in vivo BAF complex function. Our data indicate that the expression of BAF155 and BAF170 is indispensable for embryogenesis and formation of the forebrain. In cortical development, BAF complexes are essential for proliferation, differentiation, cell-cycle progression, cell survival, and layer formation.

In summary, we showed that the absence of BAF155 and BAF170 in dcKO mutants leads to the dissociation of all other BAF subunits from the complex. Subsequently, the free BAF subunits are degraded by the UPS. Thus, the generation of dcKO mutants provides a tool to investigate the roles of entire BAF complexes in development. Loss of the entire BAF complex exerts effects on global chromatin status and gene expression in neural cells. As a small part of complex molecular interactions influenced by chromatin remodeling and epigenetic machinery, we have shown here that BAF155 and BAF170 mediate the interaction between BAF complexes and H3K27demethylases, controlling the global level of H3K27me2/3 and ultimately the gene expression program in cortical development. Our findings have uncovered an epigenetic mechanism that controls corticogenesis. Understanding functional interactions between BAF complexes and other factors of epigenetic machinery during development is a challenging task for future study.

EXPERIMENTAL PROCEDURES

Transgenic Mice

Floxed *BAF155* (Choi et al., 2012), floxed *BAF170* (Tuoc et al., 2013a), *FoxG1-Cre* (Hébert and McConnell, 2000), *Emx1-Cre* (Gorski et al., 2002), *CAG-Cre* (Hayashi and McMahon, 2002), and *Nex-Cre* (Goebbels et al., 2006) mice were maintained in a C57BL6/J background. Animals were handled in accordance with the German Animal Protection Law.

Plasmids and Antibodies

A list of plasmids and antibodies with detailed descriptions is provided in the Supplemental Experimental Procedures.

MS, Peptide Assay, CoIP, ChIP-Seq and Microarray Analysis, and qPCR

Detailed descriptions are provided in the Supplemental Experimental Procedures.

IHC, FISH, and Cell-Cycle Index

IHC and determination of cell-cycle index were performed as previously described (Tuoc et al., 2009).

In Vitro and In Vivo Demethylase Assays, Complex and Protein Stability Assay, TAM, and GSK126 Treatment

Detailed descriptions of other methods are provided in the Supplemental Experimental Procedures.

Culture of Standard Cell Lines, Primary Neurons, and ESCs

Cell culture conditions as well as derivation of mouse ESCs were described previously (Pirouz et al., 2013; Tuoc and Stoykova, 2008a, 2008b).

Image Acquisition and Statistical Analysis

All images were acquired with standard (Leica DM 6000) and confocal (Leica TCS SP5) fluorescence microscopes. Images were further analyzed with Adobe Photoshop. Statistical analyses were done using Student's t test. All graphs are plotted as mean ± SEM.

ACCESSION NUMBERS

The accession number for the ChIP-seq data reported in this paper is GEO: GSE73855.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.10.046.

AUTHOR CONTRIBUTIONS

T.T. conceived, supervised, and wrote the manuscript. J.F.S., A.S., A.F., and M.K. offered suggestions for the study. R.N., L.P., K.K., and J.R. carried out IHC analyses and biochemical assays. M.P. and M.K. performed CoIP/MS, biochemical assays, and generated ESC lines. C.K. and A.F. contributed to

ChIP-seq and analysis of genome-wide data. R.W. and J.F.S. carried out FISH experiments. A.S. and R.H.S. provided transgenic lines and contributed to discussions.

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